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Genealogy and Molecular Diversity of Iranian Grapevine Progenies

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ABSTRACT

Grapes are among the world most planted horticultural crops. Since the last century, attempts have been made to improve the quality of grapes in the world. Meanwhile, the necessity of having knowledge about the history of progenies families led to the link between genealogy and breeding. Considering some previous mislabeling, in order to find out the accuracy of the controlled crosses as well as determining the possible parents and genealogy of the hybrid progenies, 23 grapevine genotypes were studied by using 14 SSRs loci. These progenies included 12 promising lines selected from 22 crosses as well as their parents that included four seedless and seven seeded cultivars from Iranian Grape Breeding Program, The highest similarity between a female parent and its progenies, which was obtained from dice similarity coefficient and cluster analysis, was about 0.65, belonging to 'Alibaba' and its three progenies (S_{54}, S_{55}, S_{40}). Results rejected any cross-selfing in female parents and also discriminated progenies from parents. Due to possible common genetic backgrounds in the parents, assigning progenies to their parents by cluster analysis or allele counting was impossible. Therefore, parentage analyses were done within likelihood based assignment approach using CERVUS 3.0 software. By this approach, true parents could be identified from candidate parents based on calculated positive and negative LOD scores. Also, by using this approach, genotyping errors, which were previously derived from low number of SSR loci or similarity in the parents' backgrounds, decreased in the final results. In addition, full sib and half sib relationships between S_{55} and S_{54} with S_{40} were obvious. Furthermore, wherever prevention of inbreeding depression is required, the results could be used to select convenient parents for backcrossing.

Keyword: Breeding, Grapevine, Likelihood based assignment, Microsatellite.

INTRODUCTION

Grapes are among the world most planted horticultural crops and a wide range of their cultivars are in use (Creasy and Creasy, 2009). Based on archeological evidence, the mountainous regions between the Caspian and Black Seas and across them were the earliest domestication regions of grapes in 5000 B.C. (McGovern *et al*., 1995; Mc Govern, 2003). Iranian grape germplasm is estimated to include about 500 cultivars, but little is known about their synonymous or homonymous genotypes (Fatahi *et al*., 2004). Some of these cultivars that possess proper horticultural traits are commercially important (Arzani *et al.,* 2009).

Controlled crosses of grapevines for cultivar improvement are well known to have been conducted before the spread of North American pest and pathogens around the world. However, crossing of *Vitis vinifera* L.

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with other grape species was not widespread until the 19th century (Owens, 2008; Creasy and Creasy, 2009). Breeding objectives varied by region and market class of the grapes. Many programs were planned to combine high quality fruit with improved disease resistance, environmental adaptation, and advances in quality attributes (Owens, 2008; Reisch and Pratt, 1996). Genealogy is a historical perspective (Crowley, 2009) and genealogists have been known as family historians (Otterstrom, 2009). The term genealogy could refer to the ancestry origin of a single gene and the whole set of genome sequences. In other words, genealogy provides a complete set of ancestors (Derrida *et al.,* 2000).

Due to perfect flower in European grapes and their self pollination behavior, emasculation of flowers in cluster is essential only before self pollination (Reich and Pratt, 1996; Bowers *et al.,* 1999). In addition, the diverse methods of determining parent-progeny relationships were used to determine new progeny's identity and also to find about their origins related to cross pollination or female parent selfing (Sefc *et al.,* 1997, 2000). Parentage analysis is important due to difficulties in grape genetic studies related to its long juvenility duration, high chromosome number (19 pairs), semi ovule sterility, and low germination of seeds, especially when progenies were used for important studies such as heritability, analysis of segregating population or gene and linkage map making (Lodhi *et al.,* 1995b; Reich and Pratt, 1996; Lahogue *et al.,* 1998; Dalbu *et al*., 2000). The ability to infer genealogical relationships among individuals in a population has opened up many areas of research on behavior, evolution, and conservation (Blouin, 2003).

Thomas *et al*. (1993) first investigated the use of microsatellite DNA for identifying grapevine cultivars. It was also demonstrated through pedigree analysis in which the microsatellite alleles were inherited in a co-dominant manner (Thomas and Scott, 1993), confirming their suitability for genetic mapping and investigation of genetic relatedness (Thomas *et al.,* 1994). Microsatellite markers are used routinely in forensic investigations dealing with paternity disputes and have recently been applicable in pedigree reconstructions in grapevines (Sefc *et al.,* 2009). A search for possible parent-offspring combinations among the microsatellite profiles of grapevines from a Portuguese collection revealed the origin of the cultivar Boal Ratinho to be the progeny of a cross between Malvasia fina and Síria (Lopes *et al.,* 1999). Microsatellite studies confirmed the former possibility, and identified the cultivar Syrah as a likely parent of Durif (Meredith *et al.,* 1999). In recent years, many more grapevine pedigrees have been discovered (Sefc *et al.,* 2009).

