

Ampelographic and Genetic Diversity Assessment of Some Local Grape Genotypes under Egyptian Conditions

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Abstract

Grape (*Vitis vinifera* L.) is assumed as one of the most valuable and popular fruit crops all over the world. In this study, ten of local grape germplasm were characterized considering phenotypic diversity and genetic features under Egyptian conditions. Selected local grapes; Baltim Eswid, Edkawy, Matrouh Eswid, Bez El-Naka, Bez El-Anza, Romy Abiad, Romy Ahmer, Gharihi, Fayoumi and Banaty, were spread geographically among seven national governorates. A total of 58 attributes were characterized grape germplasm for the distinctness of vine parts. For molecular characterization, 9 nuclear SSR loci were analysed. Data revealed a broad sense of similarity at the level of studied morphological attributes, where the highest similarity (0.93) was between Romy Ahmer and Romy Abiad. A total of 24 alleles, ranging from 124 - 253 bp in size, were detected at the nine tested loci with an average of 2.67 alleles per locus. The amplification products in all of the 9 SSRs loci showed polymorphism among the 10 grapevine cultivars. The genetic relatedness among most of the cultivars was in accordance with their identification based on ampelographic properties. Data of phenotypic and molecular analysis revealed high variability of *Vitis* germplasm in Egypt and contributed substantially to our awareness of valuable local grape genetic resources which are on the verge of extinction.

Keywords

Ampelographic, Germplasm, Grape, Identification, Molecular

1. Introduction

Grape (*Vitis vinifera* L.) is assumed as one of the most valuable and popular fruit crops all over the world [1]. According to the Food and Agriculture Organization Corporate Statistical Database [2], the estimated total production of grapes worldwide in 2021 was 73,524,196.23 metric tons, and commercial table grape production in Egypt was forecast to reach 1,435,000 metric tons where table grapes rank second after citrus fruits in terms of production quantities in Egypt. Cultivation of grape is geographically extended from Alexandria in the north to Aswan in the south, where Behira governorate represents about forty percent of the total grapes planted area in Egypt and eighteen percent of its total production [3]. The main factors that give table grapes the opportunity to be grown all over Egypt are; climate conditions, soil types and production technology.

Varieties identification and description is a very important stage in the certification program, ensuring the accuracy of breeding materials by type, germplasm improvement and preservation, and monitoring of the genetic quality. Morphological and pomological traits continue to be the first step to describing and classifying any germplasm, as well as useful tools for screening accessions for any population [4].

Ampelographic characterization according to morphological features was useful in identifying famous grape cultivars and facilitated the illustration of ambiguous denominations or the establishment of phenological relationships. Ampelography is an accepted scientific methodology for the characterization of grapevine genotypes, based on the description of several morphological, phonological and pomological characteristics [4]. This method has been standardized and expanded by many scientists for a more reasonable and accurate identification of *Vitis* materials [5] [6].

Microsatellites had been used to identify grape cultivars by [7] who proved that, microsatellite sequences that are often represented in the grapevine genome are very informative for the identification of *Vitis vinifera* genotypes. Considering the simplicity, quickness and efficiency of detecting microsatellite polymorphism even with a very little DNA quantity using PCR technique, [8] demonstrated the eligibility of microsatellites as molecular markers for genotyping in order to solve dilemmas of synonyms, homonyms or the origin of varieties, for relatedness and population genetic studies, also for molecular identification of clones and marker-assisted selection breeding purposes.

Many grape cultivars and varieties were characterized and evaluated at different regions of Egypt [4] [9]. However, most of those investigations were incompletely covered the target of local cultivars' interest. These were led to decrease

the proliferation of native cultivars in number and put them at risk of extinction. Thus, the aim of the study was to characterize ten of local grape germplasm considering phenotypic diversity and genetic features under Egyptian conditions.

