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# Molecular Markers, Yield Performance and Berry Sensory Attributes in 10 Grape Cultivars Cultivated in Assiut Governorate

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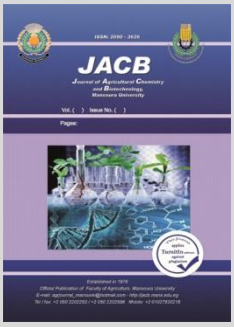


## ABSTRACT

Yield performance and berry sensory attributes in 10 grape cultivars were studied under Assiut conditions. In addition, the genetic variability and relationships among these cultivars were determined based on RAPD and SRAP molecular markers. Genetic variations were observed between the tested cultivars in all studied traits of yield and yield components as well as in berry sensory attributes. 100 sites of grape genome were amplified by RAPD and SRAP primers and used to study the interrelationships between 10 grape cultivars. The highest interrelationships were found between "Black Muscat and Provano", "Palomino and Bez El-Naka", and between "Palomino and Ruby Seedless". The lowest relatedness was found between Beauty Seedless and the other nine grape cultivars. The phenotypic data and molecular markers were effective in estimating the genetic variability between grape cultivars. The study indicated the presence of abundant genetic variability among some of the important commercial grape cultivars. Significant positive correlation was found between the phenotypic and genotypic distance indicated that the studied RAPD and SRAP markers were able to bind to effective regions in the genome.

***Keywords:*** Grape; Cultivars; Molecular Markers; RAPD, SRAP.

## INTRODUCTION

Grapes are among the most important horticultural crops in the world as well as in Egypt, so there are many grape varieties and clones in the country. During the last years, many cultivars of grapes were introduced to Egypt and the cultivated area for these new varieties and it increased rapidly. According to statistics of the Ministry of Agriculture (2017), the total area allocated for grapes in Egypt was (186,157) feddans, with a production of (1734,424) tons. To improve grapevine production, we need a wide knowledge about the genetic variability in yield components and fruit quality as well as the interrelationships between grape genotypes. The study of genetic diversity and relationships between genotypes will provide new insights into crop breeding and improvement (Henderson, and Salt, 2017).

Genetic variability and interrelationships between plant genotypes is one of the main factors for improvement of many crop plants including grapes and can be evaluated from morphological traits, pedigree analysis or using molecular markers (Pejic *et al.*, 1998). Estimates of genetic interrelationships based on morphological traits suffer from the drawback that these traits are limited in number and influenced by the environment (Maric *et al.*, 2004). Molecular markers are useful tools for determining the genetic variability and interrelationships between plant genotypes because they are unaffected by the environment and do not require prior pedigree information.

Among these molecular markers, random-amplified-polymorphic-DNA (RAPD) which introduced by Williams *et al.*(1990). RAPD-PCR is easy to use, required a very small amount of template DNA and displays informative results (Atienzar *et al.*, 2000). RAPD-PCR has become a useful tool to complement morphological, agronomic, and physiological characterization to improve assessment of genetic diversity and interrelationships between genotypes. RAPD-PCR analysis has been used by several researchers to determine the genetic distances and interrelationships among grape varieties (Qu and Lamikanra, 1996; This *et al.*, 1997; Vidal *et al.*,1999 and Su-Lan *et al.*, 2001). In addition, Sequence-related amplified polymorphism (SRAP) markers are employed in several studies to genetic variability and interrelationships among plant genotypes. SRAP markers were first developed by Li and Quiros (2001), in *Brassica*, with amplifying open reading frames (ORFs) using SRAP primers on the basis of variation in introns and conservation in exons. SRAP has been used to study genetic variations and relationships in a number of plants, including grape cultivars (Guo *et al.*, 2012 and Zhang *et al.*, 2018).

The present investigation aimed to study the yield performance and berry sensory attributes in ten grape cultivars under Assiut conditions. In addition, the genetic variability and relationships among these cultivars were studied based on RAPD and SRAP molecular markers.

## MATERIALS AND METHODS

**Plant materials:** The evaluation included ten grape cultivars which are grown in the orchard of Pomology Department at the Faculty of Agriculture, Assiut University during two successive grape seasons growing of 2017 and 2018.These cultivars were Red Roomy, Ruby Seedless (KingRuby), Thomson Seedless, Provano, Black Muscat, Palomino, Rich Baba, Bez El- Anza, Bez El -Naka and

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Beauty Seedless. The experiment was conducted on five replicates of each cultivar. Five grapevines from each cultivar were selected, therefore the whole experiment Consisted of 50 grapevines.

This experiment was arranged in a complete randomized design to study the yield performance and berry sensory attributes in 10 grape cultivars. The Following measurements were executed on each vine.

**Performance of the grape cultivars:**

**1- Initial fruit set (IFS), flower drop, and berry drop:**

Initial fruit set (IFS) was calculated according to El-Sese *et al.* (1988). Two clusters from each vine were sacked with sacks of white cheesecloth 10 days before fruit set. After two weeks of fruit set, the clusters were detached from the vines with their sacks. In the laboratory, the clusters were drawn out from the sacks on white paper sheet and they were well shacked off on it. The flowers and berries were divided into: (1 normal berries ,2) dropped berries ,3 flowers that did not set. Then the percentage of initial fruit set was calculated according to following equation: -

$$IFS\% = \frac{\text{Total No. berries/cl uster *}}{\text{Total No. flowers/cl uster *}} x100$$

***Number of normal berries + dropped berries.**

*** Number of flowers + total number of berries (normal+ dropped)**

**2- Number of setting berries**

**3- Cluster number/shoot was calculated on each selected shoot.**

**4-Yield components [Yield weight (kg)- Cluster number/vine - Cluster weight (g)].**

**5- The L/W ratio: Cluster Length (L)/width (W) ratio was calculated.**

**Berry sensory attributes:**

1- Total Soluble Solids (%) (TSS) using hand refractometer.