Iranian grape breeding program started in 1995 with evaluation of 90 cultivars and crosses were done in spring 1999. More than 1600 progenies were planted of which 381 have produced fruit since 2004. All 381 fruited progenies were evaluated for diversity, using morphological traits such as berry and cluster characteristics based on grapevine descriptor. This breeding program was performed to produce new seedless grapes with improved fruit, cluster and marketing properties (Ebadi *et al.*, 2009). Considering mislabeling of some progenies, the main purpose of this study was to study genealogical relationships among some superior progenies and their parents and, also, to find out possible parents for each one of them using parentage analysis.

MATERIALS AND METHODS

Plant Material and DNA Extraction

In this study 12 genotypes (I_{21} ،K_{67} ،R_{80} ،I_{73} ، A_{119} ،R_{84}،B_{98} ،S_{40} ،K_{93} ،S_{55} ،L_{125} ،S_{54}) as well as their possible parents including male (Sultana, Red-Sultana, Askari and Yaghuti) and female (Muscat, Ghezel, Dizmary, Rajabi, Alibaba, Alhaghi and Tabarze) cultivars were studied using SSRs markers. Female parents had big berry size and low seed per berry ratio, whereas male parents were completely seedless. Flowers were emasculated from plants that were used as

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female parents. The pollen from the four male individuals was collected and spread in equal quantities on the emasculated flowers of the female parents. The characteristics of plant materials, origin and their assumed pedigree are shown in Table 1.

Total genomic DNA was extracted from young leaf tissue after freezing in liquid nitrogen according to the protocol described earlier by Thomas *et al.* (1994).

Microsatellite Markers

Fourteen microsatellite loci developed earlier were selected for this study (Table 2). These loci were selected based on the obtained polymorphism and their position in map in order to satisfy the premise of independent segregation and also to allow the use of breeder right for identification of individuals.

Microsatellite Amplification and Detection

Polymerase chain reaction (PCR) was performed in 25 µl of mixture containing 40 *ng* DNA, 1 µl of each primer (0.4 mM), 100 µM of each dNTP, 1.5U Taq DNA polymerase, and 2.5 µl 1x reaction buffer that contained 2.1 mM $MgCl_2$, using Bio-Rad Thermal Cycler (model: i-Cycler). Thermal Cycle included a predenaturing step at 94ºC for 5 min and 35 thermal cycle (10 first cycle programmed touchdown). The time and temperature of extension step were 30 seconds and 72 ºC, respectively. Final extension occurred at 72 ºC for 7 minutes. The amplified products were separated on 6% denaturing vertical polyacrylamide gel that was stained with silver nitrate (Bassam *et al.,* 1991).

Statistical Analysis

Different genetic analyses were performed according to Nei (1978) that included polymorphic information content (PIC) and probability identity (PI). PIC and PI were calculated according to the below equations:

$PIC = 1 - \sum_{i=1}^{n} P_{ij}^{\ 2}$, where P_{ij} is the frequency of j^{th} allele from i^{th} marker.

$PI = \sum P_i^4 + \sum\sum (P_i P_j)^2$, where P_i and P_j are the allele frequencies of i and j (Ali panah *et al.,* 2006).

Cluster analyses through UPGMA method as well as Principle Component Ordination (PCO) were performed using NTSYSpc 2.02 software. The program Darwin 5.0 was used for the bootstrap analysis (Nei±Li distances; neighbor-joining tree-construction method; 300 resampled datasets).

Allele frequency, heterogenisity, effective allele number and parentage analysis were calculated using CERVUS 3.0 software (http://www.fieldgenetics.com) through a likelihood-based method (Kalinowski *et al.,* 2007). Data were collected by gel scoring based on molecular weight of every band. Later, parents and progenies were defined to the program once for female and male analysis and again for parent pair analysis. After simulation of each parent–offspring pair, the program calculated a LOD score (natural logarithm of the likelihood ratio) based on real data.

RESULTS

Microsatellite Polymorphism

The DNA templates from 23 genotypes were amplified by 14 microsatellite primers (Figure1). All the 14 microsatellite primers that were used in this study showed polymorphism and generated 78 alleles among 23 genotypes. The number of alleles varied in each locus from 3 alleles in VVMD24 to 10 alleles in VrZAG64 locus with an average of 5.86 and 5 alleles per locus on parents and progenies, respectively. The effective alleles among parents and progenies were 3.38 and 2.9, respectively (Table2).

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Table 1. The horticultural characteristics and pedigree of parental and offspring grapevine genotypes. [a]

