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Molecular characterization and genetic relationships among some grape(*Vitis vinifera* L.) cultivars as revealed by RAPD and SSR markers

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ABSTRACT

This work were focusing on estimating of intra- and inter-cultivar polymorphisms among eight seedlings (one year old) of some grape cultivars: Superior; Early Superior; Thompson seedless-1; Thompson seedless-2; Thompson seedless-3; Fayomi; Roumi Ahmer and Bez El-Anza, (collected from Agricultural Research Center (ARC) farm, Giza, Egypt), as an efficient tool in order to assess the genetic relationships and develop cultivar-specific molecular fingerprints. We have used 8 SSR pairs of primers specific for grape and 10 RAPD primers. The SSR's and RAPD's have confirmed each other and the RAPD results were more realistic comparing to SSR ones, and these analyses fit together with fruit characters. One of the major clusters contains the three cultivars, Thompson seedless-1; Thompson seedless-2 and Thompson seedless-3, that sharing the same fruit characters. Moreover, the other major cluster contains Superior; Early Superior and Fayomi, besides two other separated cultivars, Roumi Ahmer and Bez El-Anza, which showed different fruit characters [35].when combined RAPD's and SSR's data together, it has showed genetic similarity ranged between 71.3 % and 93.1%. The highest genetic similarity was 93.1% between Superior and Early Superior, and similarity of 86% between Fayomi and both of Superior and Early Superior cvs. Moreover genetic similarity ranged between 87% and 89% which were recorded among Thompson seedless-1, Thompson seedless-2 and Thompson seedless-3 cultivars. Our long term objective is to use these fingerprints to identify molecular markers that co-segregate and could be used in isolating gene(s) which controlling some important traits, thereafter can be used in breeding programs (marker assisted selection). The different RAPD and SSR primers produced a total of 122 and 43 amplicons respectively; of which 80 and 33 amplicons were polymorphic among the studied cultivars. The SSR's and RAPD's data were very promising in this aspect, because we could record very specific PCR products, especially those with Thompson seedless-1, Superior, Early Superior, Roumi Ahmer and Bez El-Anza cultivars. The outcome of this research project could help strongly the exiting pool of information on grape which may help assess national grape breeding programs.

Key Words: Grape; *Vitis vinifera* L.; Molecular markers; SSR-PCR; RAPD-PCR; DNA-polymorphism; PCR.

INTRODUCTION

Assessment of crop germplasm diversity phenotypically is usually devoid of the resolving power needed to identify an individual genotype. Identification based on morphological characters is time consuming and requires extensive field trials and evaluation [2]. In addition, morphological differences may be epigenetic- or genetic-based characters [27, 29, 30]

Grape (*Vitis vinifera* L.) is one of the oldest and most important perennial crops in the world. Anatolia has long been linked with the origin of viticulture and wine making, especially in its Eastern and Southeastern regions to which the earlier authors commonly ascribe its origin. In Turkey, a large grape germplasm, consisting of about 1200 accessions, were conserved and had so far been transferred from the different ecological zones of the country to the National Germplasm Repository Vineyard in Tekirdag [7, 10, 16].

Conventionally, genetic diversity is estimated on the basis of morphological and phenotypic characters. Assessment of crop germplasm diversity phenotypically and morphologically is usually devoid of the resolving power needed to identify an individual genotype. Estimation by biochemical markers, viz. isozyme analysis, may also be biased as only a minor portion of genome is represented by these markers [31]. Molecular markers due to their advantages against to morphological and biochemical markers such as their plentifully, independence of tissue or environmental effects, diversity identification and selection in the earlier stages of plant development, can be a useful complement to morphological and physiological characterization of plants [17].

Ampelography traditionally has been used for the identification of grape varieties. The analysis of morphological characters is a fast and inexpensive method for variety identification, especially among distantly related genotypes. However, they are strongly influenced by the environment and their interpretation is sometimes subjective, thus reliable discriminations among morphologically similar germplasm are difficult. This source of error frequently leads to mislabeling of individuals, giving rise to cultivar homonyms and/or synonyms [21]. Molecular DNA markers, on the other hand, are not influenced by the environment and their interpretation is more objective. Therefore, they are a valuable alternative for fingerprinting closely related genotypes. Among them, random amplified polymorphic DNA (RAPD) [28], restriction fragment length polyphorphism (RFLP) [5], amplified fragment length polymorphism (AFLP) [35], and simple sequence repeat (SSR) [34] have been used for cultivar identification and genetic diversity studies in grapevine. SSRs represent co-dominant, hyper-variable loci that are suitable for discriminating among closely related taxa. They have become the DNA marker of preference for a number of grapevine genetic studies [26, 34, 15, 25, 1, 13, 23, 22].