2-Titratable acidity (%) using titration by NaOH at 0.1 N and phenolphthalein as an indicator then expressed as grams tartaric acid per 100 g juice.

3- TSS/Acid ratio was then calculated.

4-Reducing sugars% were determined according to Lane and Eynom Volumetric method.

**Molecular markers:**

**DNA extraction:** Genomic DNAs were extracted from fresh leaves of 10 grape cultivars using the CTAB protocol (Lodhi *et al.* 1994). The quality and concentration of the extracted DNA which will be used in PCR reactions were determined using a 0.8 % agarose gel and a spectrophotometer according to Sambrook *et al.,* (1989). DNA dilutions were made to detect the optimum concentration for RAPD and SRAP-PCR analysis.

**RAPD and SRAP analysis:** RAPD and SRAP assays were based on the Polymerase Chain Reaction (PCR) amplification of random sites spread all over the genomic DNA. The used DNA amplification protocol was performed as described by Williams *et al.* (1990) with some modifications.

**RAPD-PCR analysis:** Seven RAPD primers (Table 1) obtained from (Metabion International AG), were tested in this experiment to amplify the template DNA. The reaction conditions were optimized and the amplification mixture (25 µl total volume) was composed of 3.0 µl 10X reaction buffer, 0.3 µlTaq DNA polymerase, 3.0 µl dNTP's mix, 2.0 µl primer, 1.0 µl Template DNA, 4.0 µl $MgCl_2$ and 11.0 µl $dH_2O$. Amplification condition were carried out in a TECHNE thermocycler (Model FTGEN5D, TECHNE, Cambridge Ltd, Duxford, and Cambridge, U.K.) with the following parameters: denaturation at 94°C for 3 min, followed by 40 cycles of 92°C for 1 min., 33°C for 1 min, 72 °C for 2 min, and a final extension at 72 °C for 10 min., then a final hold at 4ºC.

**SRAP-PCR analysis:** Seven SRAP primer combinations (Table 1) were used to study band polymorphism among the 10 grape cultivars. The SRAP-PCR reaction was carried out in 25 µL reaction mixtures which consisted of 2.5 µL of 10 × reaction buffer, 2.0 µL of 25 mM $Mg^{2+}$, 2.0 µL of 2.5 mM dNTPs, 0.5 µL of each 10 µM primer, 0.2 µL of 5 U Taq DNA polymerase and 2.0 µL of 20 ng·µL genomic DNA. All PCR reactions were carried out using a TECHNE thermocycler with the following parameters: denaturing at 94 °C for 5 min, followed by the first five cycles of 94 °C for 1 min, 36 °C for 1 min, 72 °C for 1 min and then 35 cycles of 94 °C for 1 min, 50°C for 1 min, 72 °C for 1 min and a final extension at 72 °C for 10 min.

The amplified products were separated on 1.5 % agarose gels in 1 × TAE buffer at 120 V constant voltages for 50 min. The 100 bp (Fermentas, Lithuania) DNA ladder were used to estimate the approximate molecular size of amplification DNA products. The gels were stained with ethidium bromide (EB) solution and photographed.

**Data analyses:**

The RAPD and SRAP molecular markers were visually recorded using the MVSP software package (Multivariate Statistical Package) and the DNA bands were recorded as being present (1) or absent (0). Paired comparisons between the tested samples were used to compute the genetic similarity matrix (GS) coefficient according to Nei and Li (1979). The dendrogram cluster analysis was presented as based on the GS estimates using the UPGMA (Unweighted Pair-Group with Arithmetic Mean) method.

**Table 1. RAPD and SRAP primers used in the present study.**

| PCR analysis | Primers | primer sequence 5' to 3' | Tm (ºC) |
|---|---|---|---|
| RAPD | OPY-O5 | GGC TGC GAC A | 36 |
| | OPC-18 | TGG GGG ACT C | 36 |
| | OPA-O2 | TGC CGA GCT G | 36 |
| | OPA-15 | AGA TCC AGC C | 36 |
| | OPY-O6 | AAG GCT CAC C | 36 |
| | OPW-12 | TGG GCA GAA G | 36 |
| | OPI-O5 | TGT TCC ACG G | 36 |
| SRAP | ME1 | F1:TGA GTC CAA ACC GGA AA | 50 |
| | ME4 | F2:TAG GTC CAA ACC GGA AT | 50 |
| | ME3 | F3:TAG GTC CAA ACC GGA AG | 50 |
| | ME2 | F4:TAG GTC CAA ACC GGA AC | 50 |
| | ME5 | F5:TAG GTC CAA ACC GGA CA | 50 |
| | EM3 | R3:GAC TGC GTA CGA ATT AAG | 50 |
| | EM2 | R2:GAC TGC GTA CGA ATT AAC | 50 |

## RESULTS AND DISCUSSION

**Results**

**Performance of the grape cultivars:**

The mean performance of 10 grape cultivars grown in Assiut, Egypt during two successive seasons of 2017 and 2018 are summarized in Table 2. Black Muscat, Red Roomy and Rich Baba grape (1.60) displayed the highest cluster

number/shoot (bud fertility), while Bez-El-Anza showed the lowest value (0.60).

Number of setting berries/cluster was the highest in Ruby Seedless (394.3) followed by Beauty Seedless (339.7) and Thompson Seedless (327.2), while Black Muscat (79.2) recorded the lowest value.

The highest percentage of initial fruit set (IFS%) was recorded in Rich Baba (25.92%) followed by Bez El-Anza (22.81%) and Bez El-Naka (21.05%), however, the lowest IFS% was found in Thompson Seedless (13.55%) and Ruby Seedless (13.28%) cultivars.

**Table 2. Mean values of 2017 and 2018 grape growing seasons for cluster number/shoot, number of setting berries, IFSP%, cluster number/vine, cluster weight (g), cluster length/weight (L/W) and yield weight (Kg.) of the studied grape cultivars.**

| Cultivars | Cluster number/shoot | Number of setting berries | IFSP % | Cluster number/vine | Cluster weight (g) | Cluster L/W | Yield weight (kg) |
|---|---|---|---|---|---|---|---|
| Beauty Seedless | 1.00 C | 339.70 A | 18.842 CD | 43.80 A | 226.72 EF | 1.905 A | 10.06 C |
| Thompson Seedless | 1.00 C | 327.20 A | 13.550 E | 39.60 A | 257.60 DE | 1.84 A | 10 C |
| Rich Baba | 1.60 A | 127.80 CD | 25.923 A | 19.60 C | 335.80 BC | 1.62 CB | 6.46 DE |
| Ruby Seedless | 1.40 AB | 394.30 A | 13.276 F | 39 A | 380.15 B | 1.43 CD | 15.48 B |
| Palomino | 1.20 BC | 132.80 CD | 19.678 BCD | 22.50 BC | 453 A | 1.46 C | 10.11C |
| Provano | 1.20 BC | 196 BC | 17.286 DE | 43.40 A | 449.55 A | 1.46 C | 19.34 A |
| Bez EL-Naka | 1.00 C | 101.20 D | 21.051 BC | 11.10 D | 253.15 DE | 1.59 C | 2.71 F |
| Bez EL-Anza | 0.60 D | 104.80 D | 22.810 AB | 8.50 D | 272 DE | 1.28 D | 2.33 F |
| Red Roomy | 1.60 A | 251.30 B | 15.278 EF | 25.90 B | 292.95 CD | 1.799 AB | 7.70 CD |
| Black Muscat | 1.60 A | 79.20 D | 15.227 | 23.60 BC | 186.30 EF | 1.63 BC | 4.42 EF |

Data in Table 2 revealed wide differences between the studied grape cultivars in cluster number/vine, cluster weight (g) and yield weight (kg/vine). The highest number of clusters per vine was obtained by Beauty Seedless (43.8), Provano (43.4) and Thompson Seedless (39.6) grape cultivars. Meanwhile, very low number of clusters per vine was produced by Bez El-Anza (8.5) and Bez El-Naka (11.1).

The heaviest cluster was obtained from Palomino (453.00g.) and Provano (449.55g.) followed by Rich Baba (335.8g) and Ruby Seedless (380.50g). While Black Muscat grape recorded the lowest value of cluster weight (186.30 g). However, the rest of cultivars showed intermediate values of cluster weight ranged from 226.72 g in Beauty Seedless to 292.95 g in Red Roomy cultivar.

Data concerning L/W ratio revealed that Beauty Seedless, Thompson Seedless and Red Roomy tend to be more elongate, as compared with the other studied grape cultivars.

The highest yield weight was obtained by Provano cultivar (19.34 kg/vine) followed by Ruby Seedless (15.48 kg/vine). Meanwhile, Bez El-Anza (2.33 kg/vine), Bez El-Naka (2.71) and Black Muscat (2.71) produced the lowest yield weight. In the other cultivars, grape yield ranged from 6.46 Kg/vine in Rich Baba to 10.11 Kg/vine in Palomino.

**Berry sensory attributes:**

Sensory attributes include total soluble solids percentage (TSS%), total acidity percentage (%), TSS/acid ratio and reducing sugars. Data of these characteristics are presented in Table 3.

The highest TSS% were 21.10; 20.42 and 20.14% in Beauty Seedless, Black Muscat and Thompson Seedless, respectively. While the lowest TSS% was recorded in Bez El-Anza (16.26%) and Bez El-Naka (16.30%). Total acidity percentage was highest in Provano (0.535%) and Beauty Seedless (0.531) and Thompson Seedless (0.403%) while it lowest in Bez El-Naka (0.203%) and Bez El-Anza (0.217%). The other cultivars showed moderate acidity percentages ranged from 0.285% in Palomino to 0.376% in Black Muscat cultivar.

Bez-El-Naka (90.0); Red Roomy (87.72) and BezEl-Anza (80.62) displayed the highest TSS/acidity ratio while, Beauty Seedless (40.98) and Provano (45.1) showed the lowest ratio for the same trait.

Black Muscat (18.0%); Beauty Seedless (17.6) and Thompson Seedless (17.26) revealed the highest percentage of reducing sugars, however, Palomino (12.57%) was the lowest cultivar.

**Table 3. Mean values of 2017 and 2018 grape growing seasons for TSS%; total acidity%; TSS \ acid ratio and reducing sugars% of the ten studied grape cultivars.**

| Cultivars | TSS % | Total acidity% | TSS \ acid ratio | Reducing sugars% |
|---|---|---|---|---|
| Beauty Seedless | 21.10 A | 0.531 A | 40.98 E | 17.60 AB |
| Thompson Seedless | 20.14 A | 0.403 B | 55.41 DE | 18 A |
| Rich Baba | 17.34 C | 0.374 BC | 52.37 DE | 14.70 DE |
| Ruby Seedless | 18.90 B | 0.277 CD | 74.66 C | 16.45BC |
| Palomino | 19.20 B | 0.258 D | 76.77 BC | 12.57 F |
| Provano | 16.30 D | 0.535 A | 45.10 E | 14.21 E |
| Bez EL-Naka | 18.10 C | 0.203 D | 90.00 A | 17.26 AB |
| Bez EL-Anza | 16.26 D | 0.217 D | 80.62 ABC | 14.67DE |
| Red Roomy | 17.90 C | 0.280 D | 87.72 AB | 15.60 CD |
| Black Muscat | 20.42 A | 0.376 BC | 61.26 D | 18 A |

**Diversity analysis**

**RAPD analysis:** The seven tested RAPD primers amplified a total of 48 DNA fragments from the ten grape cultivars with an average of 6.86 bands per primer (Table 4 and Figure 1). Only 9 (18.75%) of these bands were conserved among all grape cultivars which considered as species specific DNA fragments, while 39 bands were polymorphic with percentage of 81.25%. All bands generated by the primer OPC-18 were polymorphic while OPY-06 generated the lowest percentage of polymorphic bands (50%). The other primers showed high percentage of polymorphism ranged from 75.0% (OPA-02) to 85.7% (OPW-12 and OPI-05). OPC-18 amplified a maximum of 9 bands followed by OPA-02 (8 bands) while OPY-05 amplified the minimum number of DNA fragments (5 bands). The number of RAPD markers detected in grape cultivars ranged from 31 bands in Ruby Seedless to 21 bands in Beauty Seedless.

