Genetic diversity of Iranian and some of European grapes revealed by microsatellite markers

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Abstract
In order to characterize Iranian grape (Vitis vinifera L.) germplasm, 136 genotypes were collected from five grape growing regions (Azarbaijan, Qazvin, Kordestan, Khorasan and Fars) and genotyped along with 36 European cultivars using 9 sequence tagged microsatellite sites (STMS) markers. The used set of markers could distinguish all 172 genotypes under study. Altogether 84 polymorphic alleles were observed detected all the genotypes, with an average of 9.33 and 5.81 effective alleles per locus. The expected heterozygosity values were higher than those observed for all the loci. This could probably be due to the occurrence of null alleles at these loci. The usefulness of this set of markers for genotype distinction was assessed as probability of identity (PI). The estimated total PI value over all the geographic regions for this set of markers was estimated to be 5.67×10^{-9}. Comparison of samples from different grape growing regions of Iran and Europe based on various parameters using allelic data revealed similar level of genetic variation. Analysis of molecular variance (AMOVA) indicated significant difference between samples, however, no difference was observed between the Iranian and European groups. Genetic differentiation among samples based on Fst in most pairwise comparisons was significant. Cluster analysis based on coancestry coefficient matrix and principal coordinate analysis confirmed the result of AMOVA and Fst analysis.

Keywords: *Grape; Sequence Tagged Microsatellite Sites (STMS); Genetic variation; Analysis of Molecular Variance (AMOVA)*

INTRODUCTION

Grape (*Vitis vinifera* L.) is an important fruit crop in many countries. At least about 14,000 grape cultivars are listed, but the actual number of distinct cultivars is believed to be much lower, between 5,000 to 8,000 (Alleweldt, 1988). Ampelography is the traditional method of grape cultivar identification. However, this botanical method, based on morphological characters alone, is subject to environmental influence and fails to distinguish cultivars with similar appearance. Molecular markers are more precise means of genotype identification and determination of taxonomic relationship between *Vitis* species (Sefc *et al.*, 1999). Several different types of molecular markers have been used for assessment of genetic variation among grapevine cultivars (Collins *et al*., 1999; Mauro *et al*., 1992; Parfitt *et al*., 1989). Among this kind of markers, Sequence Tagged Microsatellite Sites (STMS) combine several properties of desirable molecular markers including co-dominant inheritance, high reproducibility, high information content, selective neutrality, and easy scoring in the form of allele size (Powell *et al.*, 1996; Weising *et al.*, 1998). Application of microsatellite markers in grape includes pedigree reconstruction (Bowers and Meredith, 1997), parentage analysis (Silvestrony *et al.*, 1997; Sefc *et al.*, 1997; Bowers *et al.*, 1999a), cultivar identification and diversity studies (Lopes *et al.*, 1999; Sefc *et al.*, 1999, 2000; Aradhya *et al.*, 2003), mapping (Doligez *et al.*, 2006) and synonym detection (Lopes *et al.*, 1999; Ulanovsky *et al.*, 2002). Grapevine SSR primers, have been developed by three groups and are available in the public domain (Thomas and Scott, 1993; Bowers *et al.*, 1996, 1999b; Sefc *et al.*, 1999).

About 250 grapevine cultivars belonging to *Vitis vinifera* L. are grown in Iran, mostly used as table and dried fruit (Taffazoli *et al.*, 1989). No attempts have been made so far for comprehensive characterization

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of the Iranian cultivars particularly using molecular markers. It is indeed possible that the same cultivar has been grown in various regions with different names. Moreover, the earliest "wine culture" in the world is believed to have taken place in the Transcaucasian area (McGovern, 2003), comprising modern Georgia, Armenia and Azerbaijan. The Caucasus Mountains, a natural barrier between Europe and Asia, probably limited the material exchange with Europe. Since these earliest varieties were essentially wine cultivars, they probably do not correspond to the present varieties from the Caucasian area, but it would be interesting to evaluate the relationship between the recent varieties to modern European cultivars.

The present investigation is the first report of a comprehensive diversity analysis of 136 Iranian grapevine genotypes using STMS markers. The objectives of the study were 1) to access the extend of diversity within this sample, 2) to analyze the genetic structuring of these cultivars according to their geographical origin and their relationship with European cultivars and 3) to get a first insight of the degree of synonymy among the Iranian cultivars.

The results of this study are expected to be helpful in identifying a suitable set of microsatellite markers for Iranian grape varieties and use them in grape breeding and variety identification in the context of the evolving IPR regime.

MATERIALS AND METHODS

Plant material: Cultivars from different regions of Iran (Table 1) were obtained from the grape collections of the Faculty of Agriculture, University of Tabriz, and Agricultural Research Center, Varamin (Fig. 1). Beside the 136 Iranian genotypes, a set of 36 European cultivars from the collection of "Domaine de Vassal", INRA Marseillan was used in this study. These 36 cultivars have been chosen in order to represent the main alleles at the different locus among a set of 110 cultivars from different European regions (Siret *et al.,* in preparation). There were included in this analysis in order to allow comparative studies and to facilitate the exchange (and/or pooling) of data between laboratories.

Molecular methods: DNA was extracted from fresh leaves by the procedure of Vroh *et al.,* (1996). The genotypes were analyzed using nine SSR loci: VVMD5, VVMD7, VVMD21, VVMD24, VVMD25, VVMD27, VVMD28, VVMD32, and VVMD36 (Table 2) (Bowers *et al.,* 1996, 1999b).

PCR reactions were carried out in a Perkin-Elmer 9700 thermocycler. Each 25 µl PCR reaction contained 30 ng template DNA, 20 pM of each primer, 1 unit *Taq* polymerase (Roche, Germany), 0.2 mM of each dNTP, 2.5 mM $MgCl_2$, 50 mM KCl, and 10 mM Tris-HCl. Amplification was performed following the "Touchdown" PCR profile (Don *et al.,* 1991): 3 min at 94°C; 10 cycles of 30 sec at 94°C, 30 sec at 62/66°C, decreasing by 1°C per cycle depending on the SSR primers annealing temperature, 45 sec at 72°C; 25 cycles of 30 sec at 94°C, 30 sec at 52/56°C depending on the SSR, 45 sec at 72°C; and 5 min at 72°C for final extension. PCR amplification was confirmed by running 10 µl of PCR product on 2% agarose gels. Equivalent volume of denaturing dye solution (95% Formamide, 0.5% Bromophenol blue, 0.5% Xylene cyanol) was added to the remaining 15 µl of reaction product. Three to five µl of this mixture was electrophoresed in denaturing polyacrylamide sequencing gel (Bio-Rad model Sequi-Gen GT) consisting of 6% acrylamide, 7 M urea, and 1X TBE. Silver staining was carried out using the protocol of Bassam *et al*. (1993). In the next step, the gels were treated with 10% NaOH solution and were transferred on to Wattman paper No.3 of appropriate size after washing and finally dried by Bio-Rad gel drier system (model 583) following the conditions specified by the Company. The product size was determined on the basis of migration relative to the molecular weight (mw) marker M3 ladder (Elchorm Scientific, Suiss) and Ladder VIII (Roche, Germany) that were electrophoresed in the same gel.

Statistical analysis: Microsatellite allele frequencies, the number of alleles per locus, the effective number of alleles (Ne), observed (Ho) and expected (He) heterozygosity as genetic parameters of polymorphism were calculated using the POPGENE program (Yeh and Boyle. 1997). Probability of identity (PI) (Paetkau *et al.,* 1995) and expected heterozygosity (He) were estimated based on following formula:

$$PI = \Sigma P_i^4 + \Sigma \Sigma (2P_iP_j)^2$$
$$He = 1 - \Sigma P_i^2$$

where P_i and P_j are the frequencies of the i^{th} and j^{th} alleles within each locus, respectively.

Analysis of molecular variance (AMOVA) (Excofier *et al.,* 1992) was performed using Arlequin 2.00 software (Schnider *et al.,* 2000) to partition total genetic diversity i) Between Iranian and European groups, ii) among Iranian samples and iii) among

Table 1 Cultivars investigated in this study.

EUROPE:	**QAZVIN:**	Garmak ghochan
Morrastel	Boshghabi qazvin	Neishbori ghochan
Fernao pires	Tafti-sefid qazvin	Dahmanbar ghocan
Petit arvin	Bidaneh qazvin	Raesi kashmar
Chasselas	Karelo qazvin	Rooche ghochan
Temporanillo	Keshmesh-ghermez qazvin	Anghosht-aroos ghochan
Grimposie	Molaei qazvin	Rooche kashmar
Phakri	Goje-ghermez qazvin	Keshmesh-ghermez ghochan
Moriscot tinto	Tafty-syah qazvin	Doshakhe ghochan
Pozsonyi feher	Askari qazvin	Syah ghochan
Khossaine blanc	Shahani qazvin	Hosseini ferdos
Rouchaski	Shekar-shirazi qazvin	Samarghandi lotfabad
Chatus	Yaghoti qazvin	Lael ghochan
Gouget noir	Mish-pestan qazvin	Sabze lotfabad
Romeiko	Sahebi qazvin	Sefid ghochan
Monvedro	Mesghali qazvin	Koloche ghochan
Kadarka toruk	Phakhri qazvin	Sine-kaftari ghochan
Chenin	Kori qazvin	Saadi lotfabad
Madina	Ghermez-bidaneh qazvin	Kesmesh daregaz
Bobal	Chafte-qazvin	
Cabadma alba	Sahebi-sefid qazvin	**FARS:**
Corinto bianco	Tayefi qazvin	Falati Shiraz
Tinto cao	Bidmeshki	Sarkesh Shiraz
Harlevelu	Gojetar qazvin	Syah Shiraz
Tinta pinheria		Atabaki Shiraz
Corinthe	**KORDESTAN:**	Sefid-abi Shiraz
Danlas	Kordak	Askari-sirak Shiraz
Aubun	Ahmad-alaei qorveh	Domrobahi Shiraz
Mauzac blank	Qarie heidar	Tongrlirehi shiraz
Gorgollosa	Mirzaei qorveh	Sabonati shraz
Altesse	Yaghoti qorveh	Ash-syah shiraz
Crusillon	Shahani qasre-shirin	Tokhm-kabki shiraz
Posip	Vazandaei	Askari-sefid shiraz
Crvena slaubanic	Tayefi kordestan	Yaghoti-syah shiraz
Muscat-d'Alexanrie	Sarghola	Rajabi-syah shiraz
Santa paula	Teri baghela	Askari-peikhomi shiraz
Alva	Mish-pestan qasre-shirin	Yaghoti-sefid shiraz
	Bolmazo	Rajabi shiraz
AZARBAIJAN:	Vazandaei qasre-shirin	Gholami-syah shiraz
Gaji amji	Shahani qorveh	Aeine-dorosht shiraz
Jigh jigha	Keshmesh qorveh	Askari shiraz
Garmian	Koleie qorveh	Sahebi shiraz
Nardaneh	Bidaneh qorveh	Rish-baba
Golabi ghermez		Askari-syah shiraz
Dastechin	**KHORASAN:**	Bootani shiraz
Hosseini gargar	Pirojeh kashmar	Giehee shiraz
Alhaghi	Divaneh kashmar	Gangholami shiraz
Keshmesh sefid	Sangang ghochan	Samarghandi shiraz
Shaleie	Khalili-danedar ghochan	Ghoorchashmi shiraz
Lael-bidaneh syah	Khalili-dirras mashhad	Rotabi shiraz
Shahani sefid	Sorkhe neishabor	Rajabi-sefid shiraz
Goi ozum	Keshmeshak mashhad	Thokhm-tehrani shiraz
Ali baba	Razaghi kashmar	Samarghandi sefid shiraz
Khalili	Phakhri neishabor	
Hosseini maragheh	Kolahdari bojnord	
Qezel ozum	Hozoori mashhad	
Shahani-syah tabriz	Khalili-bidaneh rezvan	
Shekar boida	Gache bojnord	
Goi-aldareh	Askari-ghermez ghochan	
Askari tabriz	Golbartabagh Ghochan	

genotypes within samples. Fst pairwise comparison was carried out as a criterion for sample differentiation. As a measure of genetic distance among pairs of samples belongs to each geographical region, the standardized Rst statistics (Slatkin, 1995) was also computed using Arlequine pakage. The matrix of pairwise distance was used as input for principal co-ordinate analysis using NTSYSpc, Version 2.02 (Rohlf, 1998).

RESULTS

Microsatellite polymorphism: The set of 172 grape genotypes (Table 1) was analyzed with the nine SSR markers. They have been selected for their ability to reveal polymorphism in 8 unlinked regions of the genome since they are located on 8 different linkage groups (Riaz *et al.*, 2003; Doligez *et al.*, 2006). Among the 9 loci, VVMD28 and VVMD36 are closely linked. These markers led to the detection of 84 polymorphic alleles (Table 3). Figure 2 illustrates the results obtained with VVMD28 for 22 cultivars.

The heterozygosity values were high for all samples but ranged from 0.69 for cultivars from Azerbaijan to 0.84 for those from Qazvin (Table 4). In all samples, except Qazvin, the level of observed heterozygosity was lower than expected but the deficiency was not statistically significant.

Cultivar identification: The proper identification and distinction of the genotypes was one of the main objectives in the present study. Detection of the most informative markers could reduce the number of loci needed for reliable genotype distinction. For this purpose, the usefulness of this set of markers was assessed as the probability of identity (PI) in different grape growing regions and over all groups (Paetkau *et al.*, 1995). PI is defined as the probability with which two randomly taken genotypes display the same SSR profile. The calculated PI values for each geographic region and for each SSR marker over all genotypes are shown in Table 5. The PI values in different samples varied from 0.07 for VVMD25 in Khorasan sample and VVMD7 in Kordestan sample to 0.5 for VVMD21 in Fars.

Figure 1. Map showing five Iran grapevine growing regions referred in the text.

Table 2. Names and sequences of applied primers (Bowers *et al.,* 1996, 1999b). Choromosomal location and mapping of primers (Doligez *et al.,* 2006).

Locus name	5′ → 3′ (Sequence)	Chromosomal location	Length	annealing temperature
VVMD5	CTA GAG CTA CGC CAA TCC AA	16	20	56
VVMD5	TAT ACC AAA AAT CAT ATT CCT AAA		24	
VVMD7	AGA GTT GCG GAG AAC AGG AT	7	20	52
VVMD7	CGA ACC TTC ACA CGC TTG AT		20	
VVMD21	GGT TGT CTA TGG AGT TGA TGT TGC	6	24	56
VVMD21	GCT TCA GTA AAA AGG GAT TGC G		22	
VVMD24	GTG GAT GAT GGA GTA GTC ACG C	14	22	56
VVMD24	GAT TTT AGG TTC ATG TTG GTG AAG G		25	
VVMD25	TTC CGT TAA AGC AAA AGA AAA AGG	11	24	56
VVMD25	TTG GAT TTG AAA TTT ATT GAG GGG		24	
VVMD27	GTA CCA GAT CTG AAT ACA TCC GTA AGT	5	27	56
VVMD27	ACG GGT ATA GAG CAA ACG GTG T		22	
VVMD28	AAC AAT TCA ATG AAA AGA GAG AGA GAG A	3	28	56
VVMD28	TCA TCA ATT TCG TAT CTC TAT TTG CTG		27	
VVMD32	TAT GAT TTT TTA GGG GGG TGA GG	4	23	56
VVMD32	GGA AAG ATG GGA TGA CTC GC		20	
VVMD36	TAA AAT AAT AAT AGG GGG ACA CGG G	3	25	56
VVMD36	GCA ACT GTA AAG GTA AGA CAC AGT CC		26	

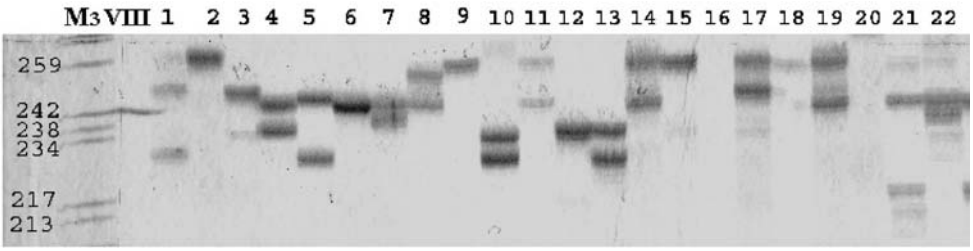

Figure 2. SSR band profile for 22 cultivars at VVMD28 locus. Amplification products were separated on denaturing polyacryamide gel and visualized by silver staining. From the left two first lanes are M3 and Ladder VIII (Roche) size markers respectively. 1-18 is Iranian genotypes and 19-22 are three European cultivars.

Genetic Diversity: A high level of genetic diversity was observed in the studied samples which are expected in view of the dioecious and outbreeding nature of wild grapes and high level of heterozygosity established during the evolution and domestication processes which have been conserved by the propagation of clones through vegetative reproduction (Olmo, 1995; Zohary, 1995). Analysis of molecular variance was performed to partition the total genetic variation into among i) individuals within sample (any individual genotypes), ii) among samples (different geographic regions) and iii) between groups (Iran and Europe) variations (Table 6). The results showed that only 0.25% of the total observed diversity is due to inter-group variation, which is not significant (P = 0.66). However Fst analysis confirmed the results of AMOVA (Table 7). Cluster analysis based on coancestory coefficient matrix grouped investigated samples in to two main groups. European cultivars along with Fars and Khorasan samples located in one group and another group consist of Azarbaijan, Qazvin and Kordestan Samples (Fig. 3).

Table 3. Genetic parameters for nine microsatellite markers in total sample that used in this study.

Locus	Na	Ne	He	Ho
VVMD5	12	7.23	0.86	0.68
VVMD7	10	6.29	0.84	0.80
VVMD21	6	3.2	0.69	0.80
VVMD24	10	6.48	0.85	0.81
VVMD25	11	6.37	0.85	0.75
VVMD27	6	4.19	0.76	0.73
VVMD28	12	7.12	0.86	0.80
VVMD32	8	6.14	0.84	0.75
VVMD36	9	5.24	0.81	0.70
Mean	9.33	5.81	0.81	0.76

The first column shows locus name, number of allels per locus (Na), effective number of allels (Ne), Expected hetrozygosity (He) and observed heterozygosity (Ho).

Table 4. Calculated genetic parameters within grapevine samples.

Sample	N	Ho	He	MNA	Ne
Europe	36	0.78 (0.01)	0.79 (0.07)	7.33 (1.42)	5.03 (1.41)
Azarbaijan	23	0.69 (0.13)	0.73 (0.12)	7.00 (1.73)	4.43 (1.40)
Qazvin	23	0.84 (0.13)	0.81 (0.12)	6.77 (1.30)	4.92 (1.76)
Kordestan	17	0.77 (0.19)	0.82 (0.06)	6.44 (1.33)	5.13 (1.36
Khorasan	40	0.72 (0.10)	0.78 (0.07)	7.33 (2.12)	4.77 (1.43)
Fars	33	0.76 (0.09)	0.76 (0.07)	6.77 (1.85)	4.35 (1.39)

The first columns showed sample size (n), observed heterozygosity (Ho), expected heterozygosity (He), mean number of allels (MNA) and effective number of allel (Ne). Values in parentheses are standard deviations.

Table 5. Calculated probability of identity (PI) for samples of each geographic regions and total sample.

Locus	Europe	Azarbaijan	Qazvin	Kordestan	Khorasan	Fars	Total
VVMD5	0.08	0.15	0.14	0.09	0.14	0.09	0.07
VVMD7	0.29	0.13	0.13	0.13	0.07	0.15	0.10
VVMD21	0.27	0.49	0.33	0.09	0.49	0.50	0.40
VVMD24	0.13	0.09	0.09	0.18	0.13	0.24	0.10
VVMD25	0.12	0.17	0.26	0.07	0.13	0.13	0.09
VVMD27	0.15	0.22	0.34	0.16	0.23	0.46	0.18
VVMD28	0.10	0.20	0.15	0.10	0.19	0.23	0.08
VVMD32	0.19	0.24	0.21	0.13	0.16	0.19	0.12
VVMD36	0.19	0.19	0.16	0.26	0.16	0.16	0.13
	5.29×10^{-8}	2.93×10^{-7}	2.41×10^{-7}	7.18×10^{-9}	9.08×10^{-8}	6.77×10^{-7}	5.67×10^{-9}

DISCUSSION

Microsatellite polymorphism: The number of observed alleles per locus ranged from 6 for VVMD21 and VVMD27 to 12 for VVMD5, and VVMD28, respectively, with an average of 9.33 alleles per locus (Table 3). The numbers of alleles are very similar to those obtained with 9 SSR microsatellite on a set of 164 European cultivars (Sefc *et al.,* 2000), but are relatively smaller than those observed on a set of 244 grape accessions both cultivated and wild by Aradhya *et al*. (2003) using 8 locus among which 5 were common with the present analysis.

The effective number of alleles varied from 3.2 for VVMD21 to 7.23 for VVMD5 (Table 3). These differences between the number of effective and of observed alleles indicated the presence of rare alleles that exist in one or a few genotypes and could be used for their identification.

The observed heterozygosity ranged from 0.68 for VVMD5 to 0.81 for VVMD24 with a mean of 0.76. The high level of heterozygosity could be due to the cross-pollinating nature of the plant and to the fact that grapes carry a heavy load of deleterious recessive genes (Olmo, 1976). Moreover, artificial selection also has a remarkable effect on this process (Sefc *et al.,* 2000). For 8 loci, it was lower than what would be expected by the random union of gametes, but most different for VVMD5. This is in contrast to reports from other similar studies (Thomas and Scott, 1993; Bowers *et al.,* 1996; Lopes *et al.,* 1999; Sefc *et al.,* 2000; Aradhya *et al.,* 2003). The heterozygosity deficiency has been found to be non-significant based on χ^2 test ($p < 0.001$). This could be due to the presence of null alleles as previously reported (Thomas *et al.,* 1994; Bowers *et al.,* 1996) but could also be due to difficulties in scoring heterozygous individuals when alleles are just one repeat apart (This *et al.,* 2004). It

Table 6. Analysis of molecular variance (AMOVA) in six samples using nine STMS markers. P-values are estimated using 1023 random permutation.

Source of variation	Degree of freedom	Sum of square	Variance component	Percent of variation	P-value
Among groups	1	10.12	0.01041	0.25	0.66
Among populations	4	32.668	0.158	5.42	<0.00
Within populations	166	673.1	4.03	96.1	<0.00
Total	171	715.75	4.22		

Table 7. Genetic differentiation between grapevine samples using pair wise values of Fst.

Sample	Europe	Azarbaijan	Qazvin	Kordestan	Khorasan	Fars
Europe	0.0000					
Azarbaijan	0.0325 *	0.0000				
Qazvin	0.0485 *	0.0115 ns	0.0000			
Kordestan	0.0448 *	0.0288 *	0.0179 *	0.0000		
Khorasan	0.0327 *	0.0313 *	0.0465 *	0.0498 *	0.0000	
Fars	0.0389 *	0.0471 *	0.0657 *	0.0679 *	0.0100 *	0.0000

* significant at 0.05 (P-value<0.05), ns nonsignificant (P>0.05). P-values are estimated using 16002 permutations.

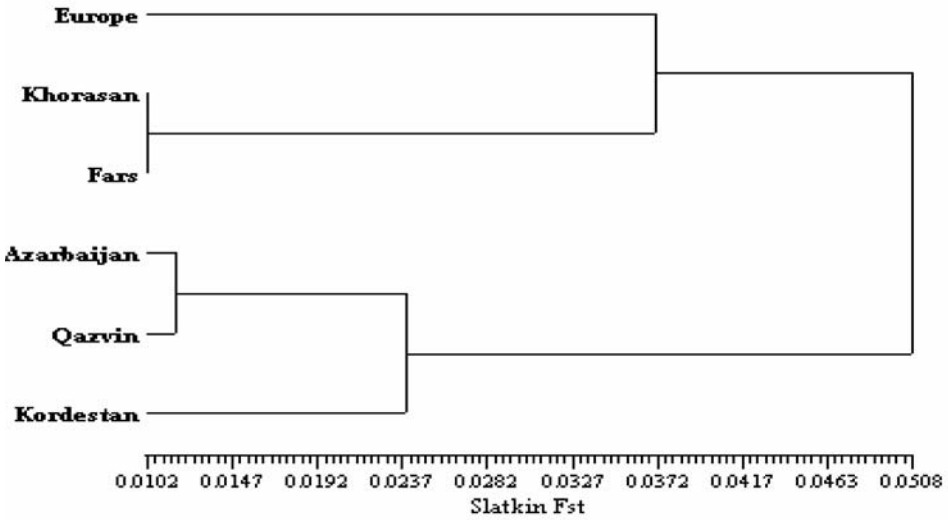

Figure 3. UPGMA dendrogram of investigated populations based on coancestry coefficient matrix using 84 different microsatellite allels in nine loci.

could also result from the history of the cultivars, some being the results of inbreeding.

Identification and distinction of the cultivars: The potential of the markers in genotype differentiation was of great interest. In this study, every single cultivar analyzed was distinct from the other ones. No synonymes have therefore been observed in the study.

The PI values in different samples varied from 0.07 for VVMD25 in Khorasan sample and VVMD7 in Kordestan sample to 0.5 for VVMD21 in Fars. The information content of a given marker differs between genotypes collected from different regions as allelic frequencies differ between grape gene pools. The high PI values were due to a low number of alleles and their uneven distribution within each sample. For example, VVMD21 in Fars had only five alleles, one of which (246 bp) has a frequency of 0.53. High PI value in VVMD21 in cultivars from Azarbaijan, VVMD25 and VVMD27 in cultivars from Qazvin, VVMD27 in cultivars from Khorasan, and VVMD28 in cultivars from Fars, were due to the presence of an allele with high frequency (data not presented). The total cumulative PI value for this set of markers was 5.67×10^{-9} that shows the high discrimination power of the selected primers. A marker revealing maximum information in one collection sample probably has a high level of discriminating power in other gene pools as well. For example, VVMD5 had low PI values in all the samples. But this is not true for all markers. For instance, VVMD21 had very high PI values (0.27-0.50) among the five samples, except cultivars from Kordestan (0.09). Therefore, based on the PI values, one could select a set of highly informative markers (with low values of PI) to study a particular sample (Table 5). Indeed, loci with uneven allele distribution could be very useful for genotype identification due to the presence of rare alleles. However the elimination of markers with high PI values may result to the loss of rare alleles.

Genetic diversity: Maximum diversity (0.82) was recorded for the samples from Kordestan, and minimum (0.73) for those from Azarbaijan. This gives a mean diversity of 0.74. The mean number of alleles (MNA) per sample varied from 6.44 (Kordestan) to 7.33 for Europe and Khorasan (Table 4). The smallest number of alleles was observed in the sample that had the smallest sample size. Therefore, this could be attributed to sampling bias since there is a positive and significant correlation between MNA and sample size ($r = 0.82$).

The results of AMOVA analysis suggests the possible presence of high genetic overlap between the Iranian and European samples. Although inter-samples diversity was significant ($P<0.00$) explaining for 5.42% of the total genetic variation, the high level of genetic diversity can be attributed to genotypes within samples which could account for of 96.1% of total diversity (Table 6). Therefore, the studied samples are valuable sources of genes, which can be utilized, in breeding programs of grapevine. Nevertheless, between sample variance was smaller compared to that of within sample, the significant between sample variation ($Fst=0.051$) indicates the samples from different geographical regions did not combined genetically due

to the migration events, mostly in form of cutting exchanges and sample from each geographic region has a distinct genetic structure. Pairwise comparisons of samples differentiation was carried out based on Fst as criteria using all the samples. Significant Fst values were observed in the case of all samples except that from Azarbaijan and Qazin (Table 7). The result of this analysis also indicated that each of the studied samples have distinct genetic constructions and differ from each other even in the case of very close samples such as Qazvin and Kordestan.

Relationship among samples: To depict among sample relationships, cluster analysis was performed based on coancestry coefficient matrix using UPGMA algorithm (Fig. 3). The resulting dendrogram clustered the studied samples into two main groups.

Group one consisted of cultivars from Europe, Khorasan and Fars in which high closeness was observed between those from Khorasan and Fars indicating the possible common origin of these collections. In the second cluster, samples from Azarbaijan, Qazvin and Kordestan were located, where the similarity between cultivars from Azarbaijan and Qazvin was higher than that of these samples with Kordestan. Principal Coordinate Analysis (PcoA) was performed based on Rst matrix (Slatkin, 1995), and scatter plot of samples against the first two principal coordinate is shown in Figure 4. Distribution of samples on the scatter plot revealed similar pattern to geographic map and dendrogram. The results of these analyses confirmed results of AMOVA analysis and Fst pair wise comparisons.

CONCLUSION

In the absence of comprehensive studies on the Iranian grape cultivars, this study showed that STMS markers are a powerful tool for cultivar identification and analysis of genetic structure. High information content of the markers enables characterization of a large number of cultivars only with combination a few loci. The nine microsatellite markers used in this study were very informative in the Iranian and European cultivars analyzed. The 136 Iranian genotypes account for approximately half of the cultivars in our collection. Genetic characterization of a larger number of genotypes will help in identifying potentially synonymous and homonymous cultivars, which will be very useful in germplasm management.

Acknowledgments

The authors are thankful to Prof. B. Sharma, IARI for his useful comments and precisely revision of manuscript. This research has been supported by ABRII, Ministry of Agriculture, Karaj, I.R. Iran.

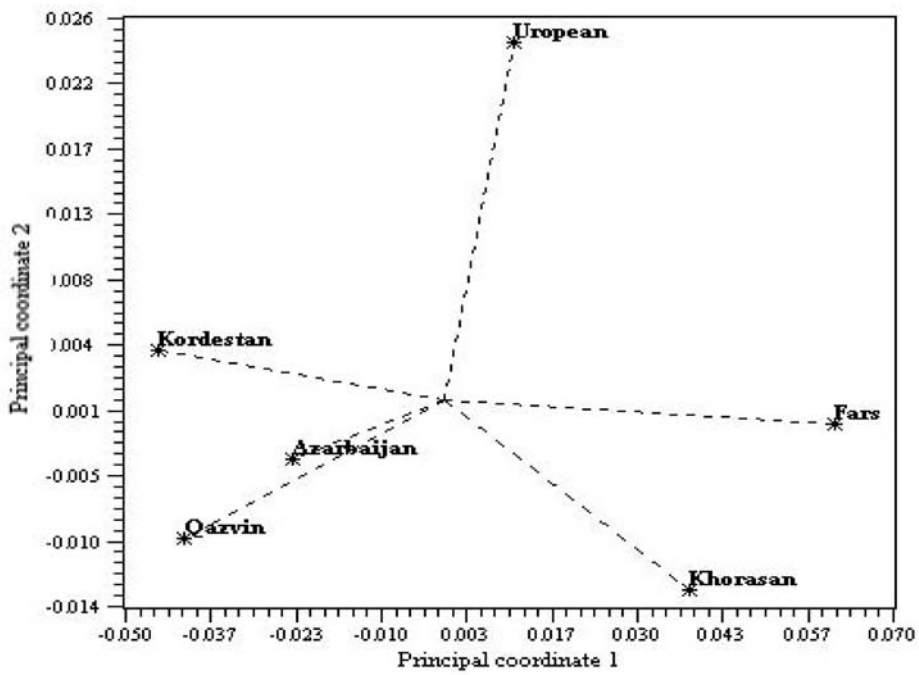

Figure 4. Scatter plot of population differentiation against two first principal coordinates based on Rst matrix.

References

Alleweldt G (1988). The genetic resources of Vitis. Genetic and geographic origin of grape, their prime names and synonyms. 2nd ed. Federal Research Center for Grapevine Breeding, Geilweilerhof, Germany.

Aradhya MK, Dangl GS, Prins BH, Boursiquot JM, Walker AM, Meredith CP, Simon CJ (2003). Genetic structure and differentiation in cultivated grapes *Vitis vinifera* L. *Genet Res Camb.* 81: 179-192.

Bassam BJ, Caetano-Anolles G (1999). Silver staining of DNA in polyacrylamide gels. *Appl Biochem Biotech.* 42: 181-188.

Bowers JE, Boursiquot JM, This P, Chu K, Johnsson H, Meredith CP (1999a). Historical genetics: The parentage of Chardony, Gamy, and other wine grapes of Northeastern of France. *Science*, 285: 1562-1565.

Bowers JE, Dangl GS, Meredith CP (1999b). Development and characterization of additional microsatellite DNA markers for grape. *Am J Enol Vitic.* 50: 243-246.

Bowers JE, Dangl GS, Vignani R, Meredith CP (1996). Isolation and characterization of new polymorphic simple sequence repeat loci in grape (*Vitis vinifera* L.). *Genome*, 39: 628-633.

Bowers JE, Meredith CP (1997). The parentage of classic wine grape: Carbet Saivignon. *Nat Genet.* 16: 84-87.

Collins GG, Symons RH (1999). Polymorphism in grapevine DNA detected by the RAPD–PCR technique. *Plant Mol Biol Rep.* 11: 105-112.

Doligez A, Adam-Blondon AF, Cipriani G, Digaspero G, Lauco V, Merdinoglu D, Meredith CP, Riaz S, Roux C, This P (2006) An integrated SSR map of grapevine based on five mapping sample. *Theo Appl Genet*. 113: 369-382.

Don RH, Cox PT, Wainwright BJ, Mattick JS (1991). Touchdown PCR to circumvent spurious priming during gene amplification. *Nucl Acid Res.* 19: 4008.

Excoffier L, Smouse PE, Quattro M (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, 131: 479-491.

Lopes MS, Sefc KM, Eiras-Dias E, Steinkellner H, Lamier da Camara Machado M, Dacamara Machando A (1999). The use of microsatellites for germplasm management in Portuguese grapevine collection. *Theor Appl Genet*. 99: 733-739.

McGovern PE (2003). Ancient wine: *The search for the origin of viticulture*. Princeton University Press, Princeton and Oxford. PP. 400

Mauro MC, Strefler M, Weeden NF, Riech BI (1992). Genetic analysis of restriction fragment length polymorphisms in *Vitis*. *J Hered.* 63: 18-21.

Olmo HP (1976). Grapes: In: Simmonds NW (ed) *Evolution of crop plants*, Longman London PP: 294-298.

Olmo HP (1995). The origin and domestication of the *Vitis vinifera* L. grape. In McGovern PE, Fleming SJ, Katz SH (ed) *The origin and ancient history of wine*. Gordon and Breach Publisher. PP: 31-43.

Paetkau D, Calvert W, Stirling I, Strobeck C (1995). Microsatellite analysis of sample structure in Canadian polar bears. *Mol Ecol.* 4: 347-354.

Parfitt DE, Arulsekar S (1989). Inheritance and isoenzyme diversity for GPI and PGM among grape cultivars. *J Amer Soc Hort Sci.* 114: 486-491.

Powell W, Machray GG, Provan J (1996). Polymorphism revealed by simple sequence repeats. *Trends in Plant Sci.* 1: 215-222.

Riaz S, Dangl GS, Edwards KJ, Meredith CP (2003). A microsatellite framework linkage map of *Vitis vinifera* L. *Theor Appl Genet*. 180: 864-872.

Rohlf FJ (1998). *NTSYS-pc, Numerical Taxonomy and Multivariate Analysis System*, Version 2.02. Exeter Software, New York.

Schneider S, Kueffer JM, Roessli D, Excoffier L (2000). *ARLEQUIN, a Software for Sample Genetics Data Analysis*, Version 2.0. University of Geneva, Geneva, USA.

Sefc KM, Steinkellner H, Wagner HW, Glossl J, Regner F (1997) Application of microsatellite markers for parentage studies in grapevine. *Vitis* 36: 179-183

Sefc KM, Regner F, Turetschek E, Glossl J, Steinkellner H (1999). Identification of microsatellite sequence in *Vitis riparia* and their applicability for genotyping of different *Vitis* species. *Genome*, 42: 367-373.

Sefc KM, Lopes MS, Lefort F, