MARKER-ASSISTED SELECTION FOR YIELD AND QUALITY TRAITS IN SOME GRAPE CULTIVARS (*Vitis vinifera* L.)

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Grapes belong to family *Vitaceae*, which are woody climbers comprising 13 genera and about 700 species worldwide. *Vitis* is the only genus with economic importance and it is divided into two subgenera, *Euvitis* Planch (38 chromosomes) and *Muscadinia* Planch (40 chromosomes). The *Euvitis* species, with hundreds of known cultivars, can be divided into three geographical groups; American group, Asian group and European & middle-Asian group (Olien, 1990; Mullins *et al*., 1992).

The most widely recognized species is *Vitis vinifera* L., which is grown all over the world, ranked as the only member in the third group and has given rise to thousands of cultivars. It is considered as the major fruit crop in the world, the second fruit crop in production after citrus and the third largest crop by area after citrus and banana.

In Egypt, grape is grown in about 160,000 feddans, which produce about 1.2 million tons with an average of 9-21 tons/feddan (Horticulture Research Institute, ARC, Egypt). Grape is able to grow in a wide range of environments and adapts to different soil types. Research in grapevine genetics is restrained by the lack of genetic stocks, high heterozygosity, inbreeding depression, large space requirements, and the relatively long juvenile period (Reisch, 1998). DNA markers technology offers great promise for plant breeding, allowing molecular breeding via marker-assisted selection (Bergamini *et al*., 2013) and to study the historical origin and genetic diversity in some genotypes of grape (This *et al*., 2006). The RAPD markers provided some useful information in studies on genetic diversity and breeding of cowpea through mutations using the iradiation (Badr *et al*., 2014). RAPD markers, which can quickly detect a large number of genetic polymorphisms, have led to the creation of genetic maps in a number of woody fruit crops including grape (Lodhi *et al*., 1997). RAPD markers have some limitations, however, including questionable reproducibility of some bands, a requirement for stringent standardization products, and some genomic factors other than heredity, such as repetitive DNA and genome size may account for pattern variation (Bach-

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mann, 1994). Microsatellite markers (simple sequence repeats or SSRs markers) were widely used in grapevine genetic research for identification of cultivars (Lin and Walker, 1998; Ibanez *et al.*, 2003), parentage analysis, genome mapping and genetic characterization of germplasm (Sefc *et al.*, 1998; 1999). Bergamini *et al.* (2013) validated the SSRs markers as a 100% effective tool for early negative selection of stenospermocarpy in *Vitis vinifera* L. crosses.

Inter simple sequence repeats (ISSRs) is a different microsatellites-based method, which does not need prior knowledge of the genome. ISSRs analysis was used to determine and describe the diversity of several plant groups (Herrera *et al.*, 2002).

ISSRs technique was used to detect genetic characterization in some species such as tomato (Shahlaei *et al.*, 2014), lentil (Lombardi *et al.*, 2014) and apple (Dhyani *et al.*, 2015).

The objectives of this study were to; develop molecular identification for different grape genotypes through DNA analysis, assess the genetic relationships among these genotypes and obtain some molecular genetic markers associated with some important economic traits in grapes.

MATERIALS AND METHODS

1. Materials

Six cultivars and five rootstocks of grapes (*Vitis vinifera* L.) were obtained from Horticulture Department, Faculty of Agriculture, Ain Shams University, Shoubra El-Kheima, Egypt for this study. Their names, origins and trait descriptions, which achieved from net site www.vitis.com, are shown in Table (1). The pictorial presentation of the grape clusters (berries) of the six grape cultivars; Thompson seedless (Banaty), Bezelanza, Flame, Superior, Early-superior, and Crimson are shown in Fig. (1). Five rootstocks (Harmony, SO4, Rogiri, Freedom, and Poulson) were used, also, in this investigation.

2. Methods

2.1. DNA molecular genetic analysis

2.1.1. Genomic DNA extractions

DNeasy^{TM} Plant Mini Kit (Qiagen Inc., cat. no. 69104) was used for DNA isolation from the leaves of the 11 grape genotypes.

2.1.2. Randomly amplified polymorphic DNA analysis (RAPD)

RAPD reactions were conducted according to the method of Michelmore *et al.* (1991) using nine random *10-mer* primers from Operon Technology (USA). Their codes, sequences and GC% are shown in Table (2). The PCR-amplification method was carried out for 42 cycles as follows: 94°C/4 min (one cycle); 94°C/1 min, 36°C/1 min, 72°C/2 min (40 cycles); 72°C/10 min (one cycle) and 4°C (infinitive). PCR products were migrated on agarose (1.2%) according to Sambrook *et al.* (1989).

2.1.3. Simple sequence repeats (SSRs) analysis

Simple sequence repeats-polymerase chain reaction (SSRs-PCR) was conducted according to Sefc *et al.* (1999) using seven pairs of primers. Their codes and sequences are shown in Table (2). The PCR-amplification method was carried out in a PCR-programmed for 47 cycles as follows: 94°C/4 min (one cycle); 94°C/1 min 50, 55 or 60°C/1 min, 72°C/2 min (45 cycles); 72°C/10 min (one cycle) and 4°C (infinitive). PCR products were migrated on agarose (4%) according to Sambrook *et al.* (1989).

2.1.4. Inter-simple sequence repeats (ISSRs) analysis

Inter-SSRs (ISSRs)-technique was used according to Zietkiewicz *et al.* (1993) using four primers. Table (2) shows the codes of these primers, their sequences and GC%.

The thermal cycler (PCR) program was applied for three main steps as follows: 94°C/4 min (one cycle), 94°C/1 min (40 cycles), 55°C/1 min (40 cycles), 72°C/2 min (40 cycle), 72°C/10 min (one cycle) and 4°C (infinitive). PCR products were migrated on agarose (1.2%) according to Sambrook *et al*. (1989).

2.1.5. Data analysis

DNA fragments were detected on UV-transillumenator, then photographed with Gel-Documentation 2000, Bio-RadTM apparatus and analyzed by diversity database V.2.1.1. Cluster analysis based on RAPD, SSRs, ISSRs and combined results of RAPD and ISSRs analyses were carried out using UPGMA computer program.