**SRAP analysis:** The seven SRAP primer combinations amplified a total of 52 DNA fragments from the ten grape cultivars with an average of 7.4 bands per primer (Table 5 and Figure 2). Only 16 (30.77%) of these bands were conserved

among all grape cultivars which considered as species specific DNA fragments, while 36 bands (69.23%) were polymorphic. The percentage of polymorphism ranged from 57.14% in ME1/EM3 to 80.0% in ME3/EM2 and ME4/EM2. The primer combinations ME3/EM2 and ME4/EM2 generated the highest number of amplified (10 bands, each) and polymorphic (8 bands, each) DNA bands. While ME2/EM2 and ME3/EM2 generated the lowest number of amplified (5 bands, each) and polymorphic (3 bands, each) bands. The number of SRAP markers detected in grape cultivars ranged from 21 bands in Beauty Seedless to 39 bands in Ruby Seedless and Black Muscat.

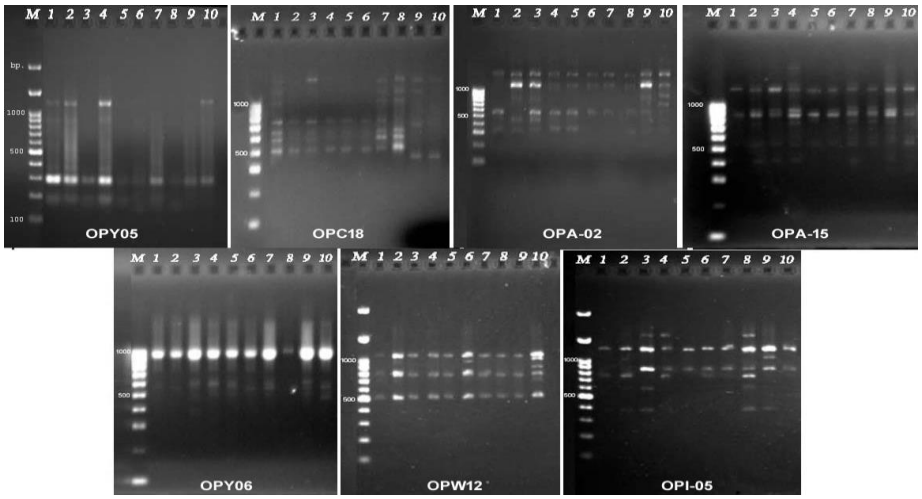


**Fig. 1. Agarose gel electrophoresis of RAPD profiles in ten grape cultivars obtained by 7 primers; M= DNA Ladder; (1): Red Roomy; (2): Thompson Seedless, (3): Ruby Seedless; (4): Bez El-Naka; (5): Bez El-Anza; (6): Beauty Seedless; (7): Palomino; (8): Rich Baba; (9): Provano and (10): Black Muscat.**

**Table 4. Number of amplified and polymorphic DNA-fragments (loci), and percentage of polymorphic bands generated from ten grape cultivars by seven RAPD primers.**

| Primers | Red Roomy | Thompson Seedless | Ruby Seedless | Bez El-Naka | Bez El-Anza | Beauty Seedless | Palomina | Rich Baba | Provano | Black Muscat | Amplified bands | Polymorphic bands | | PIC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | No. | % | |
| OPY05 | 4 | 4 | 2 | 4 | 2 | 2 | 2 | 2 | 2 | 3 | 5 | 4 | 80.0 | 0.31 |
| OPC18 | 7 | 4 | 5 | 4 | 4 | 4 | 7 | 7 | 4 | 3 | 9 | 9 | 100.0 | 0.37 |
| OPA02 | 2 | 5 | 5 | 7 | 3 | 3 | 7 | 5 | 4 | 5 | 8 | 6 | 75.0 | 0.26 |
| OPA15 | 2 | 2 | 5 | 4 | 4 | 2 | 4 | 1 | 4 | 4 | 6 | 5 | 83.3 | 0.27 |
| OPY06 | 3 | 3 | 3 | 3 | 3 | 4 | 3 | 3 | 3 | 6 | 6 | 3 | 50.0 | 0.29 |
| OPW12 | 2 | 4 | 5 | 4 | 2 | 3 | 2 | 6 | 4 | 2 | 7 | 6 | 85.7 | 0.11 |
| OPI05 | 3 | 5 | 6 | 4 | 4 | 3 | 3 | 4 | 6 | 7 | 7 | 6 | 85.7 | 0.33 |
| Total bands | 23 | 27 | 31 | 30 | 22 | 21 | 28 | 28 | 27 | 30 | 48 | 39 | 81.2 | 0.28 |