2. Materials and Methods

2.1. Plant Materials

Selected local grapes identified by growers as Baltim Eswid (Kafr El-Shiekh governorate), Edkawy (El-Beheira governorate), Matrouh Eswid (Monofiya governorate), Bez El-Naka (El-Beheira governorate), Bez El-Anza (Giza governorate), Romy Abiad (Giza governorate), Romy Ahmer (Menia governorate), Gharibi (Fayoum governorate), Fayoumi (Fayoum governorate from three different locations) and Banaty (Dakahliya and Monofiya governorates) were considered for morphological and molecular characterization (**Figure 1**). The cultivars were propagated and cultivated from 2022 onwards at the experimental field of Agricultural Research Centre, Horticulture Research Institute, Egypt.

2.2. Ampelographic Descriptions

Ampelographic data were collected during 2021 & 2022. Preparations and procedures of grape genetic resources (GRs) collection were conducted following the procedures of [10]-[16]. Grape descriptors were applied according to [17]. All morphological descriptors followed [18] terminologically as listed in **Table 2**. Data were recorded on 10-random healthy in each accession at specified plant growth stage when the attributes had expressed totally. Quantitative attributes were scored as an average of ten random accessions.

Data were described the morphological criteria and calculated average of all quantitative attributes. Data fed into the descriptive statistics with XLSTAT software to compute all attributes.

2.3. Simple Sequence Repeat (SSR) Analysis

For each sample, young leaves (1 - 2 cm of diameter) were collected and DNA was extracted using the Qiagen DNeasy Plant Mini Kit (QIAGEN, Germany) according to the manufacturer protocol, where the DNA was finally eluted with 50 µl of TE buffer. The quality of the extracted DNA was assessed by gel-electrophoresis in 1% agarose gels and the concentration of DNA was determined by means of NanoDrop Spectrophotometry (MicroDigital, Korea). Then after, the extracted DNA was stored at -80°C till further molecular analysis.

For molecular characterization using microsatellite, the samples were analysed by nine nuclear SSR loci (**Table 1**), which were previously used in genotyping of grapevine collection according to [19] [20]. This set of SSRs was selected based on their position in the linkage groups of *Vitis vinifera* to cover the whole genome and their capability to distinguish closely related grapevine genotypes.

PCR amplifications were carried out in 25 µl final volume reaction mixtures using a T100 Thermal Cycler (Bio-Rad, USA), where 3 µl of DNA template, 1 µl

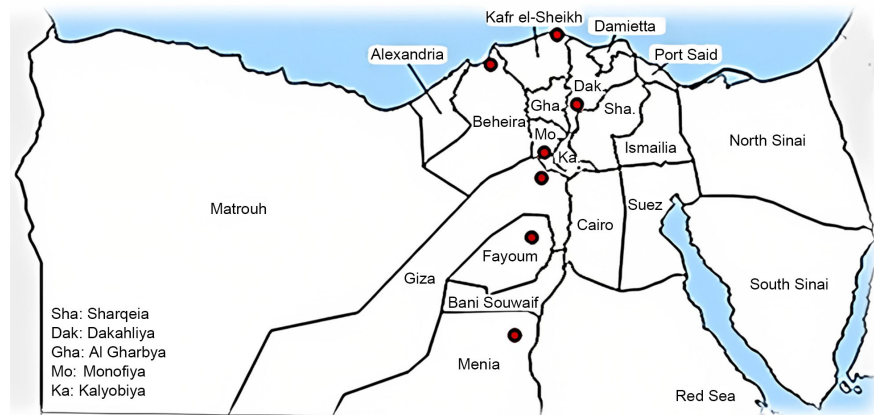


Figure 1. Geographical locations of selected local grape genetic resources.

Table 1. Sequences of Simple Sequence Repeat primers (SSRs) used for grapevine genotyping.