RESULTS AND DISCUSSION

1. Molecular identification of grape cultivars

1.1. Randomly amplified polymorphic DNA-PCR (RAPD-PCR) analysis

Nine arbitrary 10-mer oligonucleotide primers were used to amplify the genomic DNA from the 5 grape cultivars and the 5 rootstocks. The number of total amplified fragments (TFA), polymorphic fragments (PF) for each primer, amplified fragments (AF) and specific marker (SM) for each genotype are shown in Table (4). Primers produced fragments number ranged from 6 for primers; OP-B01 and OP-B13 to 9 fragments for primers; OP-B02, OP-B05, OP-B07, OP-B09, OP-B10 and OP-B11 and 12 fragments for primer; OP-B08. All these data are shown in Fig. (2a-i). All primers exhibited 100% polymorphism except primer Op-B01 which showed 83.3% polymorphism that was useful in grapes identification which agreed with Stavrakakis *et al*. (1997).

1.1.1. Genotype-specific markers based on RAPD-PCR technique

Freedom rootstock (Nematode resistant) showed one positive marker at molecular size (MS) of 300 bp with primer OP-B09 and one negative specific marker at MS of 700 bp with primer OP-B01 (Table 4). Bezelanza cultivar (sweet flavour and early season grape) exhibited two positive specific markers at MS of 950 and 876 bp with primer OP-B05. Banaty cultivar (sweet juicy flavour) showed three positive markers at MS of 1178, 100 and 1049 bp with primers OP-B07, OP-B09 and OP-B11, respectively, and one negative marker at MS of 500 bp with primer OP-B09. Flame cultivar (sweet grape with deep-red colour and early season cultivar) exhibited one negative specific marker at MS of 500 bp with primer OP-B05. Rogiri rootstock (drought tolerant and resistant to phylloxera) exhibited one positive specific marker at MS of 1042 bp with primer OP-B09 and two positive specific markers at MS of 170 and 100 bp with primer OP-B10. Poulson rootstock (drought tolerant, Nematode and phylloxera resistance) showed one positive marker with primer OP-B02.

1.1.2. Genetic similarity and cluster analysis based on RAPD fragments

The results of cluster analysis (similarity indices) based on RAPD using UPGMA computer analyses are shown in Table (3). The highest similarity value (79.5%) was recorded between Crimson and Harmony and between Rogiri and Harmony, while the lowest similarity value (44.9%) was recorded between Bezelanza and Rogiri. A dendrogram for the genetic relationships among the 10 grape genotypes are shown in Fig. (5). The ten genotypes were separated into two clusters; cluster 1 included Banaty, Bezelanza, Flame and Early-superior and cluster 2 comprised the rest of the genotypes.

Ye *et al.* (1998) showed that RAPD analysis can be used for grape cultivar identification and for discrimination among phenotypically similar grape cultivars. Miaja *et al.* (2004) found that RAPD analysis gave fast and reliable results even on young material. On the other hand, Moreavcova *et al.* (2004) reported that it was impossible to distinguish between some grape varieties by RAPD method.

1.2. Simple sequence repeats (SSRs)

SSRs have become the technique of choice to identify different genotypes and to elucidate molecular markers in plant systems due to their abundance, high degree of polymorphism and amenability (Carreno *et al.*, 2004). Seven primer pairs were used in this study to identify the 11 grape genotypes.

Amplification of SSRs primers showed different numbers of fragments (Table 4) ranged from two fragments (primer ZAG 29, Fig. 3b), 3 fragments (primer ZAG 15, Fig. 3f), 4 fragments (primer ZAG 93, Fig. 3c), 5 fragments (primers ZAG 67 and ZAG 112, Figs. 3d & e, respectively), 8 fragments (primer ZAG 25, Fig. 3g) and 10 fragments (primer ZAG 7, Fig. 3a)

1.2.1. Genotype-specific-markers

The results of amplified fragments using SSRs method for the 11 grape genotypes are shown in Table (4). Primers produced a number of fragments ranged from 2 for primer ZAG29 to 10 for primer ZAG7. Crimson cultivar showed some specific markers; seven of them as positive markers at different molecular sizes and one as a negative marker at MS of 73 bp with primer ZAG7. This cultivar also showed 2 positive markers at MS of 300, 352 bp with primers ZAG67 and ZAG25, respectively. Flame cultivar exhibited one positive specific marker at MS of 76 bp with primer ZAG112, while primer ZAG15 detected one positive marker with MS of 300 bp and one negative marker at MS of 290 bp. Banaty cultivar showed one positive specific marker with MS of 235 bp with primer ZAG67. SO4 rootstock recorded one negative marker at MS of 178 bp with primer ZAG93. Rogiri rootstock exhibited two specific markers, one was positive at MS of 290 bp and the other was negative at MS of 280 bp using primer ZAG29. Freedom rootstock, recorded positive marker at MS of 186 bp with primer ZAG67.

1.2.2. Genetic similarity and cluster analysis based on SSRs fragments

The SSRs data were used to estimate the genetic similarity among the 11 grape genotypes (Table 3). The closest relationship was recorded between cultivars Early-superior and Bezelanza (similarity index of 94.6%). On the other hand, the lowest similarity was observed between Crimson and Freedom (40.5%).

The dendrogram based on SSRs similarity matrices (Fig. 6) separated the 11 grape genotypes into two main clusters. Cultivar Crimson was placed in a separate cluster and the second cluster involved the rest of the genotypes. These genotypes were separated into four sub-clusters; one included Flame alone, the other combined Poulson and Freedom, the third sub-cluster included Harmony, Rogiri and SO4. Bezelanza, Early-superior, Superior and Banaty fell in the fourth sub-cluster.

SSRs analysis for these genotypes indicated that the high polymorphic information content (PIC) of the markers could enable us in the identification of a large number of cultivars with only a few loci, while their distribution over the genome would make them valuable tools for pedigree studies and genetic mapping. This was in agreement with Sefc *et al.* (1999). Arnold *et al.* (2002) indicated that characterized SSRs loci can be successfully used in ecological and conservation studies across related species. They postulated that SSRs markers of grapes were likely to provide useful genetic tools for population investigations of selected *Vitaceae* worldwide. Carreno *et al*. (2004) studied the degree of genomic similarity among some grape cultivars and found high biodiversity among cultivars and for the wild individuals. Merdinoglu *et al.* (2005) emphasized the potential of SSRs markers in a number of studies and for the identification and discrimination of cultivars and rootstocks, pedigree reconstruction, genetic diversity of *Vitis* species and for genetic mapping.

1.3. ISSRs amplification analysis

Amplification of four ISSRs primers showed different numbers of fragments (Table 4) ranged from 8 fragments (primer HB 15, Fig. 4b), 10 fragments (primer 17898A, Fig. 4c), 13 fragments (primer HB 10, Fig. 4a) and 14 fragments (primer 17898 B, Fig. 4d).