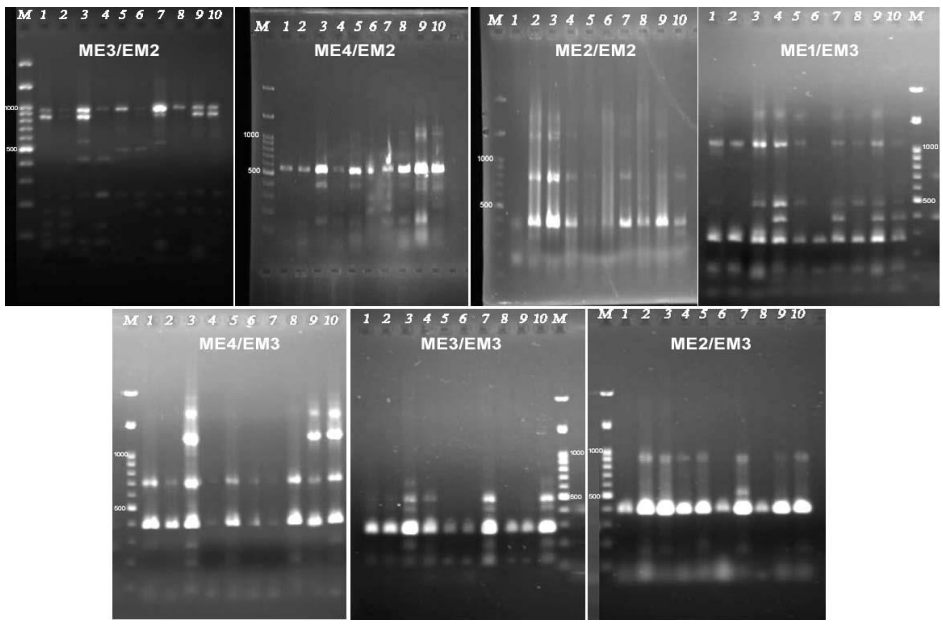


**Fig. 2. Agarose gel electrophoresis of SRAP profiles in ten grape cultivars obtained by seven primer combinations, M= DNA Ladder; (1): Red Roomy; (2): Thompson Seedless; (3): Ruby Seedless; (4): Bez El-Naka, (5): Bez El-Anza; (6): Beauty Seedless; (7): Palomino; (8): Rich Baba; (9): Provano and (10): Black Muscat.**

**Table 5. Number of amplified and polymorphic DNA-fragments (loci), and percentage of polymorphic bands generated from ten grape cultivars by seven SRAP primer combinations.**

| Primers | Red Roomy | Thompson Seedless | Ruby Seedless | Bez El-Naka | Bez El-Anza | Beauty Seedless | Palomina | Rich Baba | Provano | Black Muscat | Amplified bands | Polymorphic bands | | PIC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | No. | % | |
| ME3/ EM2 | 6 | 6 | 6 | 5 | 3 | 5 | 6 | 3 | 4 | 4 | 10 | 8 | 80.0 | 0.29 |
| ME4/ EM2 | 2 | 3 | 5 | 4 | 6 | 4 | 4 | 7 | 7 | 8 | 10 | 8 | 80.0 | 0.30 |
| ME2/ EM2 | 2 | 5 | 4 | 4 | 3 | 2 | 4 | 2 | 4 | 4 | 5 | 3 | 60.0 | 0.25 |
| ME1/ EM3 | 5 | 4 | 6 | 7 | 6 | 3 | 7 | 6 | 7 | 6 | 7 | 4 | 57.14 | 0.21 |
| ME4/ EM3 | 4 | 3 | 8 | 3 | 5 | 3 | 3 | 5 | 6 | 7 | 9 | 6 | 66.67 | 0.26 |
| ME3/ EM3 | 4 | 4 | 6 | 3 | 2 | 2 | 5 | 2 | 2 | 6 | 6 | 4 | 66.67 | 0.29 |
| ME3/ EM2 | 2 | 4 | 4 | 3 | 4 | 2 | 5 | 2 | 4 | 4 | 5 | 3 | 60.0 | 0.22 |
| Total bands | 25 | 29 | 39 | 29 | 29 | 21 | 34 | 27 | 34 | 39 | 52 | 36 | 69.23 | 0.26 |

**RAPD and SRAP combined data:** In the present investigation, two types of molecular markers, RAPD & SRAP, were used to study and determine the interrelationships between 10 cultivars of grape vine. A total of 100 DNA sites/alleles were amplified and tested in the grape cultivars with an average of 7.1 sites/primer **(**Table 6**).** Only 25 of these sites were conserved in the tested cultivars which considered as species specific DNA fragments. While the other sites showed polymorphism with a percentage of 75%. The highest number of DNA fragments was amplified from Ruby Seedless (70 fragments) followed by Black Muscat (69 bands), while the lowest number obtained from Beauty Seedless (42 fragments).

**Table 6. Total numbers of amplified and polymorphic DNA-fragments (loci), and percentage of polymorphic bands generated from ten grape cultivars by seven RAPD primers and seven SRAP primer combinations.**

| Markers | Red Roomy | Thompson Seedless | Ruby Seedless | Bez El-Naka | Bez El-Anza | Beauty Seedless | Palomino | Rich Baba | Provano | Black Muscat | Amplified bands | Polymorphic bands | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | No. | % |
| RAPD | 23 | 27 | 31 | 30 | 22 | 21 | 28 | 28 | 27 | 30 | 48 | 39 | 81.25 |
| SRAP | 25 | 29 | 39 | 29 | 29 | 21 | 34 | 27 | 34 | 39 | 52 | 36 | 69.23 |
| Grand total | 48 | 56 | 70 | 59 | 51 | 42 | 62 | 55 | 61 | 69 | 100 | 75 | 75.0 |

**PIC value:** The polymorphism information content (PIC) values of the studied markers were calculated and presented in Tables (4 and 5). In this study, PIC values for the 7 RAPD primers varied from 0.11 in OPW12 to 0.37 in OPC18 with an average of 0.28 (Table 4). The PIC values for the 7 SRAP primer combinations ranged from 0.21 in ME1/EM3 to 0.30 in ME4/ EM2 with an average of 0.26 (Table 5).