	Primer	Sequence (5'-3')	Reference
VVMD5	Forward	CTAGAGCTACGCCAATCCAA	[21]
	Reverse	TATACCAAAAATCATATTCTAAA	
VVMD7	Forward	AGAGTTGCGGAGAACAGGAT	[22]
	Reverse	CGAACCTTCACACGCTTGAT	
VVMD21	Forward	GGTTGTCTATGGAGTTGATGTTGC	[22]
	Reverse	GCTTCAGTAAAAAGGGATTGCG	
VVMD24	Forward	GTGGATGATGGAGTAGTCACGC	[22]
	Reverse	GATTTTAGGTTTCATGTTGGTGAAGG	
VVMD27	Forward	GTACCAGATCTGAATACATCCGTAAGT	[22]
	Reverse	ACGGGTATAGAGCAAACGGTGT	
VVMD32	Forward	TATGATTTTTTAGGGGGTGAGG	[22]
	Reverse	GGAAAGATGGGATGACTCGC	
VVS2	Forward	CAGCCCGTAAATGTATCCATC	[7]
	Reverse	AAATTCAAAATTCTAATTCAACTGG	
VrZAG62	Forward	GGTGAAATGGGCACCGAACACACGC	[19]
	Reverse	CCATGTCTCTCCTCAGCTTCTCAGC	
VrZAG79	Forward	AGATTGTGGAGGAGGGAACAAACCG	[19]
	Reverse	TGCCCCCATTTTCAAACCTCCCTTCC	

of each primer (Willowfort), 12.5 μ l of Cosmo PCR red master mix (Willowfort, UK) and 7.5 μ l of nuclease free water were included in each reaction. The thermal profile of PCR reaction was programmed as follows: initial denaturation at 94°C for 4 min, followed by 36 of repeated cycles where each consisted of; denaturation at 94°C for 1 min, annealing at 56°C or 60°C (only for VrZag62 and VrZag79) for 45 s and extension at 72°C for 1 min, then final extension at 72°C for 20 min [23].

Afterwards, amplicons were analysed with agarose gel electrophoresis (Bio-Rad, USA) where the products were resolved on 1.5% agarose gel in 1x TBE (10x: 0.45 M Tris-borate, 0.01 M Na₂EDTA, pH 8) and ethidium bromide staining, then photographed using Gel Documentation System (UVITEC Cambridge, UK). The molecular size of fragments was estimated by reference to the 100 bp DNA ladder (GeneDireX). Each amplification reaction was conducted twice to confirm the obtained results.

The polymorphic information content (PIC) and heterozygosity values were calculated for each SSR locus by using Gene-Calc online bioinformatics tool. PCR fragments on gels were recorded as one “1” if presence or zero “0” if absence for all samples, then a binary statistic matrix was constructed. Similarity matrices correlation between accessions were calculated using Dice coefficient, then this matrix was used to construct a phylogenetic dendrogram with Unweighted Pair-Group Average method (UPGMA) using XLSTAT software.

3. Results

3.1. Morphological Characterization of Egyptian Grape Cultivars

3.1.1. Polymorphism of Grape Morphological Criteria

A total of 58 attributes were characterized ten Egyptian grape germplasm for the distinctness of vine parts, where no morphological variations were detected for different locations of Fayoumi or Banaty cultivars. A broad sense of polymorphism is notably data as shown in **Table 2** & **Figure 2**. Some grape cultivars have a differentiate in an attribute of form of tip (Matrouh Eswid), erect hairs on internodes (Gharibi), petiole sinus limited by vein (Fayoumi), presence of seeds (Banaty) and particular flavor (Bez El-Anza). Other more germplasm shared distinctness in an attribute. These are density of erect hairs on tip, density of prostrate hairs on tip, density of erect hairs on node, density of prostrate hairs on node, density of prostrate hairs on internode, number of consecutive tendrils, density of prostrate hairs on main veins, density of prostrate hairs between veins, density of erect hairs on main veins, density of erect hairs between veins, anthocyanin coloration of main veins on upper side of blade, profile of mature leaf, length of teeth and size of grape.

3.1.2. Correlation Indices of Grape Germplasm According to Morphological Criteria

Correlation matrix of ten grape germplasm was calculated as shown in **Table 3**. Data revealed a broad sense of similarity at the level of studied morphological attributes. The lowest similarity (0.24) was scored between Gharibi and Banaty. The highest similarity (0.93) was between Romy Ahmer and Romy Abiad.

The binary data were used to estimate the ampelographic similarity among the 10 grape genotypes **Figure 3**, where they formed two distinctive clusters. The first cluster (C1) contained only Gharibi cultivar. While, the second cluster (C2) included four groups; the first group contained the only seedless cultivar (Banaty), while the second group included the three black berries cultivars (Edkawy,

Baltim Eswid & Matrouh Eswid), the third group composed of Bez El-Anza, Romy Ahmer, & Romy Abiad, and finally the fourth group contained Bez El-Naka & Fayoumi.

Table 2. Descriptors for Egyptian grape cultivars.

Trait	Baltim Eswid	Edkawy	Matrouh Eswid	Bez El-Naka	Bez El-Anza	Romy Abiad	Romy Ahmer	Gharibi	Fayoumi	Banaty
Young shoot										
Form of tip	5	5	4	5	5	5	5	5	5	5
Anthocyanin coloration of tip	1	5	0	5	1	0	0	5	0	0
Density of prostrate hairs on tip	1	0	0	0	0	0	0	7	0	0
Density of erect hairs on tip	1	0	0	0	0	0	0	7	0	0
Shoot										
Attitude	3	5	2	5	1	2	3	1	5	2
Color of dorsal side of internode	2	2	1	2	2	1	3	2	1	2
Color of ventral side of internode	1	2	2	2	2	1	1	3	1	2
Color of dorsal side of node	2	1	1	2	2	1	3	3	2	1
Color of ventral side of node	1	2	2	2	2	1	1	3	2	1
Density of erect hairs on node	1	0	0	0	1	0	0	7	0	0
Erect hairs on internode	0	0	0	0	0	0	0	1	0	0
Density of prostrate hairs on node	1	0	0	0	0	0	0	5	0	0
Density of prostrate hairs on internode	1	0	0	0	0	0	0	1	0	0
Number of consecutive tendrils	2	1	1	1	1	1	2	2	1	1
Length of tendril	3	5	5	7	1	1	3	5	5	5
Young leaf										
Color of upper surface	1	1	1	1	1	1	1	1	1	1
Density of prostrate hairs between veins	1	0	0	0	0	0	0	7	0	0
Density of erect hairs between veins	1	0	0	0	0	0	0	7	0	0
Density of prostrate hairs on main veins	1	0	0	0	0	0	0	7	0	0
Density of erect hairs on main veins	1	0	0	0	0	0	0	3	0	0
Mature leaf										
Size of blade	5	5	7	7	7	5	5	5	5	7
Shape of blade	3	2	3	4	3	3	3	2	3	3
Number of lobes	3	3	3	4	3	3	2	3	4	3
Anthocyanin coloration of main veins on upper side of blade	1	0	3	0	0	0	0	0	0	0
Profile	1	1	1	2	1	1	1	2	1	1
Blistering of blade upper surface	0	0	0	0	0	0	0	0	0	0
Shape of teeth	2	3	3	5	3	3	5	2	2	3

