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# Genetic Diversity among Iranian Grape Cultivars using Molecular and Morphological Markers

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**Abstract**

This study describes the genetic relationships among 24 Iranian grape cultivars using inter simple sequences repeat (ISSR) and morphological markers. Eleven ISSR primers generated 110 polymorphic bands which were used in the analysis. The lowest genetic similarity was observed between 'Ghzl uzum Tabriz' and two cultivars including 'Goye maleki' and 'Maie mev' (0.38) while the highest genetic similarity was found between 'Ghzl uzum Tabriz' and 'Ghara shira' (0.70). The grape cultivars were grouped into four clusters. In the morphological analysis, which included 14 traits, 9 were polymorphic. The genetic similarity coefficient ranged from -0.67 to 0.97 for this morphological analysis and the resultant dendrogram revealed three main clusters. ISSR markers could detect high polymorphism among the cultivars and the ISSR method was shown to be a suitable means of determining genetic diversity in future conservation and breeding programs.

Grapes belong to the family Vitaceae and the genus *Vitis* has been grouped into two sections, 11 series, and more than 60 species (10). *Vitis vinifera* L., the most widely cultivated species of the *Vitis* genus, is grown throughout the temperate and tropical regions of the world for fresh fruit, raisin, juice and wine production (21, 25). Grapevine (*Vitis vinifera L.*) is one of the oldest crops and is the main Mediterranean/Western Asiatic representative of the *Vitis* genus. Its domestication created cultivars suited to a wide diversity of climates, tastes and uses. With some controversy among researchers, the number of grape cultivars is generally estimated at around 5000 (2). *Vitis vinifera* has been the subject of extensive genetic studies due to its worldwide cultivation and importance. The *Vitis vinifera* genome is relatively small at approximately 500 Mbp (four times the size of *Arabidopsis thaliana* genome and one-sixth the size of maize genome) but quite complex with only about 4% of the genome transcribed (10).

Iran is a large country and a large number of economically-important grape cultivars have been developed throughout the country's history. In fact, the country is one of the leading producers of table grape varieties. Iran, with more than 300,000 ha of cultivated grapevines, is characterized as having a rich, complex and diverse viticulture heritage (23). The number of grape cultivars within the country is estimated at around 250.

Over the past centuries, historic evidence combined with ampelographic data, have frequently been used to generate hypotheses about the origin and relationships among different grapevine cultivars (18). International organizations such as the Office International de la Vigne et du Vin (OIVV), or the International Plant Genetic Resources Institute (IPGRI), have defined the main ampelographic descriptors used for cultivar identification (3, 4), even though it is recognized that morphological data are often not able to distinguish between closely-related cultivars (8). To define the existing genetic composition of grapevine selections, isozyme and DNA markers have been used by various researchers in Iran (8). However, none of these studies have used the ISSR technique for genetic

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analysis of *Vitis* material to date. In fact, molecular marker studies based on ISSR labeling are relatively few (7, 13, 19, 26), while identification and descriptions of diversity on the basis of AFLP, RAPD, RFLP and SSR methods (5, 6) have been numerous.

Inter Simple Sequence Repeat (ISSR)-PCR is a technique, which involves the use of microsatellite sequences as primers in a polymerase chain reaction to generate multi locus markers (10, 11, 26). It is a simple and quick method that combines most of the advantages of microsatellites (SSR) and Amplified Fragment Length Polymorphism (AFLP) with the universality of the Random Amplified Polymorphic DNA (RAPD) method (1, 25). ISSR markers are highly polymorphic and useful in studies on genetic diversity, phylogeny, gene tagging, genome mapping and evolutionary biology (17). ISSRs exhibit the specificity of microsatellite markers, but need no sequence information for primer synthesis, enjoying the advantage of random markers (15).

The objectives of the present study were to (a) reveal the ISSR-based genetic diversity among different types of grape cultivars, (b) analyze polymorphism level of ISSR primers used, and (c) assess the genetic relationship within cultivars based on morphological traits in Iran.

## Materials and Methods

*Plant material*

Young, tender and unbruised leaves of 24 grapevine (*Vitis vinifera* L.) genotypes were collected from the germplasm collection at the Agriculture Research Center in Azarbaijan. The commercial cultivars, their origins and main properties are presented in Table 1. Fig. 1 shows representatives of two of the studied cultivars.

*DNA extraction*

DNA for ISSR analyses was purified according to the CTAB-based protocol of Doyle and Doyle (9) with minor improvements by Kafkas et al. (16). The amplification of ISSR

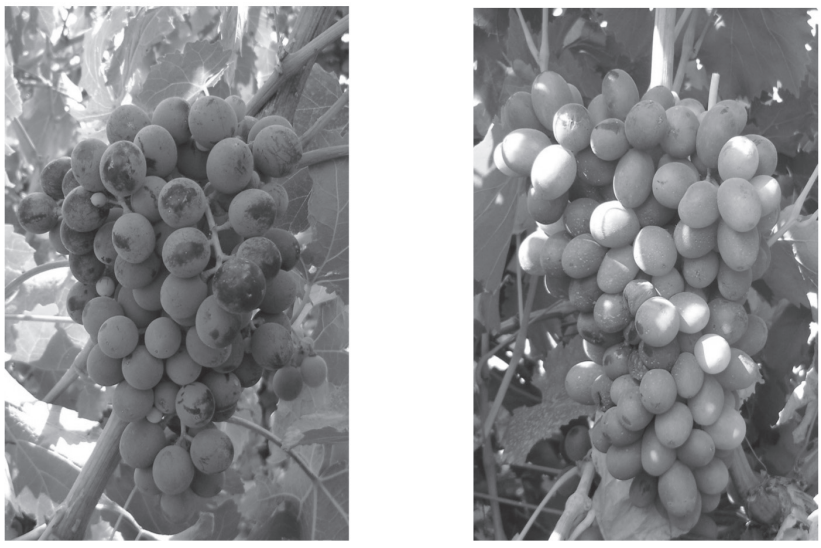


**Fig. 1.** Two cultivars of *Vitis vinifera* from Iran used in this study. A) 'Ghara shira' and B) 'Sahebi'.

**Table 1.** List of the grape cultivars with their origins, genetic composition and main properties.

| No | Cultivars | Origin | Genetic composition | Color of skin | Seed |
|---|---|---|---|---|---|
| 1 | Hosseini | Urmia | *V. vinifera* | Green/yellow | Seeded |
| 2 | H4 | Hybrid (Urmia) | *V. vinifera* 'Jighjigha'× *V. vinifera* 'Riparia Gloire' | Green/yellow | Seeded |
| 3 | Khalili sefid | Iran-unknown | *V. vinifera* | Green/yellow | Seeded |
| 4 | Shahroudi | Shahroud | *V. vinifera* | Green/yellow | Seeded |
| 5 | Fakhri | Tabriz | *V. vinifera* | Green/yellow | Seeded |
| 6 | Rezghi | Urmia | *V. vinifera* | Green/yellow | Seeded |
| 7 | Yaghoti | Iran-unknown | *V. vinifera* | Dark red/violet | Seeded |
| 8 | Ghzl uzum Tabriz | Tabriz | *V. vinifera* | Red | Seeded |
| 9 | Lale sefid | Urmia | *V. vinifera* | Green/yellow | Seeded |
| 10 | Ghzl uzum urmiye | Urmia | *V. vinifera* | Red | Seeded |
| 11 | Ghara shira | Urmia | *V. vinifera* | Rose | Seeded |
| 12 | Sahebi | Iran-unknown | *V. vinifera* | Red | Seeded |
| 13 | Shahroudi asl | Shahroud | *V. vinifera* | Green/yellow | Seeded |
| 14 | Lale siah | Urmia | *V. vinifera* | Red/grey | Seeded |
| 15 | Inak amj aii | Urmia | *V. vinifera* | Green/yellow | Seeded |
| 16 | H6 | Hybrid (Urmia) | *V. vinifera* 'Gharauzum'× *V. vinifera* 'Kober 5BB' | Red | Seeded |
| 17 | Kalati | Urmia | *V. vinifera* | Green/yellow | Seeded |
| 18 | Lale ghermez | Urmia | *V. vinifera* | Red | Seeded |
| 19 | Goye maleki | Urmia | *V. vinifera* | Green/yellow | Seeded |
| 20 | Rasha | Kurdestan | *V. vinifera* | Blue/black | Seeded |
| 21 | Yaghoti asl iran | Iran-unknown | *V. vinifera* | Green/yellow | Seeded |
| 22 | Khalili shirazi | Shiraz | *V. vinifera* | Green/yellow | Seeded |
| 23 | Maie mev | Urmia | *V. vinifera* | Green/yellow | Seeded |
| 24 | Ghara gandoma | Urmia | *V. vinifera* | Red/grey | Seeded |

loci was performed by the method Zietkiewicz et al. (27) with the minor modifications of Kafkas et al. (16). DNA concentration was determined using a Biophotometer (Eppendorf, Germany) and checked for integrity on 1% agarose gel.

*ISSR analysis*

The effects of Taq polymerase concentrations, template DNA concentrations, and different periods of time and temperatures during the annealing stage of amplification were optimized. Twenty four cultivars were analyzed using 11 polymorphic primers selected from 30 ISSR primers (Cinnagen, Iran) used in this study. The amplification reaction was carried out in 25 µL containing 3 µL genomic DNA (10 ng/µL), 2.5 µL of 10x PCR buffer, 0.4 µL primer (100 µM), 0.25 µL of Taq DNA polymerase (Fermentas, Germany), 0.5 µL of dNTPs (10 mM each of dATP, dGTP, dTTP, dCTP), 0.75 µL of $MgCl_2$ and 17.6 µL of sterile MQ water. After 3 min at 95°C pre-denaturation, 40 cycles of PCR were performed (30 sec at 95°C denaturation, 45 sec at a primer-specific annealing temperature (between 48-60°C depending on the primer used), 2 min at 72°C extension followed by 10 min at 72°C final extension. Aliquots of ISSR products were analyzed on 1.5% agarose gel with 0.5X TBE buffer. After staining with ethidium bromide, the gels were photographed using a Gel Logic 212 Pro Imaging System (Carestream, USA). ISSR markers

were scored for absence (0) and presence (1), each band being regarded as a locus. A Jaccard similarity matrix was calculated using NTSYS pc 2.02 software (21). Based on the similarity matrix, a dendrogram showing the genetic relationships among cultivars was constructed using a COMPLETE LINKAGE clustering analysis (22).

*Morphological traits*

A total of 14 morphological traits were evaluated using OIV (3, 4) grapevine descriptors. The analysis was performed at the Agriculture Research Center of Urmia. Five out of 14 traits were identical among the cultivars tested including young shoot (form of tip), mature leaves (color), mature leaves (shape of petiole sinus), bunch size and number of full seeds. Therefore, 9 polymorphic morphological traits (Table 2) were selected for description and further statistical analysis. Matrix data were obtained (Table 2), and statistical analysis performed in order to find possible relationships among the cultivars of *Vitis vinifera* studied. Analysis of the data was carried out using NTSYSpc 2.02 software and the genetic distance matrix was assessed using a CORR similarity coefficient (22). The resultant dendrogram was plotted using the COMPLATE LINKAGE method in the SAHN program (22).

## Results and Discussion

A total of 128 strong and well resolved bands were generated using the 11 ISSR primers (Table 3). 110 bands were polymorphic and the polymorphism rate was 85.48 %. The number of total bands ranged from 6 to 17 with a mean value of 11.63. Fig. 2 is representative of PCR amplified bands gen-