1.3.1. Genotype-specific markers

The results of the amplified fragments using ISSRs method for the ten grape genotypes showed some specific markers. The four primers produced fragments wit8 for primer HB15 to 14 for primer 17898B (Table 4). Flame cultivar showed one positive marker at MS of 547 bp with primer HB10, Rogiri rootstock exhibited two positive markers at MS of 907 and 373 bp with primer HB10, one at MS of 580 bp with primer HB15 and one at MS of 981 bp with primer 17898B. Freedom rootstock gave a positive marker at MS of 373 bp using primer HB10. Bezelanza cultivar recorded a positive marker at MS of 2306 bp with primer 17898A. SO4 rootstock exhibited three positive markers at MS of 3235, 2178 and 144 bp with primer 17898B. Crimson cultivar, also showed one positive marker at MS of 268 bp using primer HB15.

1.3.2. Genetic similarity and cluster analysis based on ISSRs fragments

The ISSRs data were used to estimate the genetic similarity among the ten grape genotypes (Table 3). The genetic similarity matrices ranged from 40.0% to 75.6%. The closest relationship was recorded between cultivars Banaty and Early-superior (75.6%). On the other hand, the lowest similarity was observed between Bezelanza cultivar and Rogiri rootstock (40.0%).

As shown in Fig. (7), the dendrogram based on ISSRs similarity indices separated the ten grape genotypes into two main clusters. The first cluster combined 3 genotypes into two sub-clusters; Harmony and Poulson together and Freedom alone. The second cluster was divided into two sub-clusters, the first one comprised Crimson and SO4 together and Rogiri alone. The second sub-cluster combined Banaty and Early-superior together, while each of Flame or Bezelanza genotypes were separated into different sub-clusters. This wide range of identifying the 10 grape genotypes confirmed the efficiency of ISSRs markers as agreed with Herrera *et al.* (2002) who suggested that ISSRs would be more suited than RAPD for use on a wider range of cultivars. Essadki *et al.* (2006) found that ISSRs corresponds to dominant genetic markers and a specific phenotype may reflect the occurrence of several genotypes which can’t be distinguished directly. Pasquale *et al.* (2006) confirmed the high potential of ISSRs over RAPD techniques for fingerprinting closely related accessions.

1.4. Combined identification based on RAPD and ISSRs analyses

Cluster analysis based on both RAPD and ISSRs analyses was carried out using UPGMA computer program as shown for similarity indices (Table 3) and the dendrogram of genetic distances (Fig. 8). The highest similarity index was recorded between Harmony and Poulson rootstocks (75.6%), while the lowest (43.1%) was observed between Bezelanza cultivar and Rogiri rootstock.

A dendrogram for the genetic relationships among the ten genotypes across the two technique results was carried out in Fig. (8). The ten genotypes were separated into two clusters; the first cluster was divided into two sub-clusters; the first sub-cluster contained the two cultivars Banaty and Early-superior and the second one combined Bezelanza and Flame cultivars.

The second cluster included the rest of the other genotypes which was further subdivided into two sub-clusters; Freedom alone in the first sub-cluster, the second sub-cluster contained each of SO4 or Rogiri alone, while Crimson, Poulson and Harmony were present in the third sub-subcluster. It could be concluded that among *Vitis* genotypes a combination of RAPD and ISSRs could be more reliable for the rapid identification of grapevine varieties (Herrera *et al*., 2002).

1.5. Trait-specific markers

Based on the morphological and yield-related traits, it was possible to obtain some satisfactory associations between some of these important traits and RAPD, SSRs and ISSRs markers as shown in Table (5).

From the two classes of markers; RAPD and ISSRs used in grape identification, combined systems are more preferable for the genotypes identification used in this study as a reliable approach than dependence on one system only. Molecular fingerprinting of grape cultivars would help for protection of the breeder’s rights and provide a safeguard against commercial frauds. Several investigations advocated the combined analysis concept as advantageous to single systems (Brighurst *et al*., 1981; Abdel-Tawab *et al*., 2003 and 2004). In addition, our results could contribute to the assessment of genetic relationship and biodiversity among these cultivars, which agreed with Choudhary *et al*. (2014) who used 10 ISSR primers to study the genetic variability of four grape cultivars and grouped them into two major clusters at 51 percent similarity. This would help in selecting widely divergent cultivars for the development of crosses for the improvement of grape yield and quality traits. Moreover, molecular markers elucidated in this investigation, effectively revealed minute differences between very closely-related genotypes which otherwise could not be detected at the morphological level. This was particularly evident in Superior *vs*. Early-superior genotypes. Morphological discrimination between these two cultivars in particular represents a major problem for grape growers who like to have early identification of Early-superior, and molecular identification provides a reliable tool to realize this far-reaching economic objective.

SUMMARY

The objectives of this study were to develop molecular characterization for 11 grape genotypes and rootstocks using RAPD, SSRs and ISSRs analyses and to elucidate some molecular markers associated with some quality and yield-related traits.

RAPD analysis for the ten genotypes utilizing nine random *10-mer* primers exhibited a total number of 78 fragments. All primers exhibited high levels of polymorphism (100%) and the number of bands for each primer ranged from 5 to 12. The dendrogram based on RAPD-PCR analysis divided the ten genotypes into two main clusters; cluster 1 included four genotypes and cluster 2 comprised the rest of the genotypes. Cultivar Crimson and rootstock Harmony and also rootstocks Harmony and Rogiri were closely related (79.5%), while Bezelanza and Rogiri were remotely related (44.9%).

SSRs analysis for the 11 genotypes utilizing 7 primer pairs showed a total number of 37 bands and the total number of bands for each primer ranged from 2 to 10. Genetic similarity among the 11 grape genotypes showed that the closest relationship was recorded between Early-superior and Bezelanza (similarity matrix 94.6%), on the other hand, the lowest similarity was observed between Crimson and Freedom (40.5%).

ISSRs analysis for ten genotypes utilizing 4 primers which exhibited a total number of 45 bands and the number of bands for each primer ranged from 8 to 14. The genetic similarity among the ten grape genotypes based on ISSRs indicated a closest relationship between Banaty and Early-superior cultivars (75.6%), the lowest similarity was observed between Bezelanza cultivar and Rogiri rootstock (40.0%)

The combined data of RAPD-PCR and ISSRs-PCR showed that the genetic similarity matrix between Harmony and Poulson rootstocks was 75.6%, while the lowest similarity index (43.1%) was observed between Bezelanza cultivar and Rogiri rootstock.