Continued

Length of teeth	5	3	5	5	3	5	5	5	5	5
Ratio length/width of teeth	5	3	5	5	3	5	3	5	5	3
General shape of petiole sinus	3	3	2	3	3	2	3	1	2	3
Tooth at petiole sinus	0	1	1	0	0	1	0	0	1	1
Petiole sinus limited by veins	0	0	0	0	0	0	0	0	1	0
Shape of upper lateral sinus	1	1	1	1	1	1	1	1	1	1
Depth of upper lateral sinus	5	5	3	9	5	5	5	7	5	7
Density of prostrate hairs between veins	1	0	1	0	0	0	0	3	0	0
Density of erect hairs between veins	1	0	1	0	0	0	0	3	0	0
Density of prostrate hairs on main veins	1	0	0	0	0	0	0	3	0	0
Density of erect hairs on main veins	1	0	0	0	0	0	0	3	0	0
Prostrate hairs on main veins	1	0	0	0	0	0	0	1	0	0
Length of petiole compared to middle vein	3	2	1	3	4	2	3	2	2	3
Woody shoot										
Surface	1	1	2	1	1	1	1	2	1	3
Main color	4	1	2	1	2	1	1	3	1	2
Inflorescence and fruit inflorescence										
Sex of flower	3	3	3	3	3	3	3	3	3	3
Bunch										
Size	7	7	7	5	5	7	7	7	5	1
Density	5	7	7	7	7	7	7	5	7	9
Length of peduncle	5	5	3	3	3	7	5	7	5	9
Berry										
Size	7	7	7	7	7	7	7	7	3	1
Shape	4	6	3	9	1	4	4	3	5	4
Presence of seeds	3	3	3	3	3	3	3	3	3	1
Skin color (without bloom)	6	6	5	1	1	1	4	1	1	1
Anthocyanin coloration of flesh	9	7	5	1	1	1	1	1	1	1
Juiciness of flesh	2	2	2	1	1	1	1	2	1	2
Firmness of flesh	3	2	2	1	3	3	2	3	1	1
Particular flavor	1	1	1	1	2	1	1	1	1	1
Ease of detachment from pedicel	1	2	1	3	2	3	1	1	3	2
Seed length	7	5	5	5	5	7	7	5	5	3
100-seed weight	5	9	5	7	5	7	7	7	9	1
Transversal ridges on side	0	0	0	0	0	0	0	0	0	0