**Table 2.** Morphological characterization of *Vitis vinifera* cultivars in Iran

| Clone name | OIV003 | OIV051 | OIV067 | OIV068 | OIV151 | OIV222 | OIV225 | OIV223 | OIV304 |
|---|---|---|---|---|---|---|---|---|---|
| Hosseini | 1 | 7 | 3 | 2 | 3 | 4 | 1 | 2 | 3 |
| H4 | 2 | 5 | 3 | 2 | 3 | 3 | 1 | 2 | 3 |
| Khalili sefid | 1 | 2 | 3 | 2 | 3 | 2 | 1 | 3 | 1 |
| Shahroudi | 1 | 2 | 3 | 2 | 3 | 3 | 1 | 3 | 4 |
| Fakhri | 2 | 5 | 3 | 2 | 3 | 3 | 1 | 2 | 2 |
| Rezghi | 1 | 5 | 3 | 2 | 3 | 2 | 1 | 3 | 1 |
| Yaghoti | 2 | 2 | 2 | 4 | 3 | 3 | 5 | 3 | 2 |
| Ghzl uzum Tabriz | 4 | 2 | 3 | 2 | 5 | 4 | 3 | 3 | 2 |
| Lale sefid | 1 | 7 | 3 | 2 | 3 | 3 | 1 | 4 | 3 |
| Ghzl uzum urmiye | 3 | 2 | 3 | 3 | 4 | 5 | 3 | 3 | 4 |
| Ghara shira | 2 | 2 | 3 | 2 | 3 | 3 | 2 | 4 | 4 |
| Sahebi | 1 | 4 | 3 | 2 | 3 | 5 | 3 | 0 | 4 |
| Shahroudi asl | 2 | 6 | 3 | 3 | 3 | 2 | 1 | 3 | 3 |
| Lale siah | 1 | 2 | 3 | 2 | 3 | 3 | 4 | 5 | 4 |
| Inak amj aii | 0 | 4 | 3 | 2 | 3 | 3 | 1 | 9 | 4 |
| H6 | 2 | 2 | 3 | 2 | 3 | 2 | 3 | 2 | 3 |
| Kalati | 0 | 5 | 4 | 2 | 3 | 3 | 1 | 3 | 3 |
| Lale ghermez | 2 | 2 | 3 | 2 | 3 | 4 | 3 | 4 | 4 |
| Goye maleki | 2 | 2 | 3 | 2 | 4 | 5 | 1 | 4 | 3 |
| Rasha | 2 | 7 | 4 | 2 | 3 | 2 | 6 | 4 | 4 |
| Yaghoti asl iran | 2 | 2 | 2 | 4 | 3 | 3 | 1 | 3 | 2 |
| Khalili shirazi | 2 | 5 | 3 | 2 | 3 | 3 | 1 | 3 | 2 |
| Maie mev | 2 | 7 | 3 | 2 | 3 | 3 | 1 | 3 | 3 |
| Ghara gandoma | 2 | 4 | 3 | 2 | 4 | 3 | 4 | 4 | 2 |

**Table 3.** Morphological characterization of *Vitis vinifera* cultivars in Iran

| Primer code | Sequence | $T_a$ (°C) | NTB | NPB | RP (%) |
|---|---|---|---|---|---|
| UBC807 | 5'-(AG)8T-3' | 55 | 13 | 13 | 100 |
| UBC826 | 5'-(AC)8C-3' | 55 | 14 | 14 | 100 |
| UBC827 | 5'-(AC)8G-3' | 48 | 13 | 12 | 92.30 |
| UBC835 | 5'-(AG)8YC-3' | 55 | 15 | 14 | 93.33 |
| UBC841 | 5'-(GA)8CC-3' | 55 | 8 | 5 | 62.5 |
| UBC842 | 5'-(GA)8YG-3' | 55 | 8 | 6 | 75 |
| UBC847 | 5'-(CA)8RC-3' | 55 | 10 | 10 | 100 |
| UBC848 | 5'-(CA)8RG-3' | 55 | 6 | 6 | 100 |
| UBC889 | 5'- DBD(AC)7-3' | 55 | 9 | 6 | 66.66 |
| UBC890 | 5'-VHV(GT)7-3' | 55 | 15 | 12 | 80 |
| UBC891 | 5'-HVH(TG)7-3' | 55 | 17 | 12 | 70.58 |
| Average | | | 11.63 | 10 | 85.48 |

R = (A,G) ; Y = (C,T) ;B = (C,G,T) ; D = (A,G,T) ; H = (A,G,T) ;V = (A,C,G) ; Annealing Temperature ($T_a$), Number of Total Bands (NTB), Number of Polymorphic Bands (NPB), Polymorphism Rate (PR).

erated by the UBC 891 primer. In previous studies usually 2 to 13 bands per ISSR primer were reported (13, 19, 26). Polymorphism rate varied between 62.5 and 100% and an absolute polymorphism rates of 100% was obtained with UBC 807, UBC 826, UBC 847 and UBC 848. Wu et al. (26) generated a total of 105 bands, with a 91% polymorphism rate, when analyzing 15 cultivars belonging to *V. amurensis, V. vinifera* and *Vitis* hybrids ((*V. amurensis*×*V. vinifera*) ×*V. amurensis)* when using 15 ISSR primers. These researchers also reported a polymorphism range between 60 and 100%. Similarly, Dhanorkar et al. (7) obtained a high polymorphism rate (96%) in a genetic assessment study of *V. vinifera, V. labrusca* and *V. rotundifolia* cultivars when using 13 selected ISSR primers. ISSR molecular fingerprinting of three grape varieties in Egypt produced a range in the number of

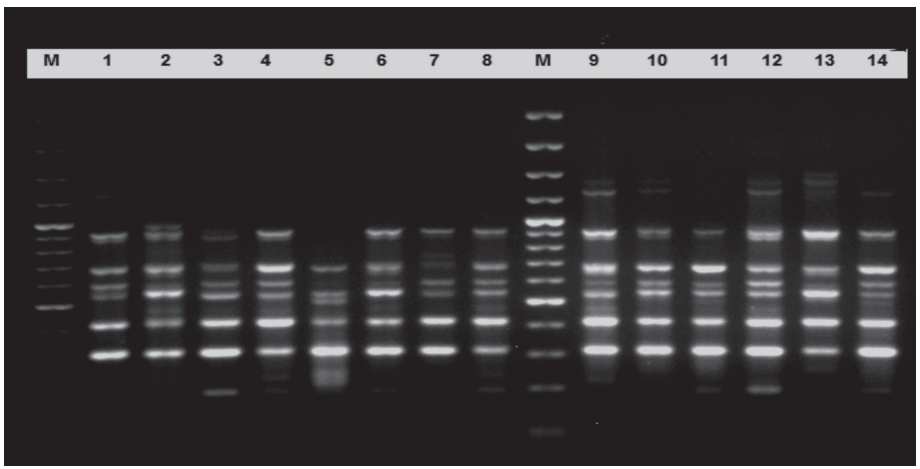


**Figure 2.** DNA fingerprinting of 14 grape cultivars using UBC 891 primer. Numbers represent cultivars listed in Table 1 and M indicates 100-3000bp DNA ladder.

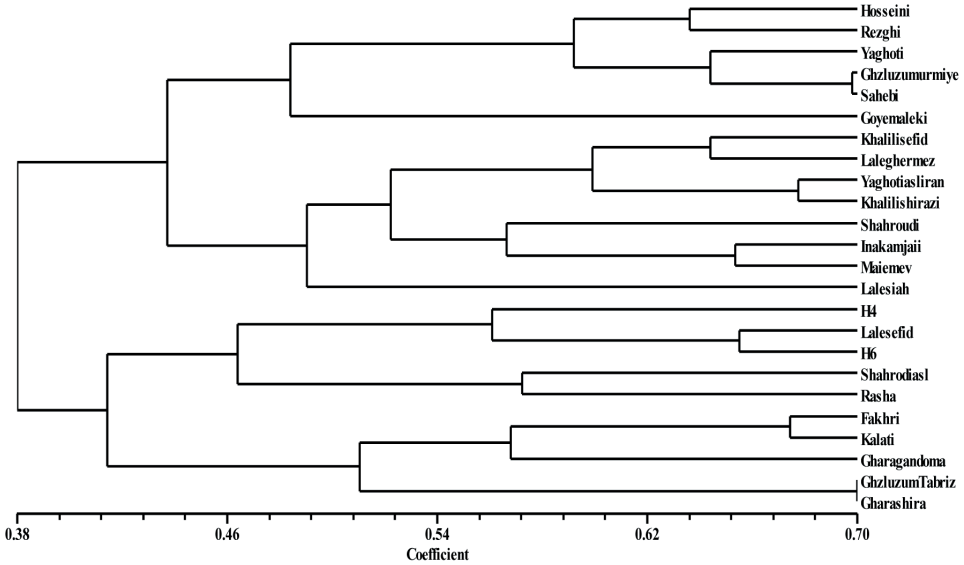


**Figure 3.** Dendrogram constructed using the ISSR marker data showing genetic relationships among 24 grapevine cultivars originating in or native to Iran.

bands per primer of between 7 and 14. The total number of polymorphic bands was recorded as 48, representing 53.9% polymorphism (12). These results suggest that ISSR primers are a powerful and reliable tool for differentiating among grape cultivars. In our study, ISSR genetic similarity values ranged from 0.38 for the most distantly related cultivars to 0.70 for the most closely related ones. The lowest genetic similarity was observed between 'Ghzl uzum Tabriz' and two cultivars from Urmia, 'Goye maleki' and 'Maie mev' with a numerical value of 0.38, followed by 0.40 between 'Ghzl uzum urmiye' and 'H4'. The highest genetic similarity was found between 'Ghzl uzum Tabriz' and 'Ghara shira' (0.70). Our results confirmed that the ISSR technique is a powerful tool in the analysis of grape germplasm and in the taxonomic investigation of genetic relationships within *Vitis* germplasm collections.

In the COMPLETE LINKAGE dendrogram, all 24 cultivars were clustered into 4 main groups (Fig. 3). Group I consisted of 'Hosseini', 'Rezghi', 'Yaghoti', 'Ghzl uzum urmiye', 'Sahebi' and 'Goye maleki'. Group II was composed of 'Khalili sefid', 'Lale ghermez', 'Yaghoti asl iran', 'Khalili shirazi', 'Shahroudi', 'Inak amj aii', 'Maie mev' and 'Lale siah'. Group III was comprised of 'H4', 'Lale sefid', 'H6', 'Shahroudi asl' and 'Rasha'. Group IV included 'Fakhri', 'Kalati', 'Ghara gandoma', 'Ghzl uzum Tabriz' and 'Ghara shira'. The twenty four grape cultivars were, therefore, clearly separated on the basis of ISSR fingerprinting. 'Ghzl uzum Tabriz' showed the strongest similarity to 'Ghara shira' among the cultivars analyzed.

Five out of 14 of the morphological traits studied appeared to be common among cultivars. Therefore, a cluster analysis using the other nine morphological traits was performed. The cultivars of *Vitis vinifera* divided into three main clusters (Fig. 4). Cluster I consisted of 'Hosseini', 'H4', 'Fakhri', 'Kalati', 'Rezghi', 'Lale sefid', 'Maie mev', 'Khalili shirazi', 'Shahroudi asl', 'Rasha' and 'Ghara gandoma'. Cluster II was comprised

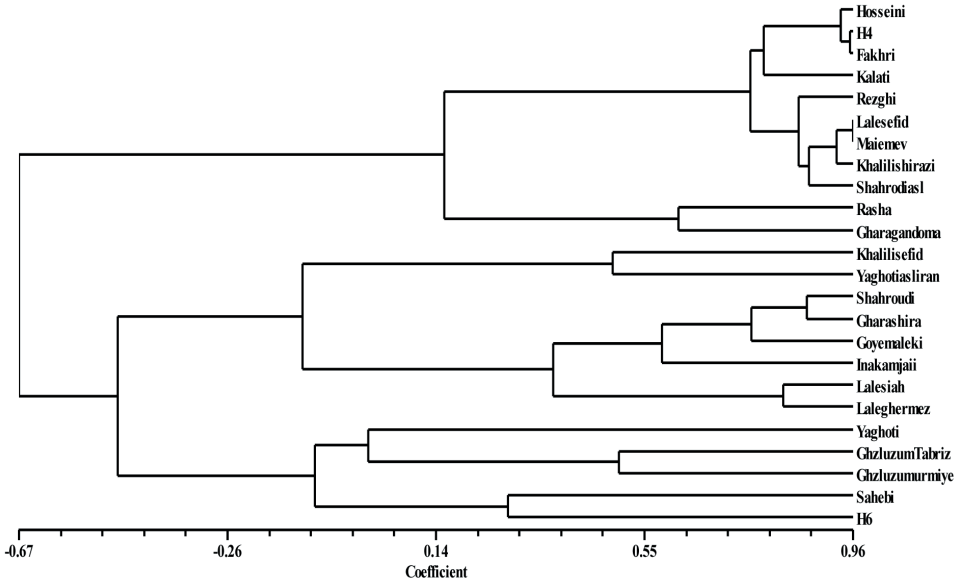


**Figure 4.** Dendrogram constructed using morphological traits based on COMPLETE LINKAGE clustering.

of 'Khalili sefid', 'Yaghoti asl iran', 'Shahroudi', 'Ghara shira', 'Goye maleki', 'Inak amj aii', 'Lale siah' and 'Lale ghermez'. Cluster III included 'Yaghoti', 'Ghzl uzum Tabriz', 'Ghzl uzum urmiye', 'Sahebi' and 'H6'. Within cluster I, 'Lale sefid' and 'Maie mev' appeared to be closest to each other. These two cultivars showed seven similar morphological traits including: young leaf color, mature leaf blade shape, mature leaf lobe number, inflorescence (sex of flower), berry size, berry skin color and physiological stage at fully maturity of the berry. Lowest morphological similarity was observed between 'H4' and 'Yaghoti' with a relatively low numerical value of -0.67 while, the highest similarity was found between 'Lale sefid' and 'Maie mev' (0.97). 'Yaghoti' appeared to be completely different from the others in three traits including young leaf color, shape of the leaf blade, and berry skin color (Table 2).

The dendrograms constructed by the two separate approaches showed some general similarities. For example, 'Khalili sefid', 'Yaghoti asl iran', 'Shahroudi', 'Inak amj aii', 'Lale siah' and 'Lale ghermez' were grouped in one cluster in both analyses. Both methods also clustered 'Yaghoti', 'Ghzl uzum urmiye' and 'Sahebi' in one group. However, ampelographic characters were insufficient to explain diversity among some of the closely related cultivars**.** Thus, we considered that ISSR genetic markers were superior over ampelographic characters to discriminate genetic variation among the Iranian grape cultivars studied.

The study of genetic relationships is important with regard to enhancing the efficiency of germplasm management in both breeding and conservation programs. High genetic diversity among 1000 domesticated grapes of *Vitis vinifera* subsp. *vinifera* based on Single Nucleotide Polymorphism (SNP) molecular marker analysis was previously reported (20) and believed to be due to introgression with the wild relative, *Vitis vinifera* subsp. *sylvestris*. Despite this high diversity, these investigators recommended extensive crossing to generate new selections in order to exploit the range of natural

genetic variation available (20). We propose that cultivars with the least similarity could be used to create the highest amounts of genetic variability in future Iranian breeding programs. Hence, crossing between 'Ghzl uzum Tabriz' and both 'Goye Maleki' and 'Maie mev' is suggested.

In conclusion, the present study reveals that PCR based fingerprinting techniques (ISSR) are informative for estimating the extent of genetic diversity as well as determining the patterns of genetic relationships. These findings should have management implications for conservation and the production of new grape cultivars in Iran.

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**MANUSCRIPTS:** These should be sent electronically in Microsoft Word format to ***Dr. Esmaeil "Essie" Fallahi at efallahi@uidaho.edu , Department of Plant, Soil, and Entomological Sciences, University of Idaho Parma Research and Extension Center, 29603 U of I lane, Parma, Idaho 83660, USA by May 30, 2013.*** The Awards Committee (4) will judge the papers, which must be acceptable for publication in a coming issue of the Journal. See a recent Journal for editorial style. Society membership is not mandatory. Paper length - *about 1000 words or three to four pages in the Journal of the American Pomological Society including photographs, charts, and tables*. Oversize manuscripts will be returned for shortening, or not considered. Publishing expenses will be covered for first place award paper(s).

**PAPER CONTENT:** Paper content should relate to cultivars of deciduous, tropical or subtropical fruits as related to climate, soil, rootstocks, a specific pomological experiment, a breeding project, history and performance of new or old cultivars, a library review pertinent to pomology, an MS or Ph.D. thesis paper.

**AWARDS PRESENTED:** At the annual meeting of The American Pomological Society (APS) which is help in conjunction with the annual meetings of the American Society for Horticulture Science. Authors of the Award winning papers are encouraged to attend the annual meeting of APS and received their Award(s). Consult your advisor on location and time of the next meeting.

**PROFESSORS, RESEARCHERS:** Please encourage your students to take part in this competition. Purpose of the contest is to encourage promising and gifted students to specialize in the field of pomology. This field is becoming short of leaders in the professional, industrial and orchard management areas. Winning papers should assist the author(s) in job seeking or a promotion!

**Deadline for Submission is May 30, 2013**