In general, the molecular genetic studies of the 11 grape genotypes proved to be effective tools for the identification of these genotypes. In aaddition, such studies provided some molecular markers associated with some economically important traits in grape vine.

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Table (1): Code number, names of the 11 grape genotypes (six cultivars and five rootstocks), their origins and genotype specific traits.

Code number	Genotype names	Origin	Specific traits
(Cultivars)			
1	Bezelanza	Local	Light green color, large elongated berries, very sweet flavor and an early season cultivar.
2	Thompson seedless (Banaty)	Local	Light green color, oblong berries, sweet juicy flavor and medium season cultivar.
3	Flame	Foreign	Round, crunchy, sweet grape with a deep-red color and early season cultivar.
4	Superior	Foreign	Clear green color. The bunches are filled with berries, crisp texture refreshingly sweet taste and early season cultivar.
5	Early-superior	Foreign	Large seedless berries, clear green color, crisp texture, refreshingly sweet taste and very early season cultivar.
6	Crimson	Foreign	Red and crisp berries, sweetly tart, spicy flavor and late season cultivar
Rootstock			
7	Harmony	Unknown	Drought tolerant, Nematode resistance with medium resistance to phylloxera.
8	SO4	*V. berlandieri* x *V. riparia*	Resistance to Nematode and phylloxera
9	Rogiri	*V. berlandieri* x *V. rupestris*	Drought tolerant, Resistance to phylloxera with medium resistance to Nematode
10	Freedom	1613 x Dog Ridge	It is a strong rootstock with Nematode resistant.
11	Poulson		Drought tolerant, Resistance to Nematode and phylloxera

Table (2): Primer names, their sequences and GC% used for RAPD, SSRs and ISSRs analyses.

RAPD

Primer name	Sequences (5'→3')	GC %	Primer name	Sequences (5'→3')	GC %
OP-B01	GTTTCGCTCC	60%	OP-B09	TGGGGGACTC	70%
OP-B02	TGATCCCTGG	60%	OP-B10	CTGCTGGGAC	70%
OP-B05	TGCGCCCTTC	70%	OP-B11	GTAGACCCGT	60%
OP-B07	GGTGACGCAG	70%	OP-B13	TTCCCCCGCT	70%
OP-B08	GTCCACACGG	70%			

SSRs

Primer code	Forward primer sequence (5'→3')	Reverse primer sequence (5'→3')
ZAG 7	GTGGTAGTGGGTGTGAACGGAGTGG	AACAGCATGACATCCACCTCAACGG
ZAG 15	GGATTTTGGCTGTAGTTTTGTGAAG	ATCTCAAGCTGGGCTGTATTACAAT
ZAG 25	CTCCACTTCACATCACATGGCATGC	CGGCCAACATTTACTCATCTCTCCC
ZAG 29	ATAACCAGGACAAGTTATTCAAGCC	ACCCAATTGACCTATCTTTTATGCTG
ZAG 67	ACCTGGCCCGACTCCTCTTGTATGC	TCCTGCCGGCGATAACCAGCTATG
ZAG 93	TATGGAGGGACCGAGGTGGGCTAGG	GCACTCTTCGACGTTAAACAAAGCC
ZAG112	CGTTTAAAGCCAGCTGAATCTTGGG	TGGCTCCATACTGCTTCACGTAGGC

ISSRs

Primer	Sequences (5'→3')	GC %
17898A	CACACACACACAAC	50%
17898B	CACACACACACAGT	50%
HB10	GAGAGAGAGAGACC	57%
HB15	GTGGTGGTGGC	73%

Table (3): Similarity indices among the 10 grape genotypes based on RAPD, SSRs, ISSRs and combined RAPD and ISSRs data analyses.

RAPD									
Genotypes	Banaty	Bezelanza	Flame	Early- superior	Crimson	Harmony	SO4	Rogiri	Poulson
Bezelanza	0.500								
Flame	0.628	0.718							
Early-superior	0.615	0.654	0.679						
Crimson	0.487	0.551	0.474	0.590					
Harmony	0.513	0.474	0.474	0.667	***0.795***				
SO4	0.526	0.538	0.513	0.577	0.679	0.679			
Rogiri	0.487	***0.449***	0.500	0.667	0.641	***0.795***	0.603		
Poulson	0.462	0.526	0.526	0.590	0.744	0.769	0.628	0.692	
Freedom	0.462	0.500	0.474	0.487	0.641	0.641	0.577	0.615	0.692

SSRs										
Genotypes	Banaty	Bezelanza	Flame	Superior	Early- superior	Crimson	Harmony	SO4	Rogiri	Poulson
Bezelanza	0.919									
Flame	0.703	0.730								
Superior	0.892	0.919	0.757							
Early-superior	0.919	***0.946***	0.730	0.919						
Crimson	0.432	0.459	0.459	0.486	0.514					
Harmony	0.703	0.784	0.676	0.703	0.784	0.568				
SO4	0.784	0.811	0.703	0.784	0.757	0.432	0.811			
Rogiri	0.649	0.730	0.730	0.703	0.676	0.459	0.838	0.811		
Poulson	0.730	0.757	0.649	0.784	0.757	0.541	0.757	0.784	0.703	
Freedom	0.703	0.676	0.676	0.757	0.730	***0.405***	0.730	0.757	0.676	0.757

ISSRs									
Genotypes	Banaty	Bezelanza	Flame	Early- superior	Crimson	Harmony	SO4	Rogiri	Poulson
Bezelanza	0.711								
Flame	0.733	0.622							
Early-superior	***0.756***	0.600	0.667						
Crimson	0.733	0.578	0.644	0.578					
Harmony	0.622	0.600	0.578	0.556	0.578				
SO4	0.711	0.467	0.622	0.556	0.711	0.511			
Rogiri	0.644	***0.400***	0.467	0.622	0.644	0.533	0.622		
Poulson	0.533	0.511	0.578	0.511	0.711	0.733	0.644	0.578	
Freedom	0.578	0.600	0.444	0.422	0.533	0.689	0.556	0.533	0.600

Table (3): Continued

Combined RAPD and ISSRs									
Genotypes	Banaty	Bezelanza	Flame	Early- su-perior	Crimson	Harmony	SO4	Rogiri	Poulson
Bezelanza	0.577								
Flame	0.667	0.683							
Early-superior	0.667	0.634	0.675						
Crimson	0.577	0.561	0.537	0.585					
Harmony	0.553	0.520	0.512	0.626	0.715				
SO4	0.593	0.512	0.553	0.569	0.691	0.618			
Rogiri	0.545	***0.431***	0.488	0.650	0.642	0.699	0.610		
Poulson	0.488	0.520	0.545	0.561	0.732	***0.756***	0.634	0.650	
Freedom	0.504	0.537	0.463	0.463	0.602	0.659	0.569	0.585	0.659

Table (4): Number of amplified fragments and specific markers of the grape genotypes based on RAPD, SSRs and ISSRs.