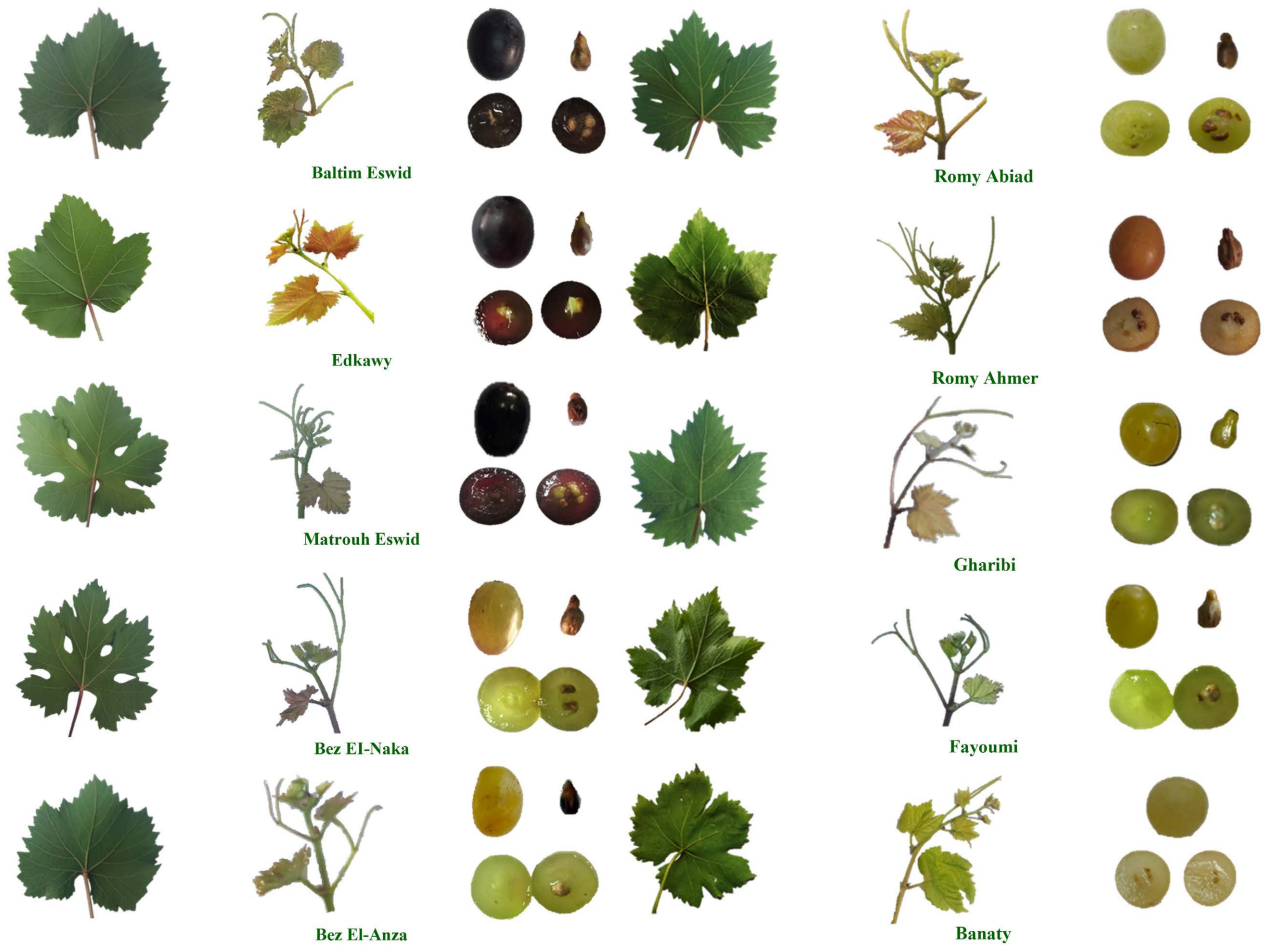


Figure 2. Morphological features of selected local grape germplasm.

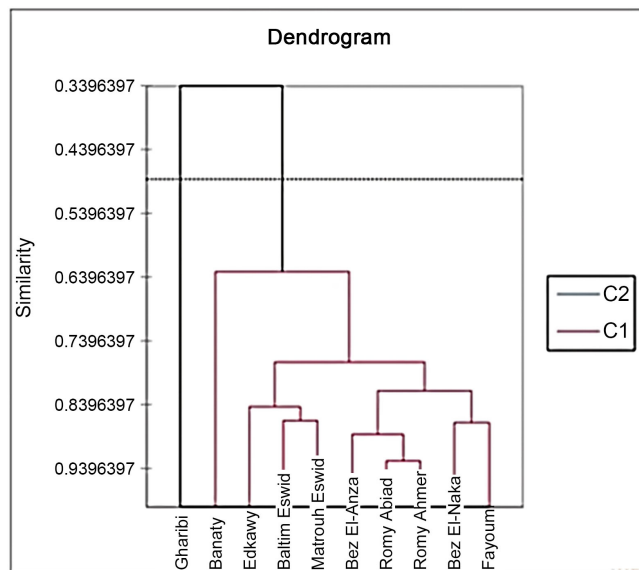


Figure 3. Phylogram generated by UPGMA using 58 morphological attributes for 10 grapevine cultivars cultivated in Egypt. The tree was constructed based on similarities among the cultivars according to Pearson correlation coefficient. C1 and C2 correspond to clusters 1 and 2, respectively.

Table 3. Correlation matrix of grape cultivars according to morphological criteria.