The superiority of molecular markers over ampelometry for the characterization of grape cultivars is well established. In grape, molecular markers like RFLP, RAPD [19, 38], microsatellite or SSR [32] and AFLP [24] are widely used for characterization of cultivars, parentage analysis, identification of clones, establishing the genetic relationship and molecular mapping. Microsatellites and AFLP are the two very useful classes of molecular markers. Commercial varieties of grape are extensively analyzed with molecular markers, however only limited reports are available on molecular characterization of grape rootstocks [11]. This study was undertaken to characterize grape rootstocks available in Indian Grape Germplasm at our Centre, using SSR and AFLP markers. The random amplified polymorphic DNA (RAPD) technique has several distinct advantages: the cost per reaction is low; only a small amount of

plant material is required for DNA extraction; and the method does not require prior knowledge of the sequence of the genome. Theoretically, the polymorphisms revealed by the RAPD markers are mainly due to nucleotide substitutions or insertions/deletions [33]. The RAPD technique has been successfully used for identification of grapevines [6, 18].

MATERIALS AND METHODS

Eight seedlings (one year old) of grape cultivars (Superior; Early Superior; Thompson seedless-1; Thompson seedless-2; Thompson seedless-3; Fayomi; Roumi Ahmer and Bez El-Anza) samples were collected from Agricultural Research Center (ARC) farm, Giza, Egypt. The selected seedlings were treated as follows:

1. Isolation and purification of genomic DNA from the different grape cultivars:

Fresh young healthy grape leaves were collected from the studied grape cultivars and grounded to powder with liquid nitrogen using a mortar and pestle. Genomic DNA was isolated from leaf samples using the procedure described by DNeasy Plant Mini Kit protocol (QIAGEN, Germany).

2. Establishment of Molecular Marker techniques:

2.1. Performing SSR's and RAPD's techniques:

2.1.1. SSR analysis: PCR reaction and condition.

Extracted grape genomic DNA was PCR-amplified using 8 pair primers (table 1) flanking SSR sequences, previously cloned and sequenced in grape [5] . The SSR primers product was manufactured in the laboratories of the Midland Certified Reagent Company Inc. of Midland, Texas, USA. The amplification performed in a 25 µl reaction volume containing about 3µl (10ng/ul) genomic DNA, 2 µl primer / each (F and R) (Operon Technologies Inc.) and 18 µl master mix (Promega).The PCR temperature profile was applied through a Gene Amp® PCR System 9700 (Perkin Elmer, England).

Table1. List of the 8 SSR primers used in this study, grape simple sequences repeat (SSR) markers assayed and polymorphism obtained in the studied grape genotypes

No.	SSR Locus	Primer Sequences (5' → 3')	Length in bases	Temperature of annealing $^{\circ}c$
1	VVMD5-F	ctagagctacgccaatccaa	20	56
	VVMD5-R	tataccaaaaatcatattcctaaa	24	
2	VVMD6-F	atctctaaccctaaaaccat	20	52
	VVMD6-R	ctgtgctaagacgaagaaga	20	
3	VVMD7-F	agagttgcggagaacaggat	20	52
	VVMD7-R	cgaaccttcacacgcttgat	20	
4	VVMD14 F	catgaaaaaatcaacataaaagggc	25	56
	VVMD14 R	ttgttacccaaacacttcactaatgc	26	
5	VVMD21 F	ggttgtctatggagttgatgttgc	24	56
	VVMD21 R	gcttcagtaaaaagggattgcg	22	
6	VVMD24 F	gtggatgatggagtagtcacgc	22	56
	VVMD24 R	gattttaggttcatgttggtgaagg	25	
7	VVMD25 F	ttccgttaaagcaaaagaaaaagg	24	56
	VVMD25 R	ttggatttgaaatttattgagggg	24	
8	VVMD27 F	gtaccagatctgaatacatccgtaagt	27	56
	VVMD27 R	acgggtatagagcaaacggtgt	22	

The thermal cycler was programmed with an initial step of 5 min at 94° C; the amplification reaction was carried out using 40 cycles of 40 s at 94° C, an annealing step of 1 min at specific annealing temperature for each primer as mentioned in Table 2, and an elongation step of 1min at 72°C; and finally a 7 min extension at 72° C. The amplification products were detected by

electrophoresis on 8% Polyacrylamide non-denaturing gels in 1X TBE buffer at 95 volts. 100bp DNA ladder Marker was used (fermants).PCR products were visualized on UV light and photographed using a gel documentation system (Bio-Rad® Gel Doc-2000).