RAPD																							
Genotypes			Banaty		Bezelanze		Flame		Early su-perior		Crimson		Harmony		SO4		Rogiri		Poulson		Freedom		TSM
Primers code	Primer TAF	PF	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	
OP-B01	6	5	4	-	5	-	2	-	5	-	5	-	5	-	3	-	2	-	3	-	2	1(-)	1
OP-B02	9	9	4	-	3	-	3	-	4	-	5	-	3	-	6	1(+)	2	-	3	1(+)	0	-	2
OP-B05	9	9	4	-	8	2(+)	5	1(-)	6	-	3	-	3	-	3	-	3	-	2	-	2	-	3
OP-B07	9	9	4	1(+)	6	-	6	-	6	-	4	-	4	-	3	1(+)	3	-	3	-	2	-	2
OP-B08	12	12	3	-	7	-	7	-	6	-	6	-	3	-	5	-	2	-	3	-	4	-	0
OP-B09	9	9	4	1(+) 1(-)	3	-	4	-	3	-	2	-	2	-	3	-	3	1(+)	2	-	5	1(+)	4
OP-B10	9	9	2	-	4	-	3	-	4	-	5	-	5	-	4	-	7	2(+)	4	-	2	-	2
OP-B11	9	9	3	1(+)	5	-	6	-	4	-	5	-	4	-	3	-	3	-	5	-	4	-	1
OP-B13	6	6	4	-	2	-	3	-	4	-	3	-	3	-	3	-	3	-	3	-	1	-	0
Total	78	77	32	4	43	2	39	1	42	0	38	0	32	0	33	2	28	3	28	1	22	2	15

TAF= Total amplified fragments, PF= Polymorphic fragment for each primer, AF= Amplified fragments,
SM= Specific markers including either the presence or absence of a fragment and TSM= Total number of specific markers.

Table (4): Continued

SSRs																									
Genotypes			Banaty		Bezelanze		Flame		Superior		Early superior		Crimson		Harmony		SO4		Rogiri		Poulson		Freedom		TSM
Primers code	Primer TAF	PF	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	
ZAG7	10	10	3	-	3	-	2	-	3	-	3	-	7	(+)7 (-)1	1	-	3	-	1	-	3	-	2	-	8
ZAG93	4	4	2	-	3	-	4	-	3	-	3	-	4	-	3	-	1	(-)1	3	-	2	-	1	-	1
ZAG29	2	2	1	-	1	-	1	-	1	-	1	-	1	-	1	-	1	-	1	(+)1 (-)1	1	-	1	-	2
ZAG67	5	5	3	(+)1	2	-	1	-	1	-	1	-	1	(+)1	1	-	1	-	1	-	1	-	1	(+)1	3
ZAG112	5	4	2	-	2	-	2	(+)1	2	-	2	-	3	-	3	-	2	-	2	-	4	-	2	-	1
ZAG15	3	3	1	-	1	-	2	(+1) (-1)	1	-	1	-	1	-	1	-	1	-	2	-	1	-	1	-	2
ZAG25	8	7	4	-	5	-	3	-	3	-	4	-	6	(+)1	3	-	3	-	3	-	4	-	1	-	1
Total	37	35	16	1	17	-	15	3	14	-	15	-	23	10	13	-	12	1	13	2	16	-	9	1	18

TAF= Total amplified fragments, PF= Polymorphic fragment for each primer, AF= Amplified fragments,
SM= Specific markers including either the presence or absence of a fragment and TSM= Total number of specific markers.

Table (4): Continued

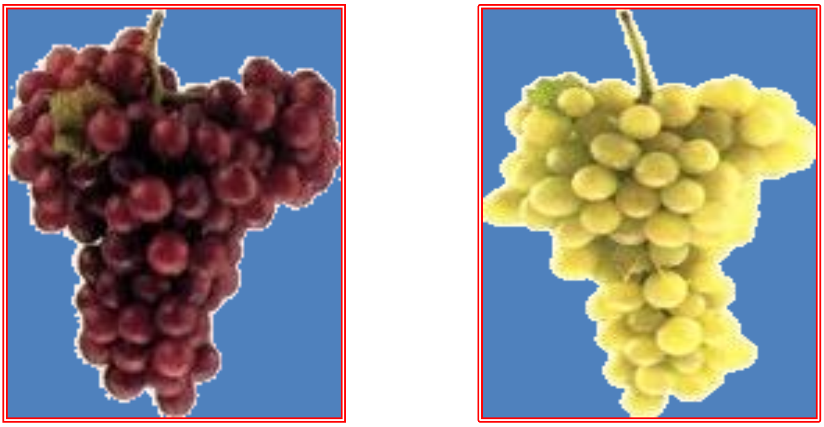
ISSRs																							
Genotypes			Banaty		Bezelanze		Flame		Early superior		Crimson		Harmony		SO4		Rogiri		Poulson		Freedom		TSM
Primers code	Primer TAF	PF	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	
HB10	13	13	5	-	4	-	7	(1+)	5	-	5	-	2	-	6	-	6	(2+)	2	-	3	(1+)	4
HB15	8	8	1	-	3	-	1	-	3	-	5	(+1)	2	-	3	-	4	(+1)	4	-	1	-	2
17898A	10	10	6	-	8	(1+)	7	-	3	-	4	-	4	-	6	-	1	-	5	-	5	-	1
17898B	14	14	2	-	4	-	7	-	2	-	4	-	5	-	6	(+3)	1	(+1)	4	-	4	-	4
Total	45	45	14	-	19	1	22	1	13	-	18	1	13	-	21	3	12	4	15	-	13	1	11

TAF= Total amplified fragments, PF= Polymorphic fragment for each primer, AF= Amplified fragments,
SM= Specific markers including either the presence or absence of a fragment and TSM= Total number of specific markers.