Cultivars	Baltim Eswid	Edkawy	Matrouh Eswid	Bez El-Naka	Bez El-Anza	Romy Abiad	Romy Ahmer	Gharibi	Fayoumi	Banaty
Baltim Eswid	1.00									
Edkawy	0.85	1.00								
Matrouh Eswid	0.86	0.84	1.00							
Bez El-Naka	0.65	0.82	0.74	1.00						
Bez El-Anza	0.72	0.73	0.80	0.77	1.00					
Romy Abiad	0.78	0.79	0.82	0.80	0.89	1.00				
Romy Ahmer	0.81	0.84	0.85	0.83	0.88	0.93	1.00			
Gharibi	0.35	0.31	0.28	0.36	0.37	0.42	0.37	1.00		
Fayoumi	0.68	0.81	0.78	0.87	0.77	0.89	0.86	0.35	1.00	
Banaty	0.52	0.55	0.60	0.69	0.66	0.68	0.65	0.24	0.70	1.00

3.2. Molecular Characterization of Egyptian Grape Cultivars

3.2.1. SSR Analysis of Grape

A total of 24 alleles, ranging from 124 - 253 bp in size, were detected at 9 tested loci with an average of 2.67 alleles per locus **Table 4**. The amplification products in all of the 9 SSRs loci showed polymorphism among the 10 grapevine cultivars, neither Fayoumi nor Banaty samples collected from different locations were genetically identical. The total number of alleles per locus ranged from 2 for VVMD5, VrZAG62 and VrZAG79 to 3 for the rest. The primer heterozygosity ranged from 0. 0.34 (VrZAG62) to 0.60 (VVMD7). The average PIC value of the 9 SSR markers used is 0.43 with the highest values of 0.52 for VVMD7, VVMD21 & VVMD24, and 0.51 for VVMD27, and the lowest of 0.28 for VrZAG62, where informative primers are shown by PIC values ≥ 0.5 [24], whereas primers with higher PIC values are superior to be use as molecular markers.

3.2.2. Correlation Indices of Grape Germplasm According to Genetical Criteria

The dendrogram based on SSR similarity indices as shown in **Figure 4**, separated the 10 grape genotypes into two distinctive clusters. The first cluster (C1) included, the two black berries cultivars (Baltim Eswid & Matrouh Eswid) in a group, and the two cultivars which belongs to El-Beheira governorate (Edkawy & Bez El-Naka) in another one. The second cluster (C2) included two main groups, the first group contained Fayoumi and Bez El-Anza, while the second group was composed of the only seedless cultivar (Banaty) in a sub-group and Romy Ahmer, Gharibi & Romy Abiad in another one.

4. Discussion

Plant descriptors is a key vital to utilization for various specialists [25] [26] and can help for “1” managing the strategy of collections, “2” the capacity to manage in gene bank, and “3” facilitating the exchange of the GRs and information [27]. These results are in the same line with [4] [9]. [28] evaluated ten grape cultivars

and hybrids grown in South region of turkey. [9] characterized ten cultivars grown under Assuit climatic conditions morphologically. [4] evaluated six newly-introduced grape cultivars under Egyptian conditions.

This investigation supported the utilization of morphological descriptors as an approach of measuring genetic similarity [26]. The association between procedures suggested that, the morphological traits will continue to be useful to inexpensively identify diverse germplasm. The results between various approaches may be due to contravention data recording and between the procedures of biostatistics also. The measuring variability and its structure might still require more investigation under various eco-environment conditions and decisions on a specific, optimal, and sampling strategy.

Table 4. PCR results statics as revealed by 9 SSR markers among the 10 grape cultivars.