2.1.2. RAPD analysis: PCR reaction and condition.

A total of 10 random primers were used, (Table 2). The amplification performed in a 25 µl reaction volume containing about 3µl (10ng/µl) genomic DNA, 3 µl primer (Operon Technologies Inc.) and 19µl master mix (Promega). The PCR temperature profile was applied through a Gene Amp® PCR System 9700 (Perkin Elmer, England).The thermal cycler was programmed with an initial step of 5 min at 94° C; the amplification reaction was carried out using 40 cycles of 40 s at 94° C, an annealing step of 1 min at 36° C, and an elongation step of 1min at 72°C; and finally a 7 min extension at 72° C. The amplification products were resolved by electrophoresis in a 1, 5 % agarose gel containing ethidium bromide (0.5µg/ml) in 1X TBE buffer at 95 volts. @x 174 Marker cutting by *hae*III (OP-A03 & OP-A11 & OP-B07) and100bp DNA ladder (for the rest primers) were used (fermants).PCR products were visualized on UV light and photographed using a gel documentation system (Bio-Rad® Gel Doc-2000).

Table2: List of random primers used for RAPD analysis

Primer No.	Primer name	Primer sequence
1	OP-A03	5'-AGT CAG CCA C-3'
2	OP-A07	5'-GAA ACG GGT G-3'
3	OP-A11	5'-CAA TCG CCG T-3'
4	OP-A15	5'-TTC CGA ACC C-3'
5	OP-B02	5'-TGA TCC CTG G-3'
6	OP-B07	5'-GGT GAC GCA G-3'
7	OP-B16	5'-TTT GCC CGG A-3'
8	OP-B19	5'-ACC CCC GAA G-3'
9	OP-G19	5'-GTC AGG GCA A-3'
10	OP-G20	5'-TCT CCC TCA G-3'

3. Data scoring and statistical analysis:

To ensure the absence of artifacts, bands were carefully selected from replicated amplifications. Amplified bands were designated by their primer code and their size in base pairs. Data were recorded as discrete variables: 1 for the presence and 0 for the absence of a similar band. Only intense and reproducible bands appearing on the gel will be scored.

Band scoring was analyzed using Gene Tools-gel analysis software of SYNGENE (Beacon House, Nuffield Road, Cambridge, UK). Genetic relatedness among genotypes were studied by UPGMAM (Unweighted Pair Group Method with Averages Mean) cluster analysis and Principal Component analysis [37] using diversity database software which provided by Bio-Rad manufacture.

RESULTS AND DISCUSSION

SSR-PCR fingerprinting of grape cultivars:

The use of SSRs molecular markers was aiming to show fast and reliable discrimination of any variations. In this kind of molecular markers, the DNA of eight cultivars of grape were used as templates for eight pairs of primers. The SSR-PCR results, using primers pair (VVMD-5) are illustrated in Fig. (1-A). The molecular size of two PCR products ranged from 350 bp to 450 bp.

One common band of 450 bp was observed in all cultivars, and exhibited 50% monomorphism, while the second fragment showed polymorphism among the 8 cultivars.