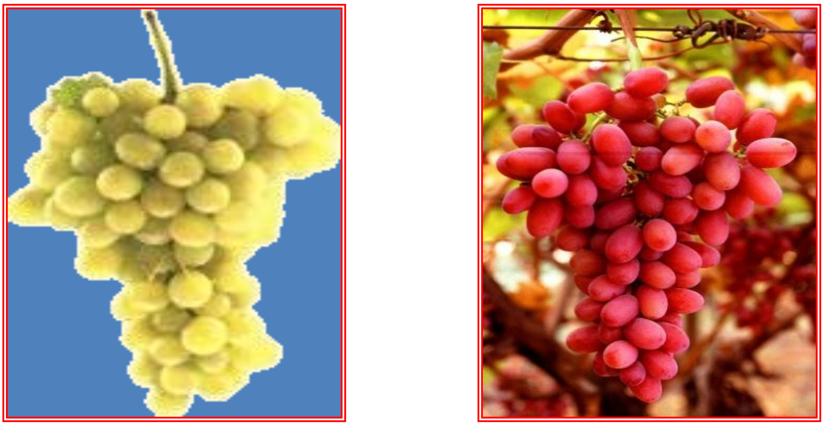
Table (5): Trait specific markers based on RAPD, SSRs and ISSRs analyses for grape genotypes.

Trait	Primers			MS of specific markers (bp)			Genotypes
	RAPD	SSRs	ISSRs	RAPD	SSRs	ISSRs	
Nematode resistance	OP-B01 OP-B09	ZAG67	HB10	700 300	186	2731	Freedom rootstock
Very sweet flavor and early season cultivar	OP-B05 OP-B05		17898A	950 876		2306	Bezelanza cultivar
Sweet flavor, deep red colour and early season cultivar	OP-B05	ZAG15 ZAG15 ZAG112	HB10	500	300 290 76	547	Flame cultivar
Sweet juicy flavor, and medium season traits	OP-B07 OP-B11 OP-B09 OP-B09	ZAG67		1178 1049 500 100	235		Banaty cultivar
Drought tolerant and resistance to phylloxera	OP-B09 OP-B10 OP-B10	ZAG29 ZAG29	HB10 HB10 HB15 17898B	1042 170 100	290 280	907 373 580 981	Rogiri rootstock
Drought tolerant, Nematode and phylloxera	OP-B02			200			Poulson
Spicy flavour, crisp berries and late season cultivar		ZAG7 ZAG7 ZAG67 ZAG25	HB15		689-121 73 300 352	1589	Crimson cultivar
Nematode and phylloxera resistance	OP-B02 OP-B07	ZAG93	17898B 17898B 17898B	1000 700	78	3235 2178 144	SO4 (rootstock)

Thompson seedless (Banaty) Bezelanza

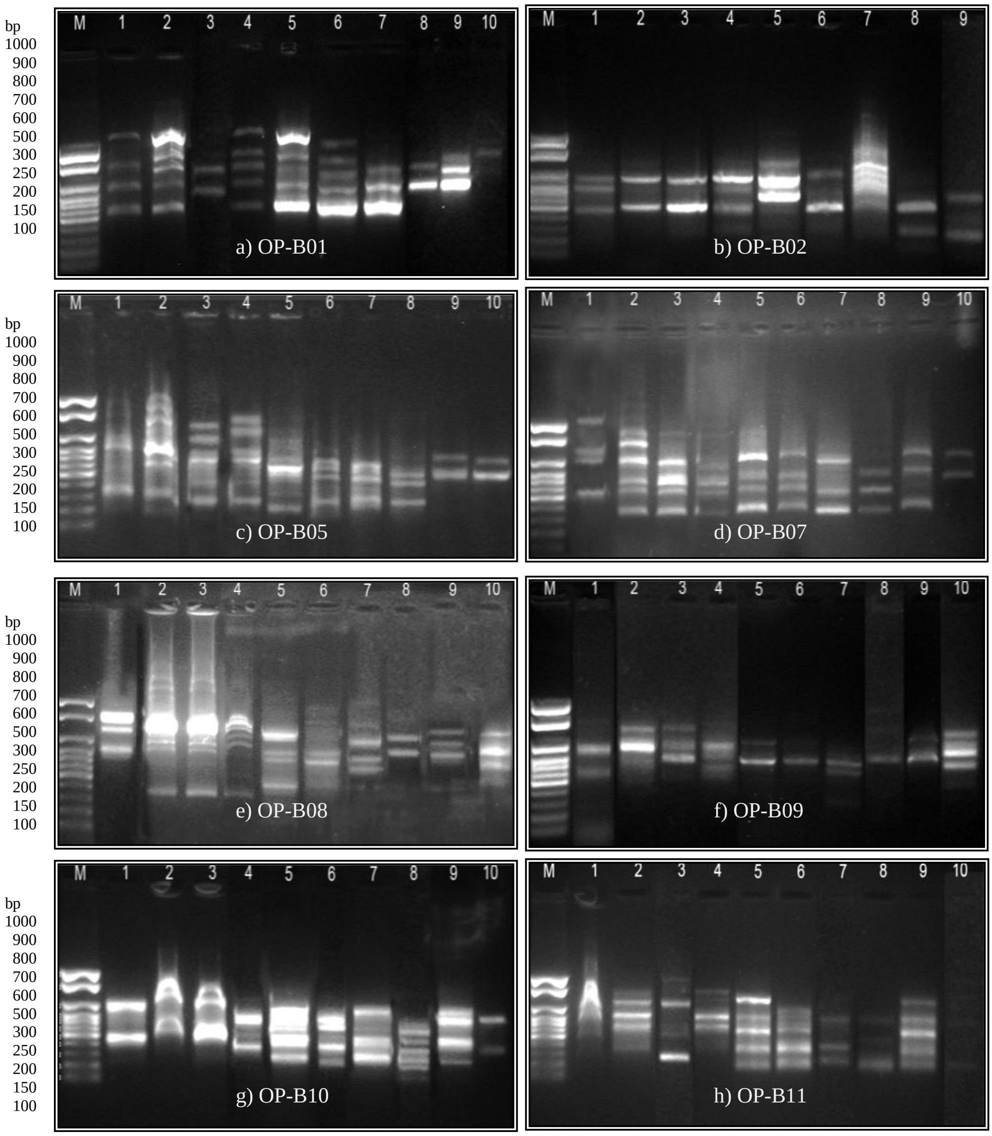


Flame seedless Superior seedless



Early superior (seedless) Crimson seedless

Fig. (1): The morphology of clusters (berries) of the six grape cultivars.



a) OP-B01

b) OP-B02

c) OP-B05

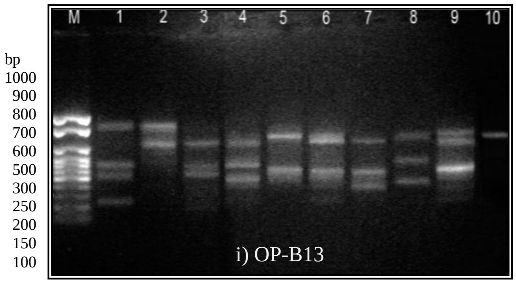
d) OP-B07

e) OP-B08

f) OP-B09

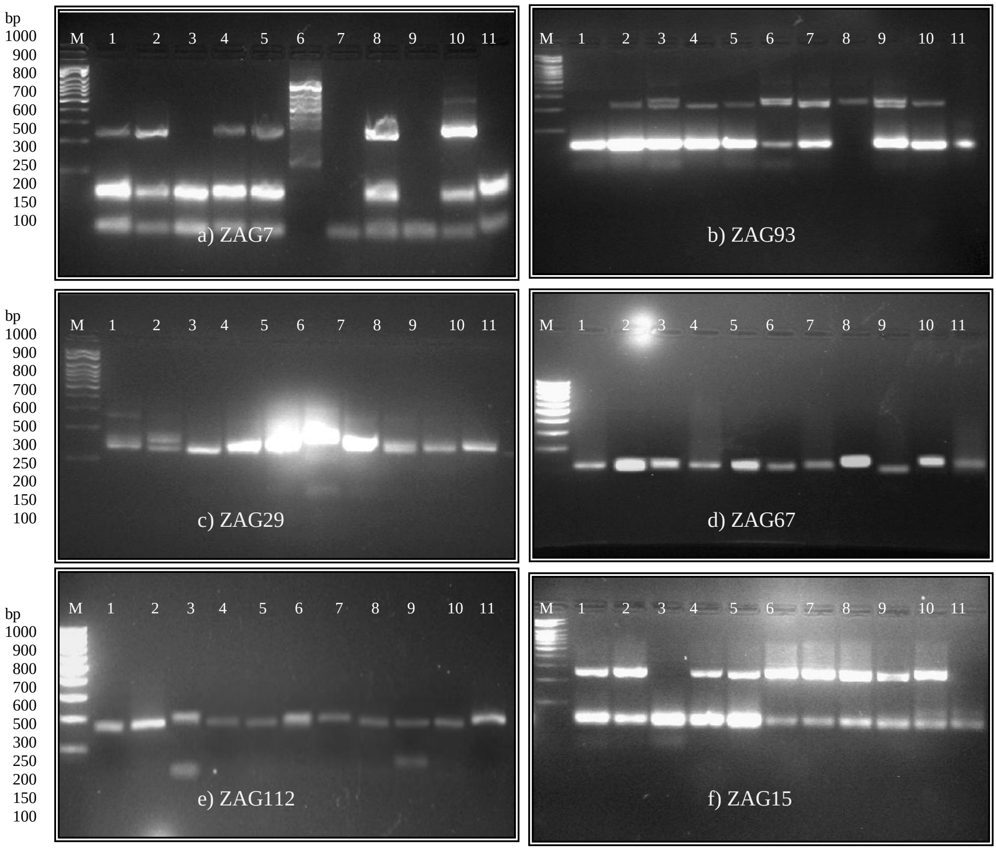
g) OP-B10

h) OP-B11



i) OP-B13

Fig. (2 a-i): DNA polymorphism using 9 primers for RAPD-PCR technique with the 10 grape genotypes; lanes 1 to 5 represent cultivars Banaty, Bezelanza, Flame, Early-superior and Crimson, respectively, Lanes 6 to 10 represent rootstocks, Harmony, SO4, Rogiri, Poulson and Freedom, respectively and M = DNA size standard.



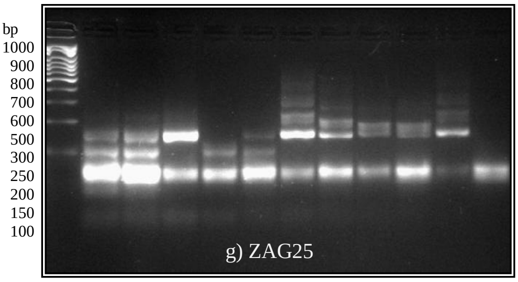
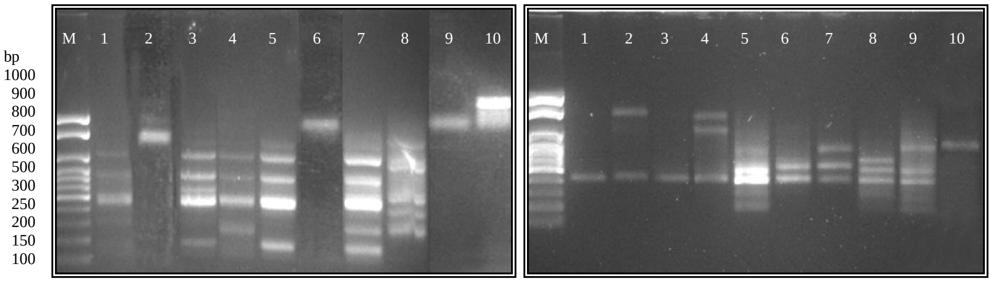
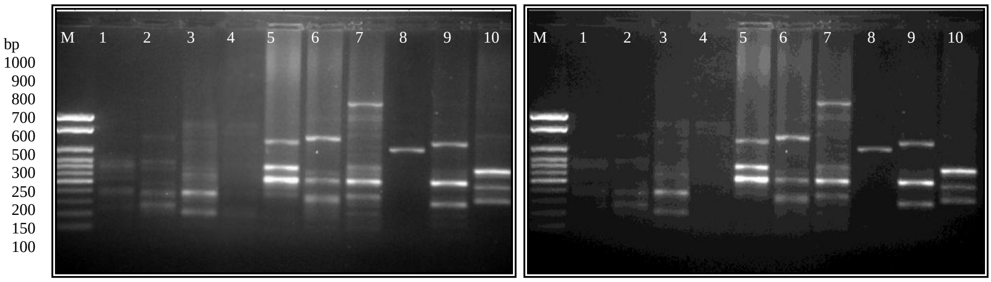


Fig. (3 a-g): DNA polymorphism using 7 primers for SSRs-PCR technique with the 10 grape genotypes; lanes 1 to 5 represent cultivars Banaty, Bezelanza, Flame, Early-superior and Crimson, respectively, Lanes 6 to 10 represent rootstocks, Harmony, SO4, Rogiri, Poulson and Freedom, respectively and M = DNA size standard.



a) HB10

b) HB15



c) 17898A

d) 17898B

Fig. (4 a-d): DNA polymorphism using 4 primers for ISSRs-PCR technique with the 10 grape genotypes; lanes 1 to 5 represent cultivars Banaty, Bezelanza, Flame, Early-superior and Crimson, respectively, Lanes 6 to 10 represent rootstocks, Harmony, SO4, Rogiri, Poulson and Freedom, respectively and M = DNA size standard.