Primer	No. of alleles	Allele size (bp)	Polymorphic information content	Primer heterozygosity
VVMD5	2	212 - 224	0.34	0.43
VVMD7	3	213 - 242	0.52	0.60
VVMD21	3	211 - 232	0.52	0.59
VVMD24	3	182 - 195	0.52	0.59
VVMD27	3	160 - 173	0.51	0.59
VVMD32	3	223 - 248	0.34	0.38
VVS2	3	124 - 137	0.46	0.52
VrZAG62	2	163 - 177	0.28	0.34
VrZAG79	2	243 - 253	0.34	0.43

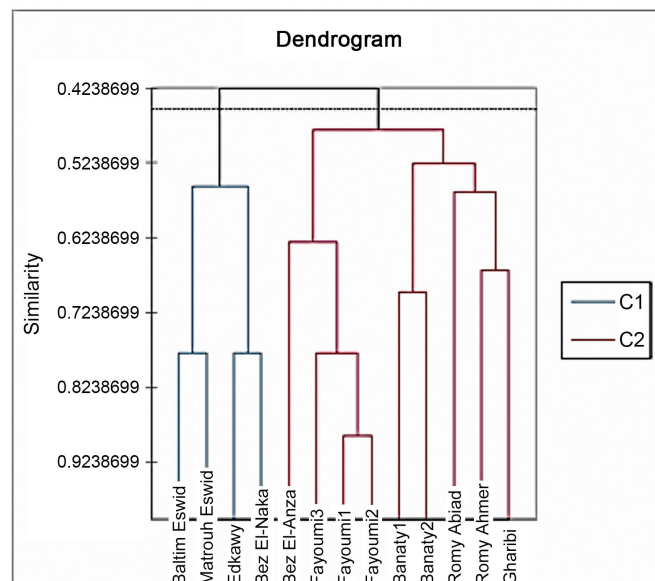


Figure 4. Phylogram generated by UPGMA using 9 microsatellite markers for 10 grapevine cultivars cultivated in Egypt. The tree was constructed based on genetic similarities among the cultivars according to Dice coefficient. C1, C2 and C3 correspond to clusters 1, 2 and 3, respectively.

Simple Sequence Repeat (SSR), as one of various marker systems, was reported by [29] as the most preferential and reliable marker for genotyping in grapevine due to its high polymorphism, abundance in the genome, co-dominant nature and inheritance in Mendelian manner.

The genetic relatedness among most of the tested cultivars was in accordance with their identification based on ampelographic properties. For example, the two black berries cultivars (Baltim Eswid & Matrouh Eswid) were represented close relatedness using both ampelographic and molecular tools recording 0.86 and 0.78, respectively. On the other hand, according to both ampelographic and genetic relatedness, the two cultivars which belongs to Fayoum governorate (Gharibi & Fayoumi) affiliated under two different groups, while Gharibi instead shared one group under the same cluster using molecular tools with Romy Abiad which is the closest using ampelographic properties (0.42). Additionally, both ampelographic and molecular tools confirmed the closest related to Romy Abiad is Romy Ahmer (0.93 and 0.60, respectively), while both belongs to different governorates (Giza and Menia, respectively).

Although microsatellite loci proved to be a useful tool to differentiate among grapevine cultivars based on their genotypic diversity, this method showed that the genetic relatedness among the characterized cultivars was not always in accordance with their classification based on ampelographic properties. [30] proposed that this may be due to the tested SSR markers are not part of the genome coding for these morphological traits. It was suggested that, further studies targeting more SSR loci as well as other genetic markers such as Inter Simple Sequence Repeats (ISSR) and Amplified Fragment Length Polymorphism (AFLP) might further strengthen the genetic relatedness among the identified cultivars of grapevine.

The potential of both phenotypic and molecular tools for solving problems of cultivar identification was reported by many authors. [31] explained that, the absence of a comprehensive germplasm database and problems in naming of various cultivars are between many factors makes these tools suitable for genotyping purposes.

5. Conclusion

In this investigation, ten cultivars that barely exist in old Egyptian vineyards were identified, recovered and characterized. Data of phenotypic and molecular analysis revealed high variability of *Vitis* germplasm in Egypt and contributed substantially to our awareness of valuable local grape genetic resources that are on the verge of extinction, which in turn reinforces the protection of endangered local grape biodiversity. Moreover, utilization of native genetic resources described here may contribute to the development of viticulture in Egypt.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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