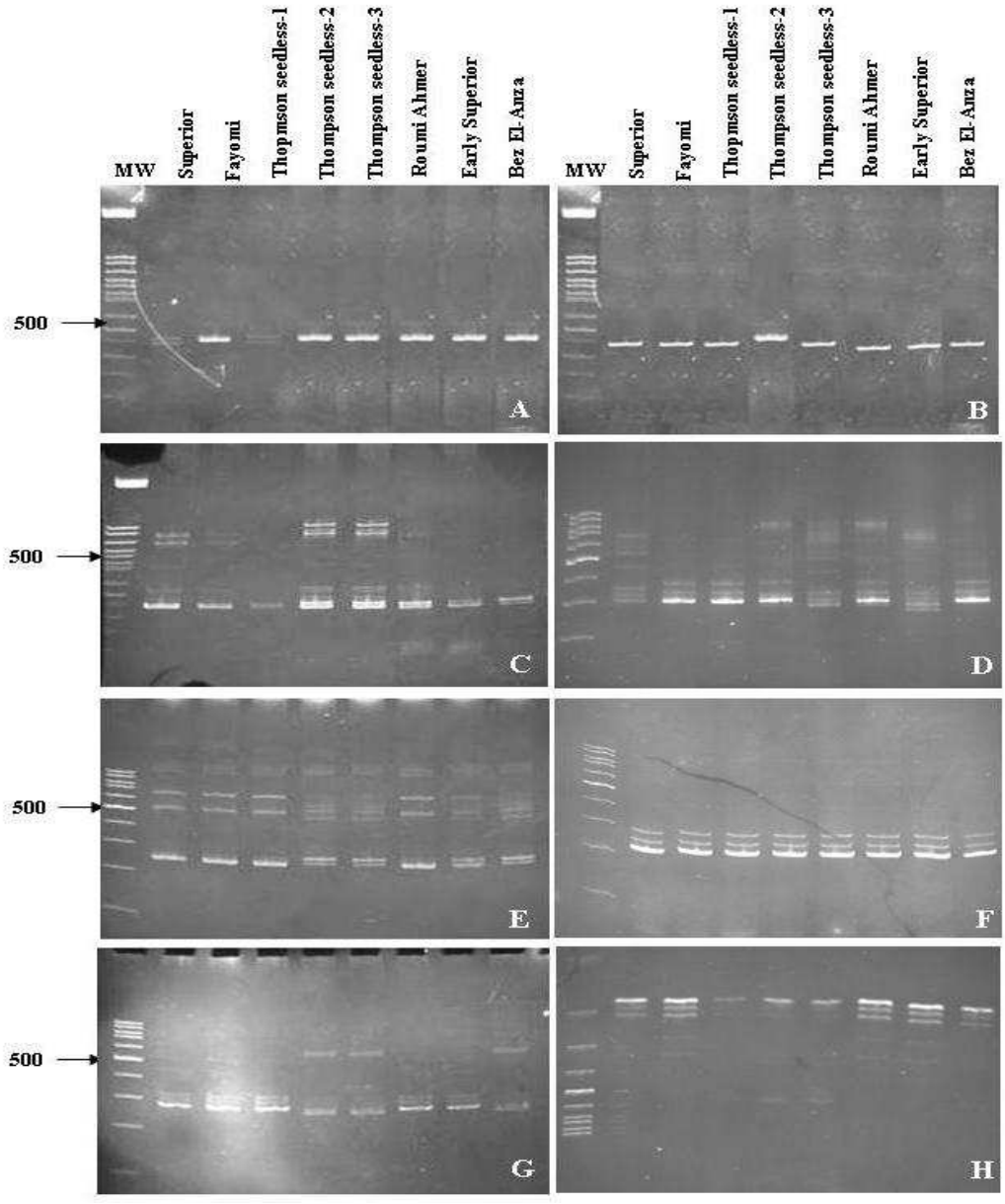

Figure 1: SSR fingerprinting of 8 local Egyptian grape cultivars (*Vitis vinifera* L.). (A)PrimerVVMD-5; (B)Primer VVMD-6; (C)Primer VVMD-7; (D)Primer VVMD-14 (E) Primer VVMD-21; (F)Primer VVMD-24; (G)Primer VVMD-25(H)Primer VVMD-27

While, with primers pair (VVMD-6), a total of three bands in the eight used cultivars are illustrated in Fig. (1-B) and molecular size ranged from 350 bp to 450 bp. The 3 fragments were polymorphic among the eight cultivars. These pair of primers was very important in our study, they showed a very interesting products. Thompson seedless-2 cultivar, exhibited a unique product with molecular size 450 bp. Moreover, Superior and Fayomi cultivars were similar together, and differ than the other four cultivars in the other two product bands, these two bands ranged between 350 bp and 380 bp. The results of SSR-PCR with primers pair (VVMD-7) are depicted in Fig. (1-C). The molecular size of these products ranged from 80 bp to 850 bp. The

primers gave eight bands; seven of them were polymorphic among the different eight grape cultivars. These pair of primers were very specific to Thompson seedless-1 cultivar, because there was only a unique product for this cultivar with molecular weight 250 bp.. Eleven bands in the eight used cultivars with primers pair (VVMD-14) are illustrated in Fig. (1-D) and molecular size ranged from 180 bp to 800 bp. These pair of primers were very important in our study; they showed a specific product for Superior, Early Superior, Fayomi, Thompson seedless-1, Thompson seedless-2 and Roumi Ahmer cultivars. SSR reaction using primer pair (VVMD-21) is illustrated in Fig. (1-E). The results revealed that these primers pair product had five fragments with size ranged from 250 bp to1100 bp., three of them were polymorphic. Grape cultivars, Thompson seedless-2 and Thompson seedless-3 were very similar in all polymorphic and monomorphic product bands, on the other side the other four cultivars, Roumi Ahmer and Bez El-Anza were similar as well in all products.

A total of three bands in the used eight cultivars with primers pair (VVMD-24) are illustrated in Figures (1-F) with molecular size ranging between around 220 bp. to 290 bp. All three fragments were not very informative in our study because they did not show any polymorphism level among the different cultivars. The SSR-PCR results, using Primers pair (VVMD-25) are illustrated in Fig. (1-G). The molecular sizes of the five PCR products ranged between the regions of 220 bp to 550 bp. The five fragments showed polymorphism among the 8 cultivars with 100% polymorphism. The three cultivars Superior, Early Superior and Fayomi, were similar in the PCR products with this pair of primers. Also Roumi Ahmer and Bez El-Anza cultivars showed specific products.

Moreover, with primers pair (VVMD-27), a total of six bands in the eight used cultivars are illustrated in Figures (1F), with molecular size ranging between around 450 bp. to 1500 bp. five of the six fragments were very informative in our study because they did not show polymorphism level among the different cultivars. Among these five polymorphic band, there is one product size was very specific for only two cultivars, Thompson seedless-2 and Thompson seedless-3.

RAPD-PCR fingerprinting of grape cultivars:

The use of RAPD's molecular markers was aiming to show fast and reliable discrimination of any variation. In this kind of molecular markers the DNA of eight cultivars of grape were used as templates for four RAPD primers. The RAPD-PCR results, using primer (OP-A03) are illustrated in fig. (2-A). The molecular size of 16 PCR products ranged from 384 bp - 1733 bp. five common bands were observed in all cultivars, and exhibited almost 30% monomorphism, while the remained11 fragments had showed around 70% polymorphism among the 8 cultivars. While, with primer (OP-B07), a total of eleven bands in the eight used cultivars were showed in fig (2-B). The molecular size of 11 PCR products ranged from 300 bp - 1500 bp. Only three fragments showed polymorphism among the eight cultivars, and recording around 27% polymorphism among the cultivars. This primer has showed a specific PCR product of 550 bp with the Roumi Ahmer cultivar. A total of fifteen bands in the eight used cultivars with primers (OP-A11) are exhibited in fig. (2-C). The molecular size of 15 PCR products ranged from 349 bp - 2091 bp. four common bands were observed in all cultivars, and exhibited almost 35% monomorphism, while the other 11 fragments have showed around 65% polymorphism among the 8 cultivars.

The results of RAPD-PCR with primer (OP-A15) are depicted in fig. (2-D). The PCR products were 13 fragments. Twelve fragments showed polymorphism among the eight cultivars, exhibiting more than 93% polymorphism. Only one product was monomorphic among the

cultivars. A total of twenty bands in the eight used cultivars with primers (OP-B02) are illustrated in fig. (2-E). The molecular size of 20 PCR products ranged from 343 bp - 2169 bp. nineteen out of the 20 fragments have showed more than 95% polymorphism among the 8 cultivars.

Ten PCR fragments with primer (OP-A07) are depicted in fig. (2-F). Eight of them showed 80% polymorphism among the grape cultivars. The fragments ranged between 250 bp. to 1600 bp.

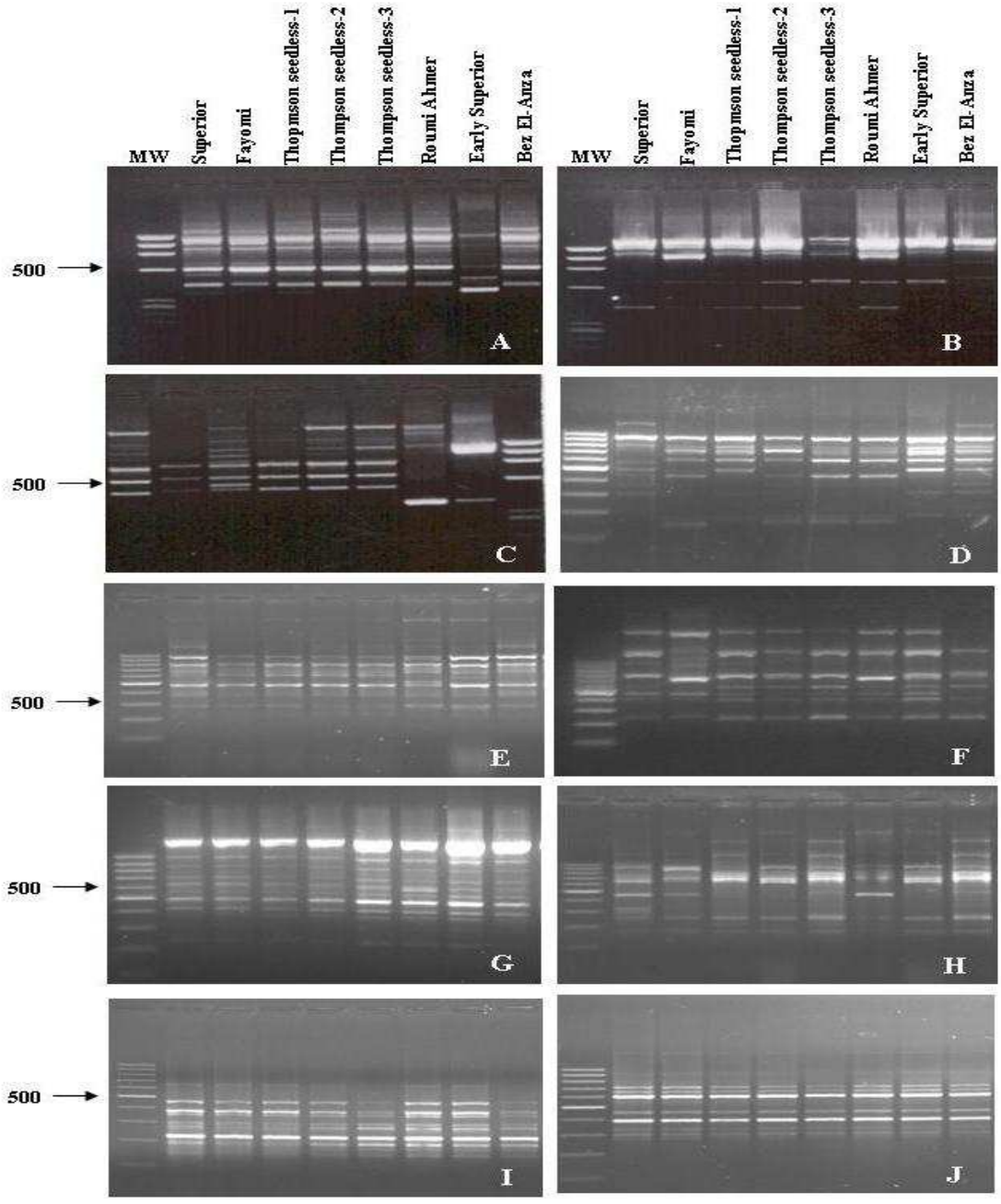

Figure 2: RAPD fingerprinting of 8 local Egyptian grape cultivars (*Vitis vinifera* L.). (A)Primer OP-A03; (B)Primer OP-A07; (C) Primer OP-A11; (D)Primer OP-A15; (E) Primer OP-B02; (F)Primer OP-B07; (G)Primer OP-B16 ; (H)Primer OP-B19; (I)Primer OP-G19 and (J)Primer OP-G20

A total of twelve bands in the eight used cultivars with primers (OP-B16) are illustrated in fig. (2-G). The molecular size of 20 PCR products ranged from 400 bp - 1661 bp.. Nine out of the 12

fragments had showed more than 75% polymorphism, while the other 3 fragments have showed around 25% monomorphism among the 8 cultivars. These primers had showed a specific product for the Roumi Ahmer and Early Superior cultivars. RAPD's reaction using primer (OP-B19) is exhibited in fig. (2-H). The molecular size of 11 PCR products ranged from 180 bp - 1700 bp. seven out of the 11 fragments had showed more than 65% polymorphism among the 8 cultivars. These primers had showed a specific product for the Superior cultivars, with 300 bp. length. While, with primer (OP-G19), a total of eight bands in the eight used cultivars were showed in fig (3-I). The molecular size of 8 PCR products ranged from 150 bp - 450 bp. Moreover, a total of six bands in the eight used cultivars with primers (OP-G20) are exhibited in fig. (3-J). The molecular size of 6 PCR products ranged from 300 bp - 700 bp. 100% of the PCR products with these two primers were monomorphic.

Genetic relationships as revealed by SSR and RAPD markers:

The SSRs in this study showed a genetic similarity ranged from 71% to 96% (data not shown). The highest genetic similarity were revealed by the SSR analysis was 96% between cultivar Thompson seedless-1 and Thompson seedless-2 cultivars, and 94% between cultivars Superior and Early Superior. On the other hand, the lowest genetic similarity was 71% between Superior and Thompson seedless-1 cultivars.

Meanwhile the RAPD's product showed a genetic similarity ranged from 46.2% to 93.5% (data not shown). The highest genetic similarity revealed by the RAPD's analysis was 93.5% among Thompson seedless-1 and cultivar Thompson seedless-3, and 89.3% between cultivars Thompson seedless-1 and Thompson-2 cultivars. On the other hand, the lowest genetic similarity was 46.2% between Bez El-Anza and Roumi Ahmer cultivars.

The RAPD's data have confirmed The SSR's data, when combined both data together. It has showed genetic similarity ranged between 71.3 % and 93.1% (Table 3).The highest genetic similarity was 93.1% between Superior and Early Superior, and similarity of 86% between Fayomi and both of Superior and Early Superior cultivars. Moreover genetic similarity ranged between 87% and 89% was recorded among Thompson seedless-1, Thompson seedless-2 and Thompson seedless-3 cultivars.

Table (3): Genetic similarity (GS) matrices computed according to Dice coefficient from SSR and RAPD markers of eight Grape cultivars

	Thompson seedless-1	Thompson seedless-2	Early Superior	Superior	Thompson seedless-3	Fayomi	Bez El-Anza	Roumi Ahmer
Thompson seedless-1	100	87.1	83.2	83.1	87.0	83.4	73.1	79.0
Thompson seedless-2		100	81.1	81.0	89.0	81.2	71.0	77.3
Early Superior			100	93.1	83.8	86.2	72.7	74.4
Superior				100	83.0	86.0	72.9	74.0
Thompson seedless-3					100	87.1	71.3	71.9
Fayomi						100	73.9	79.4
Bez El-Anza							100	73.6
Roumi Ahmer								100

Cluster analysis as revealed by SSR and RAPD markers:

SSR results in (figure: 1) showed that in a dendrogram the eight cultivars cluster comprised two subclusters with 21 % dissimilarity (data not shown). One subcluster includes (Superior and Early Superior), with 94% genetic similarity. The other subcluster included five cultivars (Thompson seedless-1, Thompson seedless-2, Thompson seedless-3, Bez El-Anza, and Roumi Ahmer), with similarity starts from 82% until 96%. While the other cultivar Fayomi was falling outside of both of two subclusters. These results demonstrated that Fayomi cultivar is genetically faring from the other seven grape cultivars. While the five cultivars, (Thompson seedless-1, Thompson seedless-2, Thompson seedless-3, Bez El-Anza, and Roumi Ahmer), although they belong to different farms, but they came from the same genetic origin. Also (Superior and Early Superior) cultivars despite collected from different farms but they showed the highest similarity (96%). While RAPD's product in figure (2) showed that in a dendrogram the eight cultivars cluster comprised two major sub clusters with 19 % dissimilarity. One subcluster includes (Superior, Early Superior and Fayomi), with genetic similarity starts from 80%. The other subcluster including three cultivars (Thompson seedless-1, Thompson seedless-2 and Thompson seedless-3), with similarity starts from 91% until 93.5%. While the other two cultivars Roumi Ahmer and Bez El-Anza were falling outside of both of two sub clusters (data not shown). This means that the environmental and biotic conditions are not very powerful in causing genetic variations among Grape cultivars. Moreover, the classification of these varieties might be was based on morphological traits which is not so powerful comparing with the molecular markers. Also the results confirmed the logic results, when the Superior and Early Superior showed high similarity, because they had the same fruit properties, but some speciation pressures could cause the 4% of differences between the two cultivars.

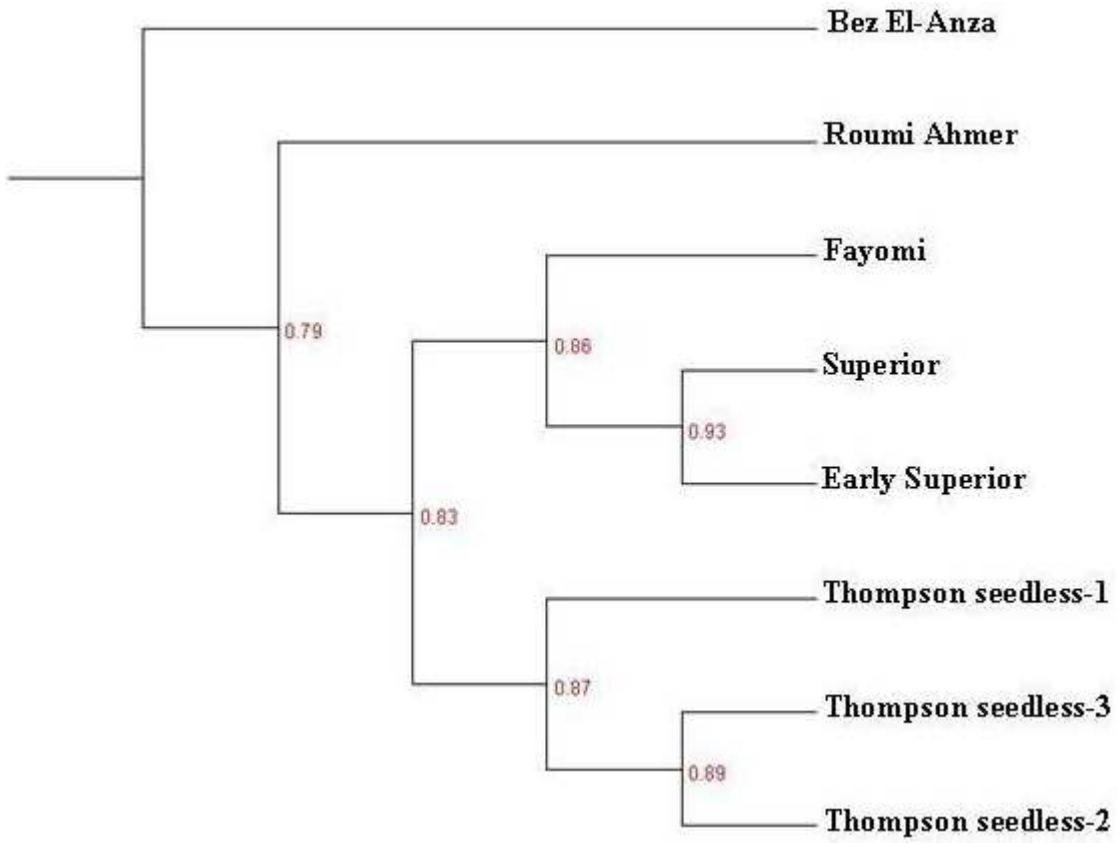

Figure 3: SSR's and RAPD's Phylogenetic analysis among eight Grape cultivars.

The RAPDs results have confirmed some of the SSR products when combined together. The analysis has showed more realistic clustering for the eight grape cultivars comparing with the SSR's, when the clusters contain several cultivars that had the same fruit characters. One of the major clusters contains the three cultivars, Superior, Early Superior, and Fayomi, that sharing the same fruit characters (figure 3). Moreover, the other major cluster, contains Thompson seedless-1, Thompson seedless-2 and Thompson seedless-3 cultivars, which are also sharing the same fruit characters (http://www.alkherat.com).

Although the eight grapevine varieties were collected from the same place, the overall positioning in the dendrogram reveals that they are not genetically identical, based on microsatellites and RAPD's. It is obvious that the varieties cultivated in different ecological conditions of Egypt have attained different genetic profiles during the time.

Most important criteria for the selection of marker were: Amount of available information, simple process or work, less expenses, high rate of doing work. In attention to the fact that RAPD marker don't need the use of radioactive elements and having sample sequence in plant genome such as possibility of the use of general primers those can be applied in every species of plant therefore RAPD marker is the suitable marker for the study of genetic variations and relativity rate and also it has the ability to assess the reservoirs of inheritance of the plant species.

The mean number of alleles per locus obtained, 4.1, is within the range of other works carried out in grape, 3.4 alleles per locus in 25 cultivars [3], 3.0 alleles per locus in 28 cultivars [36], 4.2 alleles per locus in 27 cultivars [9]and 4.5 alleles per locus in 50 cultivars [12]. However it is lower than the 7.3 alleles per locus obtained by [4] in 212 peach cultivars. The relatively lower polymorphism detected in this work compared to [4] can be due to two main reasons. One is the method used for the detection of the microsatellite markers. The works cited above used polyacrylamide gel electrophoresis [9] or capillary electrophoresis [4] and, in this work, we have used agarose gel electrophoresis, which could be underestimating the microsatellite polymorphism. A second reason for the relatively low SSR polymorphism observed can be due to the narrow geographical origin of the cultivars studied, given that all of them are local cultivars, whereas in other works the genotypes studied have a very diverse geographical origin.

From this study, we can recognize that microsatellites occur in the grape genome at frequencies comparable to those seen in other plant species. CT repeats occur at least once in every 100 kb in grape; compared to once in every 120 kb in apple [14] and every 225 kb in rice [40]. In concurrence with previous observations, CA repeats were less frequent, occurring once in every 420 kb in grape. In apple and rice, CA repeats occur every 190 kb and 480 kb respectively. Of the microsatellite motifs we have evaluated in grape, the AGG repeat motif was found to be the least common, occurring once in every 700 kb. Low frequencies of occurrence of trinucleotide repeats have also been reported by other researchers; in apple trinucleotide repeats occur every 3 Mb [40], while in wheat tri nucleotides was as much as ten-times less frequent than dinucleotide repeats [20]. The number of alleles observed for microsatellites in grape appeared to be relatively low (one to four) when compared to other species such as apple (one to nine) [14]. However, the polymorphism level in grape germplasm is still quite satisfactory (average heterozygosity=0.45) for most genetic studies. Our estimates of grape microsatellite heterozygosity and general utility agree well with those of [8]. Since the markers generated from microsatellite sequences identify significant levels of polymorphism, are highly transportable, and occur in reasonable abundance, it is evident that microsatellites have significant potential for genetic mapping, map merging, and cultivar identification in grape.

CONCLUSION

Detection of plants in advanced generations with diverse identity to one parent obviously shows the multidirectional effects of selection made by breeders on genetic diversity of plant material at molecular level [31]. The fruit characterization for the eight studied cultivars was collected from http://www.alkherat.com website [39]. We have used two different molecular markers, SSR's and RAPD's to estimate of *intra*- and *inter*-cultivar polymorphism among these cultivars. The SSR an RAPD analysis could complement each other, when the eight cultivars have clustered into two major groups. The RAPD's analysis was more realistic results when the RAPD's dendrogram analysis has showed two major groups, and this analysis fit together with fruit characters. One of the major clusters contains the three cultivars, Thompson seedless-1, Thompson seedless-2 and Thompson seedless-3, which sharing the same fruit characters. Moreover, the other major cluster contains Suprior, Early Superior and Fayomi cultivars, which are also sharing the same fruit characters (Figure 3).

Comparing study between RAPD's profile and different fruit traits of the eight different grape cultivars has showed interesting correlations. Moreover SSR's have showed very specific PCR products especially cultivars Superior, Early Superior, and Roumi Ahmer. These products were very specific to the fruit characters of different cultivars, and explaining the clustering analysis of RAPD's and SSR's.

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