**Genetic similarity and UPGMA analyses:**

The highest genetic similarity (GS) for RAPD markers was found between Bez El-Anza and each of Bez El-Naka (0.73) and Beauty Seedless (0.72), while the lowest GS was found between Black Muscat and each of Rich Baba (0.35) and Red Roomy (0.39) (Table 7, above diagonal). The dendrogram grouped the 10 cultivars into four main clusters within a branched-off 0.472 GS **(**Fig. 3A**)**. Red Roomy and Thompson Seedless were grouped together in the 1st cluster within 0.613 GS. In the 2nd cluster, Bez El-Naka and Bez El-Anza grouped together firstly with 0.733 GS and then with Palomino at 0.686 GS and Ruby Seedless within a branched-off 0.664 GS. The 3rdcluster included Rich Baba and Beauty Seedless within a branched-off 0.633 GS. Provano and Black Muscat were grouped together in the 4th cluster at 0.629 GS.

Based on SRAP data, the highest GS was between Bez El-Anza and Rich Baba (0.806) and the lowest GS was between Ruby Seedless and Beauty Seedless (0.395) (Table 7, below diagonal). On Dendrogram, the cluster analysis separated the tested grape cultivars into four main clusters and one branch within a branched-off 0.498 GS **(**Fig. 3B**)**. Beauty Seedless was separated from the other cultivars in a single branch. Red Roomy and Thompson Seedless were grouped together in the 1st cluster within 0.742 GS. In the 2nd cluster, Bez El-Naka and Palomino grouped together at 0.733 GS. Provano and Black Muscat found in the 3rd cluster at 0.78 GS and then grouped with Ruby Seedless at 0.677 GS. Bez El-Anza and Rich Baba found in the 4th cluster at0.806si GS.

The interrelationships between the ten grape cultivars based on the combined data of RAPD and SRAP markers are more reliable than single marker and illustrated in the UPGMA clustering analysis **(**Fig. 4A**).** The dendrogram grouped the 10 cultivars into four main clusters and one branch within a branched-off 0.533 genetic similarity.

**Table 7. Similarity percent of SRAP (below diagonal) and RAPD (above diagonal) markers among 10 grape cultivars.**

| Cultivars | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1- Red Roomy | -- | 0.61 | 0.50 | 0.61 | 0.61 | 0.57 | 0.59 | 0.59 | 0.43 | 0.39 |
| 2- Thompson seedless | 0.742 | -- | 0.66 | 0.68 | 0.58 | 0.55 | 0.53 | 0.57 | 0.50 | 0.46 |
| 3- Ruby Seedless | 0.561 | 0.619 | -- | 0.65 | 0.66 | 0.53 | 0.69 | 0.64 | 0.57 | 0.53 |
| 4- Bez El-Naka | 0.636 | 0.706 | 0.619 | -- | 0.73 | 0.59 | 0.71 | 0.61 | 0.50 | 0.46 |
| 5- Bez El-Anza | 0.543 | 0.568 | 0.659 | 0.611 | -- | 0.72 | 0.67 | 0.56 | 0.58 | 0.49 |
| 6- Beauty Seedless | 0.586 | 0.515 | 0.395 | 0.515 | 0.515 | -- | 0.58 | 0.63 | 0.41 | 0.42 |
| 7- Palomina | 0.639 | 0.703 | 0.659 | 0.703 | 0.537 | 0.528 | -- | 0.65 | 0.53 | 0.45 |
| 8- Rich Baba | 0.625 | 0.514 | 0.571 | 0.600 | 0.806 | 0.548 | 0.525 | -- | 0.49 | 0.35 |
| 9- Provano | 0.513 | 0.615 | 0.659 | 0.615 | 0.703 | 0.447 | 0.619 | 0.649 | -- | 0.63 |
| 10- Black Muscat | 0.524 | 0.619 | 0.696 | 0.545 | 0.581 | 0.429 | 0.622 | 0.571 | 0.780 | -- |

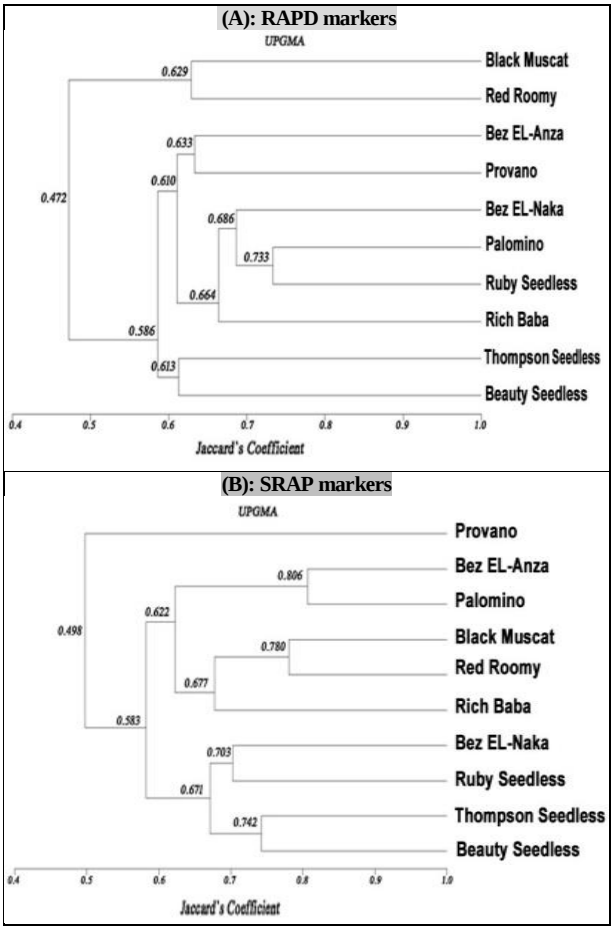


**Fig. 3. Dendrograms demonstrating the relationships among ten grape cultivars based on data recorded from (A) seven RAPD primers and (B): seven SRAP primer combinations.**

Red Roomy and Thompson seedless were grouped together in the 1st cluster within 0.677 GS. In the 2nd cluster, Bez El-Naka and Palomino grouped together firstly with 0.704 GS and then with Ruby Seedless at branched-off 0.652 GS. The 3rd cluster included Bez El-Anza and Rich Baba within a branched-off 0.683 GS. All cultivars in these three clusters showed an overall 0.600 GS and Beauty Seedless found in a single branch and joined these clusters at this similarity. Both Provano and Black Muscat were grouped together in the 4th cluster at 0.711 GS.

**Association between RAPD, SRAP and yield traits:**

The correlation (r) and the Mantel test statistic were calculated to measure the degree of relationship between the similarity matrices obtained with RAPD, SRAP and all agronomical-chemical traits data. Insignificant correlation (0.231, $P > 0.05$) was found between RAPD and SRAP data. On the other hand, significant but low to moderate correlations were found between the dissimilarity matrix generated from the yield traits (yield & chemical) data and each of RAPD ($r = 0.5504$, $P < 0.05$), SRAP markers ($r = 0.316$, $P < 0.05$) and combined RAPD-SRAP data ($r = 0.447$, $P < 0.05$). These results will be evident when comparing a dendrogram based on all studied traits (yield and chemicals) with the dendrogram based on combined data of both RAPD and SRAP markers (Fig 4A&B). In the two dendrograms, some cultivars like Tomson Seedless and Beauty Seedless are found in the same cluster while other cultivars showed diffenent distribution.

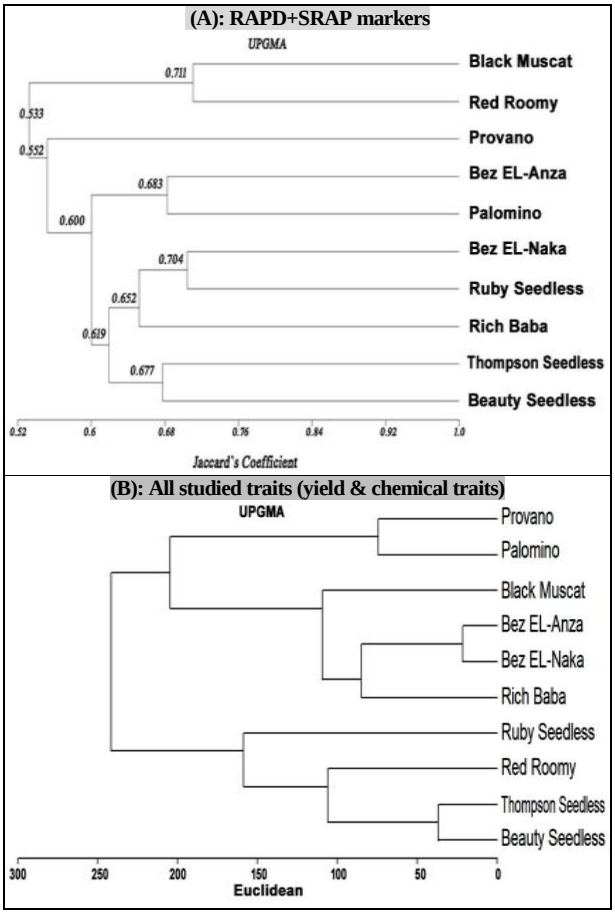


**Fig. 4. Dendrograms demonstrating the relationships among ten grape cultivars based on data recorded from (A) all RAPD and SRAP markers and (B): all studied traits (yield & chemical traits).**

**Discussion**

The results revealed that there are great differences between the studied grape cultivars in all the studied traits including yield components and berry sensory attributes, which can be used as distinct characteristics between them. Aziz (1984) found great variations in grape yield components and its quality attributes. Shellie (2007) reported that, grape cultivars differed in yield components, vegetative vigor, TSS, TA and pH and vary by genotype and environments. Thakur *et al*. (2008) and Shellie *et al*., (2014) demonstrated that the variability identified between grape cultivars for phenology, cluster attributes, yield and berry quality provide a useful guide for cultivar selection. Sharma *et al*. (2018) suggested that, various factors affect quality parameters of different grape cultivars consists of external and internal factors. Performance of germplasm in relation to yield and quality parameters has own importance. Total yield, grape juice and its quality is affected by various external factors (environmental conditions) and internal factors (genetic makeup) along with manipulation in growing conditions result in yield and juice quality.

In the present investigation, seven RAPD primers and seven SRAP primer pairs were used to study the genetic differences and interrelationships among 10 grape cultivars. A total of 100 reproducible bands were amplified using all tested primers with an average of 7.14 bands per primer. Forty-eight of these bands were generated by RAPD primers

while the rest (52 bands) obtained by SRAP primer combinations. Liu *et al.* (2012) used SSR and SRAP markers to analyze the genetic diversity of 26 grape genotypes and obtained a total of 223 bands by 12 SRAP primer pairs of which 208 bands were polymorphic (93.3%). Vidal *et al.* (1999) used RAPD technique to assess genetic relationships among 32 white grapevine cultivars and obtained 204 DNA bands by 33 primers. Zhang *et al*. (2018) used SRAP markers to assess genetic relationships among 39 grape genotypes and obtained 135 bands by 25 primer combinations, with an average of 5.52 bands per primer pair.

Further, RAPD markers showed % of polymorphic bands (81.25%) higher than SRAP markers (69.23%) with an overall average of 75.0%. These results were in agreement with those obtained by Zhang *et al.* (2018) who reported a polymorphism efficiency of 79 % using SRAP markers.The high polymorphism existing among the 10 grape cultivars indicating that the tested RAPD and SRAP markers were able to reveal genetic diversity in grapes (Zhang *et al*., 2018).This polymorphism level is higher than in some earlier studies with grape (Su-Lan *et al*., 2001) (RAPD, 68.7%) and (ErgüL *et al*., 2004) (AFLP, 33.7 %). While using12 SRAP primer pairs with 26 grape genotypes, Liu *et al*. (2012) found the % of polymorphism was 93.3%.

Generally, it was reported that cultivars polymorphism can arise from the changes in nucleotide order that prevent amplification by mismatch at one priming site; or priming site deletion; insertions that make the priming site too distant to support amplification. Insertions or deletions changed the size of the amplified product (Powell *et al.*, 1996). Polymorphism also considered as a useful selection tool in monitoring alien genome introgression in plant breeding programs.

The PIC values of markers indicate the utility of DNA markers for molecular breeding, gene mapping and evaluation of the germplasm (Peng and Lapitan, 2005). In this study, the average PIC value was 0.28 in RAPD markers and was 0.26 in SRAP markers. These moderate values of PIC for the RAPD and SRAP markers could be attributed to the diverse nature of grape genotypes and/or highly informative RAPD and SRAP markers. Similar results and conclusion were obtained by Nagaty *et al*. (2011) in wheat.

The highest similarity in RAPD markers were found between Bez El-Anza and each of Bez El-Naka (0.73) and Beauty Seedless (0.72). While, based on SRAP markers the highest similarity was between Bez El-Anza and Rich Baba (0.806). This was expected since the two systems of markers tested different sites of the genome, in which RAPD primers amplified random sites (Williams *et al.* 1990) while SRAP primer pairs amplified the open reading frames (ORFs) (Li and Quiros 2001).

The lowest similarity was observed between Black Muscat and each of Rich Baba (0.35) and Red Roomy (0.39) using RAPD markers, and between Ruby Seedless and Beauty Seedless (0.395) using SRAP markers. Zhang *et al.* (2018) reported that the similarity coefficients of SRAP polymorphism varied from 0.463 to 0.981 among 39 grape genotypes analysed.

More reliable results could be obtained from the combined analysis of RAPD and SRAP data. In the present study, 100 sites of grape genome were amplified by RAPD and SRAP primers and used to study the interrelationships between 10 grape cultivars. The highest interrelationships were found between Black Muscat and Provano (0.711) and between Palomino and each of Bez El-Naka (0.704) and Ruby Seedless (0.652). Bez El-Anza and Rich Baba also showed a relatively high interrelationship (0.683) followed by Red Roomy and Thompson Seedless (0.677). The lowest relatedness was found between Beauty Seedless and the other grape cultivars. Powell *et al.* (1996) reported that several factors might affect the estimates of genetic relationships between individuals i.e., number of markers used, distribution of markers in the genome (genome coverage) and the nature of evolutionary mechanisms underlying the variation measured. Guo *et al*. (2012) used SRAP to analyse genetic relationships among eight species of Chinese wild grapes. Using 25 SRAP primer pairs, Zhang *et al.* (2018) found that the dendrogram classified the 39 grape accessions in 21 clades of 3 main clusters.

The results also revealed insignificant correlation between RAPD and SRAP data. The main reason for the difference between RAPD and SRAP results is that the two markers targeted different parts of the genome (Souframanien and Gopalakrishna, 2004). Meanwhile, the significant correlations between the dissimilarity matrix of yield traits (yield & chemical) data and each of RAPD, SRAP markers and combined RAPD-SRAP data results indicating that the studied RAPD and SRAP markers were able to bind to effective regions in the genome. This finding supported that the studied RAPD and SRAP markers as well as the studied yield traits were found to be useful for the assessment of genetic diversity in grape, and the characterization based on yield traits and molecular markers will be a useful tool to the breeders to choose genotypes with appropriate traits. Similar conclusion was reached by Zarkti *et al*. (2010). Vidal *et al*. (1999) reported that the UPGMA cluster analysis based on RAPD markers classified the 32 white grapevine cultivars according to their common cultivation area and ampelographic characters. Liu *et al.* (2012) found that the UPGMA dendrogram obtained using SRAP data was almost consistent with conventional morphological classification.

## CONCLUSION

The phenotypic data and molecular markers were effective in estimating the genetic variability between grape cultivars. The study indicated the presence of abundant genetic variability among some of the important commercial cultivars. Significant positive correlation found between the phenotypic and genotypic distance indicated that the studied RAPD and SRAP markers were able to bind to effective regions in the genome.

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## الواسمات الجزيئية وأداء المحصول وصفات جودة الحبات في عشرة أصناف من العنب المزروعة بمحافظة أسيوط

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تمت دراسة أداء المحصول وصفات جودة الحبات في عشرة أصناف من العنب تحت الظروف البيئية لمحافظة أسيوط. بالإضافة إلى ذلك، تم تحديد الاختلافات الوراثية وعلاقات درجة القرابة بين هذه الأصناف بناءً على الواسمات الجزيئية RAPD وSRAP. أظهرت النتائج وجود اختلافات وراثية بين العشرة اصناف المختبرة في جميع الصفات المدروسة من المحصول ومكوناته وكذلك في صفات جودة الحبات. تم تضخيم 100 موقع من جينوم العنب بواسطة بادئات RAPD و SRAP واستخدمت لدراسة علاقات القرابة بين عشرة أصناف من العنب. وجدت أعلى علاقات قرابة بين "بلاك موسكات، بروفانو"، بين "بالومينو ويز الناقة، وكذلك بين "بالومينو، روبي سيدلس". أقل درجة قرابة وجدت بين صنف بيوتي سيدلس وأصناف العنب الأخرى. كانت بيانات الصفات المظهرية والواسمات الجزيئية فعالة في تحديد الاختلافات الوراثية بين أصناف العنب العشرة محل الدراسة. أشارت الدراسة إلى وجود تنوع وراثي كبير بين بعض الأصناف التجارية الهامة للعنب. وجد ارتباط معنوي موجب بين البعد الوراثي والصفات المظهرية لأصناف العنب المختبرة مما يدل على أن واسمات RAPD وSRAP المدروسة كانت قادرة على الارتباط بمناطق فعالة في الجينوم.