Row	Genotype	Berry density	Berry attachment	Seedlessness	Hypothetical parents	Origin in horticultural research station, the university of Tehran
1	*Askari*	*medium*	*easy*	*Seedless*	*Pollinator (male)*[b]	*Old grape collection*
2	*Yaghuti*	*Very dense*	*medium*	*Seedless*	*Pollinator (male)*[b]	*Old grape collection*
3	*Sultana*	*medium*	*medium*	*Seedless*	*Pollinator (male)*[b]	*Old grape collection*
4	*Red-Sultana*	*medium*	*medium*	*Seedless*	*Pollinator (male)*[b]	*Old grape collection*
5	Muscat	loose	Very difficult	Seeded	Emasculated (female)[c]	Old grape collection
6	Ghezel	loose	Very difficult	Seeded	Emasculated (female) [c]	Old grape collection
7	Dizmary	medium	difficult	Seeded	Emasculated (female) [c]	Old grape collection
8	Rajabi	loose	Very difficult	Seeded	Emasculated (female) [c]	Old grape collection
9	Alibaba	loose	difficult	Seeded	Emasculated (female) [c]	Old grape collection
10	Alhaghi	Very loose	difficult	Seeded	Emasculated (female) [c]	Old grape collection
11	Tabarze	loose	medium	Seeded	Emasculated (female) [c]	Old grape collection
12	I21	medium	medium	Seedless	Rajabi × Yaghuti	Hybrids collection
13	K67	loose	difficult	Seedless	Muscat × Red-Sultana	Hybrids collection
14	R80	Very loose	difficult	Seedless	Rajabi × Askari	Hybrids collection
15	I73	Very loose	medium	Seedless	Rajabi × Sultana	Hybrids collection
16	A119	medium	difficult	Seedless	Muscat × Askari	Hybrids collection
17	R84	Very loose	difficult	Seedless	Rajabi × Askari	Hybrids collection
18	B98	Very loose	medium	Seedless	Rajabi × Sultana	Hybrids collection
19	S40	Very dense	medium	Seedless	Unknown	Hybrids collection
20	K93	Very loose	medium	Seedless	Dizmary× Sultana	Hybrids collection
21	S55	medium	medium	Seedless	Tabarze× Yaghuti	Hybrids collection
22	L125	Very loose	difficult	Seedless	Rajabi × Red-Sultana	Hybrids collection
23	S54	loose	difficult	Seedless	Tabarze × Yaghuti	Hybrids collection

[a]: The horticultural characteristic is from Erfani *et al*., 2008.

[b]: The pollen from male individuals was used after collecting the pollen from the four male plants and spreading equal quantities on the emasculated flowers of the female plants.

[c]: Female flowers were emasculated from plants that were used as female parents.

Table 2. Summary of microsatellite primer pairs used for genealogy and molecular diversity of Iranian grapevine progenies.

SSR Loci	Sequence 5` ⟶ 3`	Allele size (bp)	Primer length F	Primer length R	Linkage Group	Source
VVS2	CAGCCCGTAAAGTGTCCATC	129-155	21	25	11	Thomas & Scott 1993
VVS2	AAATTCAAAATTCTAATTCAACTGG					
VVS4	CCATCAGTGATAAAACCTAATGCC	167-186	24	22	8	Thomas & Scott 1993
VVS4	CCCACCTTGCCCTTAGATGTTA					
VVMD5	CTAGAGCTACGCCAATCCAA	226-246	20	24	6	Bowers et al. 1996
VVMD5	TATACCAAAAATCATATTCCTAAA					
VVMD7	AGAGTTGCGGAGAACAGGAT	233-263	20	20	7	Bowers et al. 1996
VVMD7	CGTTCCTTCACACGCTTGAT					
VVMD14	CATGAAAAAATCAACATAAAAGGGC	222-250	25	26	5	Bowers et al. 1999
VVMD14	TTGTTACCCAAACACTTCACTAATGC					
VVMD24	GTGGATGATGGAGTAGTCACGC	208-215	22	25	14	Bowers et al. 1999
VVMD24	GATTTTAGGTTCATGTTGGTGAAGG					
VVMD25	TTCCGTTAAAGCAAAAGAAAAAGG	243-275	24	24	11	Bowers et al. 1999
VVMD25	TTGGATTTGAAATTTATTGAGGGG					
VVMD27	GTACCAGATCTGAATACATCCGTAAGT	173-194	27	22	5	Bowers et al. 1999
VVMD27	ACGGGTATAGAGCAAACGGTGT					
VVMD36	TAAAATAATAATAGGGGGACACGGG	244-315	25	26	3	Bowers et al. 1999
VVMD36	GCAACTGTAAAGGTAAGACACAGTCC					
VMC4A1	ATGCGACCTTAATAAATTGGGAA	265-275	23	23	9	Digasper et al. 2005
VMC4A1	AAGCTACCGTTGTATGAGGGAGA					
VMC4H6	GTATAGAACCACGCATCCAACA	152-168	22	22	9	Digasper et al. 2005
VMC4H6	CCCTTAGTTTCCTCGTGCTTTT					
VMC4G6	CCTTGAAGAGATGAGTTTGCTA	114-176	22	22	6	Digasper et al. 2005
VMC4G6	TATTTAACTTTGTGCCTCTGCT					
VrZAG21	TCATTCACTCACTGCATTCATCGGC	190-214	25	25	4	Sefc et al. 1997
VrZAG21	GGGGCTACTCCAAAGTCAGTTCTTG					
VrZAG64	TATGAAAGAAACCCAACGCGGCACG	137-197	25	25	10	Sefc et al. 1997
VrZAG64	TATGAAAGAAACCCAACGCGGCACG					

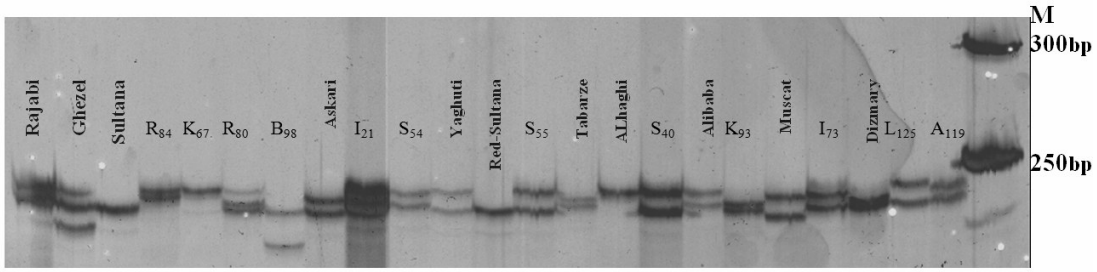

Figure 1. Poly acrylamide gel electrophoresis of microsatellite alleles stained with silver nitrate for VVMD5 locus. First column showed size marker, red lines showed parental (Askari, Yaghuti, Sultana and Red-Sultana) and maternal (Rajabi, Ghezel, Tabarze, Alibaba, Muscat, Alhaghi and Dizmary) cultivars and black lines included 12 progenies from their crossing.

PI value varied from 0.07 in VVS2 locus to 0.57 in VVMD27 locus, while two loci (VrZAG64 and VMC4A1) with mismatching were not considered in PI calculation (Table3).

Principal components ordination was carried out. Results showed that the first five components with Eigen values greater than one could define 71 percent of the total variance. The first and second components of PCO analysis included 45.96 and 8.28 percent of the variation, respectively, constituting 54.24 percent of the total variance. Results of two dimensional plot analyses determined four groups of genotypes that were close to each other besides a female (Ghezel) and a progeny (B_{98}) that were separated from others (Figure 2).

Cluster and Bootstrap Analysis

Genotypes were clustered based on UPGMA method using Dice similarity coefficient and the reliability of the dendrogram was obtained from repeated bootstrap analysis (Figure 3).

All studied genotypes were divided into five groups. Four groups consisted of parents and progenies that were close to each other. All of the progenies were located at similarity distance of 0.65% from the nearest parent.

The first group consisted of a female parent, Rajabi, as well as three progenies (I_{21}, I_{73} and B_{98}). Rajabi was the closest female parent to progenies with 50% similarity. The highest similarity (60%) was between I_{73} and B_{98}, whereas I_{21} showed less similarity to them (Figure 3.b1). Taberze (female parent) was separated from the rest of this group by similarity level of 35%. Gezel (female parent) was in the next group solely. The third group was divided into two major subgroups with a similarity coefficient higher than 50% (Figure 3 b.3 and4).

In the first subgroup, two male cultivars, namely, Sultana and Red-Sultana, showed 96% similarity and 100% bootstrap value. Among the genotypes, L_{125} was close to the Sultana and Red-Sultana, according to the similarity index (Figure 3. b3).

The second subgroup consisted of S_{54} and S_{55} with similarity value of 86% (Figure 3. b4), while the other genotypes showed lower similarity. On the other hand, S_{40}, as a progeny with 70% similarity to S_{55} and S_{54}, and Alibaba and Alhaghi, as female parents, were located in this group. Alibaba showed higher similarity to the progenies of this group with the value of 65%.Yaghuti was the most similar and the closest male parent to this group. K_{67} and Yaghuti, the male parent of $K_{67,}$ which were located in this subgroup, showed the highest similarity coefficient with the value of 72% (Figure 3, b4).

In the fourth group, a high similarity was observed between R_{84} and R_{80} as progenies with a value of 66%. Askari as a male parent and Dizmary as a female parent along with K_{93} were located in another subgroup along with Muscat and A119 with a similarity coefficient value of 60% (Figure 3, b5).

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Table 3. The genetic parameters obtained with 14 SSR primers used in 23 grapevine cultivars/ genotypes (11 parents and 12 progenies).

Locus name	Allele number		Observed heterozygosity		Expected heterozygosity		Polymorphic Information content		Effective alleles		Probability Identity
	progeny	parents	Progeny	parents	progeny	parents	progeny	parents	progeny	parents	total
VVMD5	6	6	1	0.64	0.87	0.81	0.7	0.75	3.37	3.95	0.14
VVMD7	4	5	0.5	0.46	0.76	0.84	0.68	0.77	3.13	4.26	0.15
VVMD14	5	7	0.67	0.82	0.74	0.84	0.66	0.77	2.96	4.35	0.13
VVMD24	3	3	1	0.55	0.65	0.65	0.56	0.55	2.25	2.24	0.35
VVMD25	5	5	0.83	0.64	0.82	0.73	0.75	0.64	4.03	2.8	0.24
VVMD27	5	6	1	1	0.79	0.82	0.73	0.75	3.64	4.03	0.57
VVMD36	5	5	0.75	0.91	0.79	0.77	0.71	0.69	3.47	3.22	0.19
VVS2	7	9	1	0.82	0.85	0.91	0.79	0.85	4.78	6.62	0.07
VVS4	2	4	0.33	0.64	0.39	0.52	0.31	0.45	1.44	1.82	0.36
VMC4G6	3	6	0.5	0.64	0.58	0.68	0.49	0.61	1.95	2.55	0.26
VMC4H6	6	7	0.83	1	0.76	0.83	0.69	0.77	3.23	4.26	0.15
VMC4A1	5	5	0.67	0.91	0.84	0.78	0.77	0.70	4.41	3.36	-
VrZAG64	9	10	0.75	1	0.88	0.89	0.82	0.84	5.65	6.1	-
VrZAG21	3	4	0.83	0.82	0.63	0.64	0.52	0.73	2.07	3.66	0.26
Mean	5	5.86	0.76	0.77	0.73	0.76	0.66	0.70	2.9	3.38	

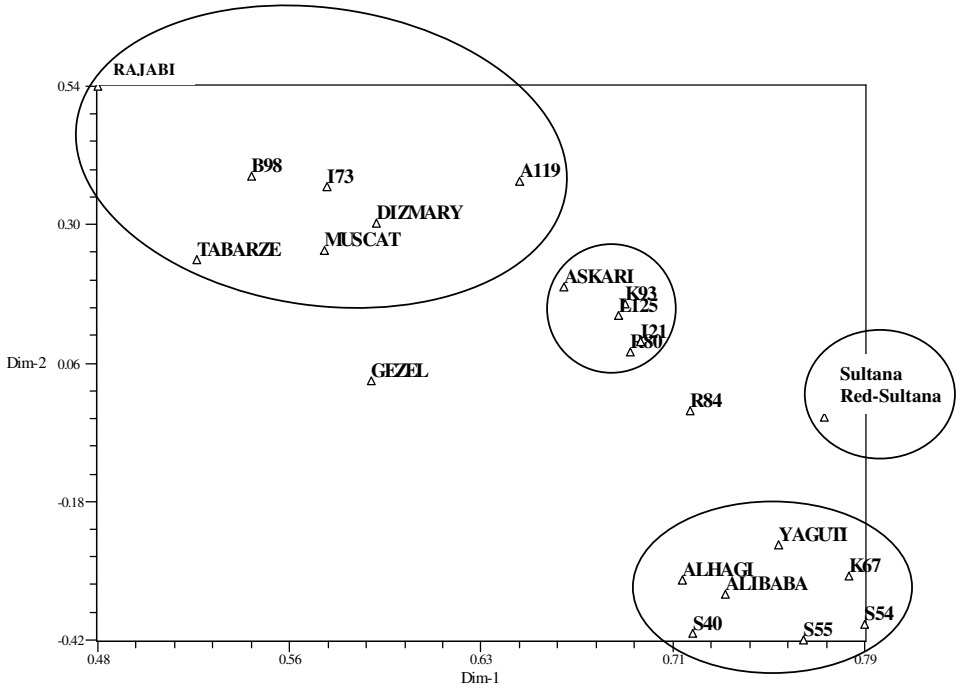

Figure 2. Two-dimensional plots of Principal component ordination for 14 SSR primers in parental (Askari, Yaghuti, Sultana and Red-Sultana) and maternal (Rajabi, Ghezel, Tabarze, Alibaba, Muscat, Alhaghi and Dizmary) grapevines cultivars and their 12 progenies.

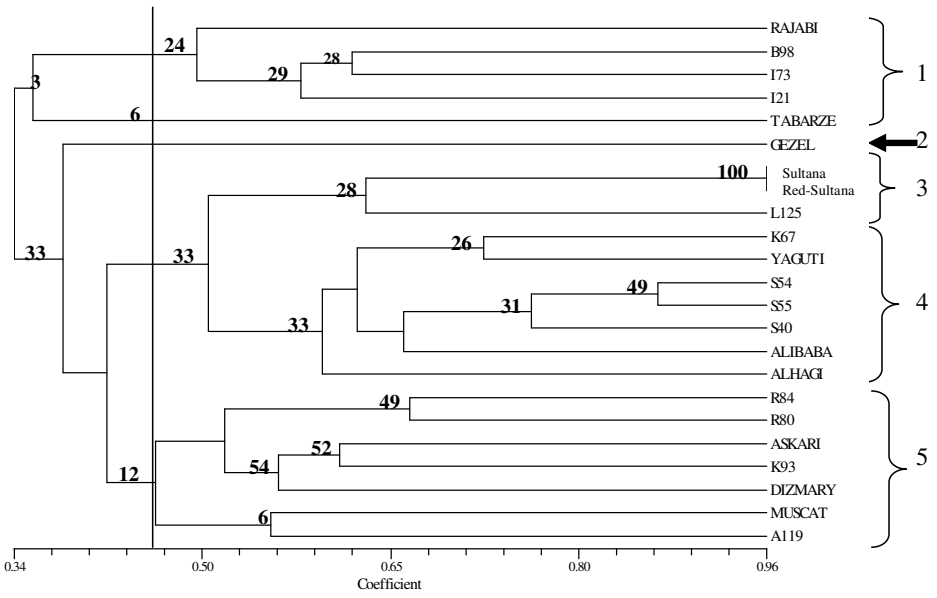

Figure 3. UPGMA dendrogram based on Nei's distance showing genetic relationships among the 23 grapevine that divided them to 5 group included parental (Askari, Yaghuti, Sultana and Red-Sultana) and maternal (Rajabi, Ghezel, Tabarze, Alibaba, Muscat, Alhaghi and Dizmary and their 12 progenies) . Numbers on the branches are bootstrap values (%) obtained from 300 replicate analyses.

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The bootstrap value varied from 3% to 100%. The least value was observed for a group consisting of two female parents (Tabarze and Rajabi) as well as three progenies, I_{21}, I_{73} and B_{98}, whereas the highest value was in a group including Sultana and Red-Sultana as male parents.

Parentage Analysis

Three methods of parentage analysis including maternity (pollen receptor parent), paternity (pollinator), and parent pair analysis were carried out by CERVUS 3.0 software. At first, some simulation parameters were defined to the software (Table 4), then, categorical and fractional allocations were used for likelihood based assignment approaches. The categorical allocation assigned the entire offsprings to a particular parent, whereas the fractional technique distributed offsprings among some most probable parents. Later, maternity, paternity and parent pair analysis were done to find and select the highest LOD score and precision. Finally, results for all progenies were compared (Table 5) and progenies were assigned to their parent(s) (Figure 4).

The assignment of some progenies such as I_{21} and B_{98} resulted in a negative LOD score (Table 5).

Results showed that, if the parents have similar genetic backgrounds, the software could introduce the close parent in addition to the true parent (closest) even for the control genotypes (Table 5).

This software introduced male and female parents of progenies with positive LOD score and significant confidence. Schematic relationships of all parents and their progenies are demonstrated in Figure 4.

DISCUSSION

Microsatellite markers used in this study were realized to be useful for recognition, identification, and discrimination of genotypes in grape as suggested by previous studies (Thomas *et al*., 1993; Bowers *et al.*, 1996; Fatahi *et al.,* 2004). The 78 alleles and their frequency showed good diversity among genotypes, which could be applicable to polymorphism studies. Our results showed that the most useful markers in this study were VVMD27, due to its 100% heterozygosity and high PIC value demonstrated in both parents (0.75) and progenies (0.73), and VVS2 and VrZAG64, due to their highest number of alleles, effective alleles, and polymorphic information contents.

Therefore, the potential of the above mentioned markers to identify each cultivar were considerable. Hence, through selecting discriminating markers, the numbers of markers required to discriminate cultivars can be reduced.

In this study we also found some rare alleles for cultivars and genotypes. They could be used as indices for registration and identification of specific cultivar as well as breeder rights protection.

Moreover, combination of markers with

Table 4. The parameters used in simulation with the CERVUA3.0 software and the values used in simulations for 23 grapevine genotype and cultivars (11 parents and 12 progenies).

Parameters	Value used
Number of candidate female	7
Proportion of candidate female sampled	1
Number of candidate males	4
Proportion of candidate males sampled	1
Proportion of loci typed	0.854
Rate of typing error	0.01
Relaxed confidence level	80%
Strict confidence level	95%

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Table 5. Comparison of three parentage methods for 3 superior genotypes. The LOD Score (natural logarithm of the likelihood ratio) showed in blow of the result of each method. [a]

Final result		Likelihood based assignment			
		Parent pair	female (M)	male (F)	Progenies
(M)	(F)	(M)×(F)	(M)	(F)	
(Tabarze or Rajabi)×(Sultana or Red-Sultana)		Rajabi×Yaghuti	Tabarze	Sultana (Red-Sultana)	B_{98}
		-0.197 -0.229	-0.13	-3.61	LOD
(Rajabi or Ghezel) ×Yaghuti		Rajabi×Yaghuti	Ghezel	Yaghuti	I_{21}
		-0.135 -5.33	-0.115	-0.034	LOD
Alibaba×Yaghuti		Alibaba×Yaghuti	Alhaghi	Yaghuti	S_{54}
		2.97 -5.26	-1.26	0.42	LOD
Alhaghi×Yaghuti		Alhaghi×Yaghuti	Alhaghi	Yaghuoti	S_{40}
		4.33 -1.6	-4.33	3.21	LOD

[a] negative LOD scores for parents and their progenies aroused from the fact that Iranian grape cultivars, used as parents, were sharing similar genetic backgrounds (For more detail see the text).

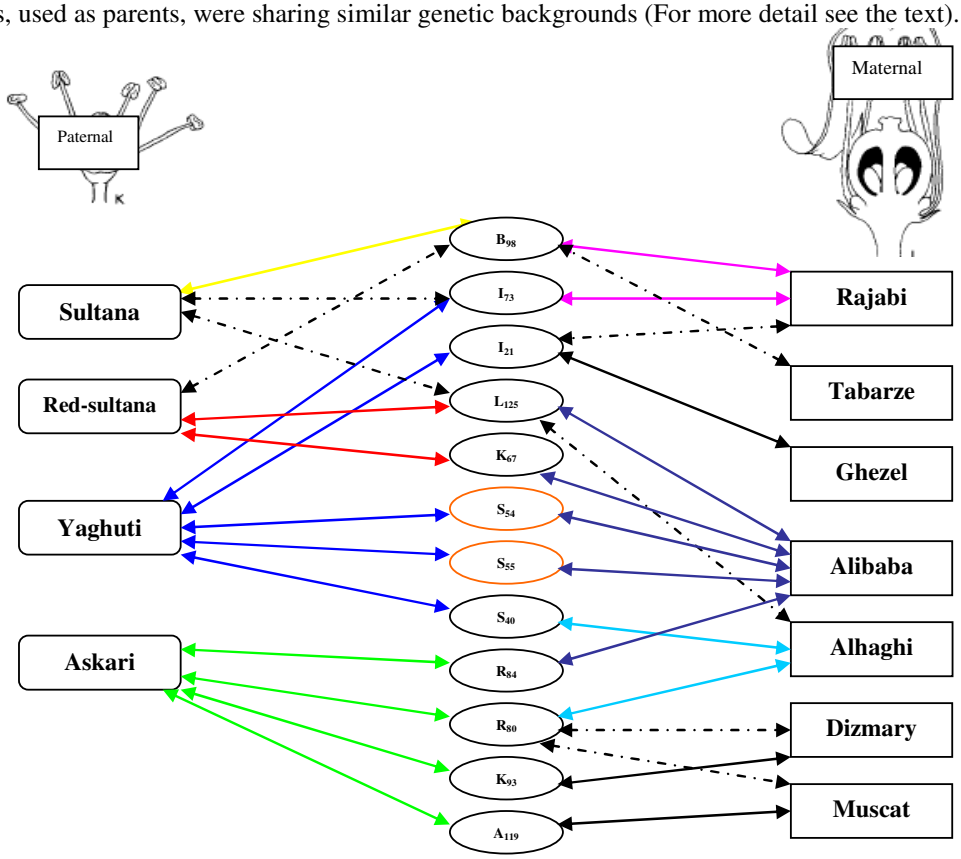

Figure 4. Final results of Parentage analysis for four male parents (pollinator), seven female parents (pollen receptor) and 12 superior genotypes. Bulk lines show categorical allocation results and dot lines show fractional allocation results based on 14 microsatellite primers.

low PI could be used in discrimination and registration of new cultivars. PI value is the probability that showed the most variation value between genotypes and high discrimination power of markers (Alipanah *et al.,* 2006). Results showed that in some loci the observed heterozygosity was greater than expected, but the mean of the observed

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heterozygosity was slightly lower than the mean expected. Some factors such as null allele or crossing between similar individuals in breeding program could result in increasing homozygosity in progenies. Production of heterozygous genotypes to benefit heterosis was the final goal of this breeding program.

Low distribution of molecular markers in the genome is the result of high values of the first components of PCO analysis. However, proper distribution of markers on chromosome could be due to low amounts of variation that is explained by the first few components of PCO. In evaluation of genetic diversity, uniform and appropriate distribution of markers may cover the entire genome. Therefore, if markers are selected from different parts of the genomes, the correlation between them will be low and higher number of components would be significant to describe their total variations.

The results of cluster analysis revealed that microsatellite markers were capable of discriminating progenies from their parents (Figure 3), except for two male parents, Sultana and Red-Sultana, which were morphologically indistinguishable in all characters except their berry color. Previous studies suggested that microsatellite markers were not able to distinguish diversity among berry color mutation in grapes (Fatahi *et al.,* 2003; Sefc *et al.,* 1998; Lopez *et al.,* 1999). Moreover, similarity coefficient of L_{125} with Sultana and Red- Sultana were 65% and 60%, respectively, reflecting more similarity between L_{125} and Sultana. This could be related to genotyping error. However, in order to determine this relationship, evaluation with more loci number is recommended.

Several groups and subgroups were observed in cluster (Figure 3), showing that our superior progenies originated from different crosses among seedless and seeded cultivars. Results showed that superior progenies used in this study were hybrid due to accuracy in emasculation and controlled pollination and the similarity coefficient being lower than that needed to support selfing in female parents: according to Fatahi *et al.* (2003), in self-fertilization, the similarity between progeny and parent must be greater than 70%. Cluster analysis indicated that hybrid genotypes of S_{54} and S_{55} were full sib, which originated from a single cross with the same parents. They also have a common male parent with S_{40} and K_{67} genotypes and created half sib, due to different female parents. Hampel *et al.* (2001) reported high bootstrap values and short terminal branches for the *Tritrichomonas foetus*/*suis,* suggesting that they were close relatives (clonal) created by radiation, as has been found in this study for Sultana and Red-Sultana. The relatively lower bootstrap values and long terminal branch could have resulted from genetic recombination (sexual reproduction), which suggests more ancient radiation (Hampl *et al.* 2001).

Although, cluster analysis discriminated progenies and parents from each other, it was unable to determine the parent of each progeny. In this study, some of the groups and subgroups included progeny(ies) and a female parent (Figure 3, b.1, 2 and 5), but none of them could be used to assign progeny genotype(s) to pair parents.

Kalinowski *et al.* (2007) suggested that relationship estimation is notoriously vulnerable to genotyping error that can be caused by contamination, allelic dropout, microsatellite stutter, null alleles or human error. To solve these problems, convenient software such as CERVUS 3.0 should be used since, by using likelihood based assignment approaches, they are capable of distinguishing and considering the probability of errors occurrence. Otherwise, more loci numbers should be studied.

In this study, parents of some of the progenies were confirmed by the use of likelihood based assignment. However, in some other cases, new parents were suggested. Assignment approach was not able to introduce precise parents when there was allele similarity for parents in certain loci. Therefore, in such cases, the program introduced the most similar parents instead

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of just one male or female parent (Table 5). For example, for progeny I_{21}, among seven female candidate plants, two cultivars, namely, Rajabi and Ghezel, were introduced as the final possible female parents. Considering their negative LOD scores, these two cultivars probably had common ancestor and genetic backgrounds .

Our results showed that negative LOD scores in the output of likelihood based assignment approaches for parents and their progenies stemmed from the fact that the Iranian grape cultivars used as parents were sharing similar genetic backgrounds. Accordingly, it seems that distance based analysis would not identify parents of grape progenies.

Assignment results introduced new parents that had genetic backgrounds similar to the previously known parents but showed different morphology. It would be useful to decrease inbreeding depression while crossing progenies with their parents. It is well known that backcrossing of progeny with heterozygous female parents results in inbreeding depression. Thus, in order to maintain heterozygosity level, breeders tend to use a cultivar that is close to the female parent. Fractional allocation will determine the best female parent for backcrossing in grapevine breeding program.

For the most accurate maternity, chloroplast SSR (cpSSR) markers could be used as a useful tool that demonstrates utility in studying genetic relationships, germplasm management, evolutionary studies, and analysis of the material from introgression and somatic-fusion experiments (Siragusa and Carimi 2009).

As a general conclusion, our results indicate that highly polymorphic microsatellite markers could be used for genetic diversity study, testing the accuracy in the results of controlled crossing, and in determination of the relationship between progenies and their parents. The effect of low genome coverage or the incidence of errors in the results of microsatellite markers can be tested and improved by using some complementary software. Then, by applying likelihood-based assignment approach to the test data, determination of the original or close parents of the progenies and a precise genealogy could be possible.

The results of this study can be employed to avoid off-type occurrences for better genealogy, to discriminate progenies from each other and from their parents, and to find proper female parents for the next backcrossing programs.

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تبارشناسی و بررسی تنوع مولکولی نتاج حاصل از انگور ایرانی

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چکیده

انگور یکی از رایج‌ترین محصولات باغبانی جهان است. از قرن گذشته تاکنون تلاش‌های زیادی برای اصلاح انگور در دنیا صورت گرفته است که اغلب تبارشناسی نیز با آن همراه بوده تا تاریخچه افراد معلوم باشد. بدلیل بروز برخی اشتباهات در نامگذاری ژنوتیپ های برتر دورگ و بمنظور تایید صحت تلاقی‌های کنترل شده، تعیین والدین و شجره آنها، ۲۳ ژنوتیپ انگور شامل ۱۲ ژنوتیپ برتر حاصل از ۲۲ تلاقی کنترل شده و والدین آنها که شامل چهار رقم بیدانه و هفت رقم دانه دار انگور برنامه اصلاحی انگور ایران، توسط ۱۴ نشانگر ریزماهواره مورد مطالعه قرار گرفتند. بیشترین تشابه یک والد مادری با نتاج به میزان ۰/۶۵ و بین رقم علی‌بابا و سه ژنوتیپ S_{40} ,S_{55}, S_{54} بدست آمد. نتایج بروز خودگرده افشانی در والدین مادری را مردود دانسته و نتاج را والدین تفکیک نمود. اما به دلیل پس زمینه مشترک ژنتیکی در جمعیت والدین، امکان انتساب نتاج به والدین شان از طریق تجزیه کلاستر و شمارش آلل‌ها امکان پذیر نبود. بنابراین تجزیه شناسایی والدین بر اساس رویکردهای انتساب مبتنی بر درست نمایی و با استفاده از نرم افزار CERVUS 3.0 صورت گرفت. با استفاده رویکردهای انتساب و بر مبنای مقادیر مثبت و منفی امتیاز LOD والدین واقعی از والدین کاندید شناسایی شدند. نتایج نهایی نشان داد خطای ژنوتیپ‌یابی که می‌تواند مربوط به تعداد کم مکان ریزماهواره و یا تشابه پس زمینه ژنتیکی والدین باشد، کاهش یافت. علاوه براین روابط خواهر و برادری تنی و ناتنی بین ژنوتیپ های S_{55}وS_{54} با S_{40} مشخص گردید. همچنین با استفاده از این نتایج و نیز بدلیل تشابه پس زمینه ژنتیکی ارقام انگور ایرانی، امکان تعیین بهترین والد جهت انجام تلاقی برگشتی نیز فراهم گردید.

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