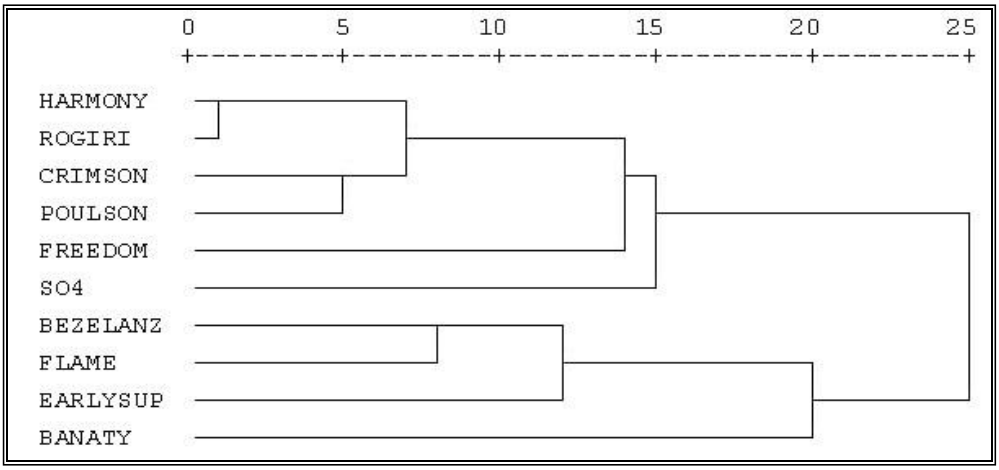


Fig. (5): Dendrogram for the genetic distances among the ten grape genotypes based on similarity indices data of RAPD analysis.

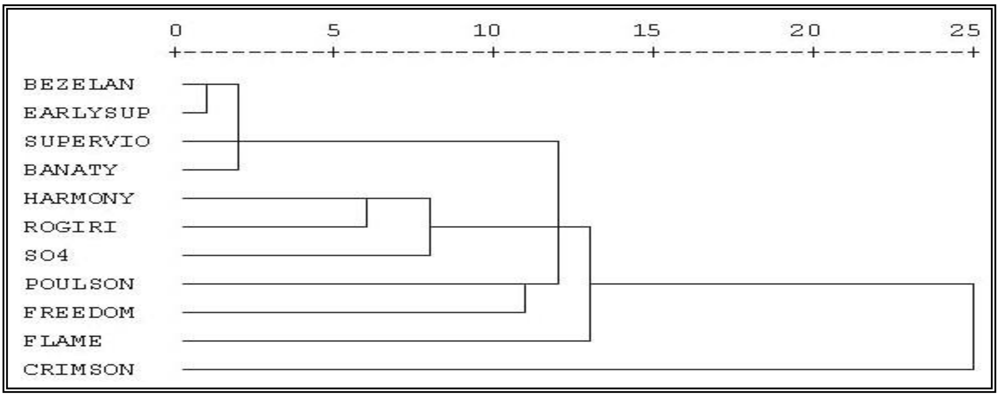


Fig. (6): Dendrogram for the genetic distances among the 11 grape genotypes based on similarity indices data of SSRs analysis.

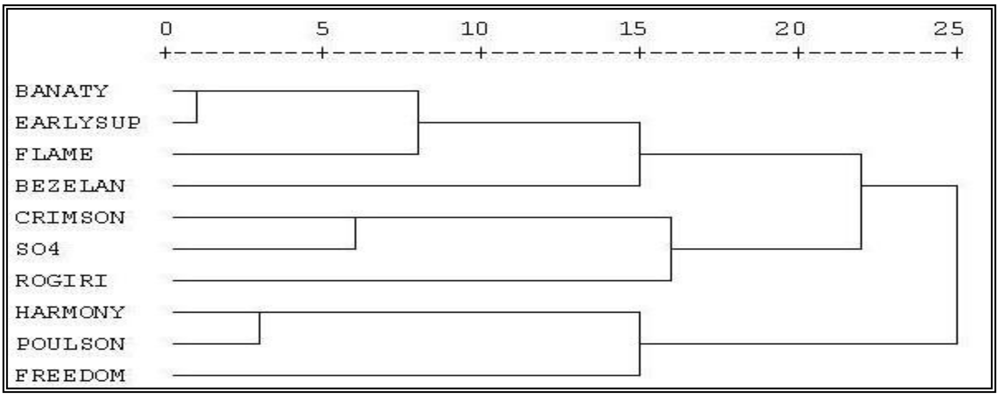


Fig. (7): Dendrogram for the genetic distances between the ten grape genotypes based on similarity indices data of ISSRs analysis.

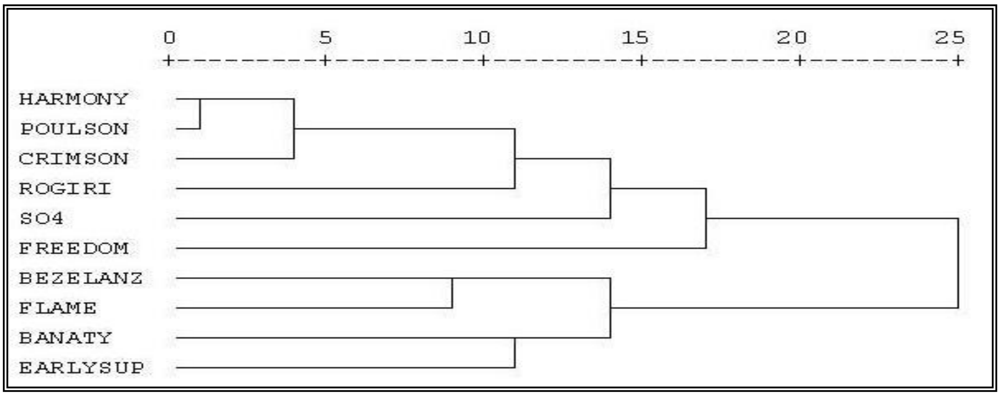


Fig. (8): Dendrogram for the genetic distances between the ten grape genotypes based on RAPD and ISSRs analysis.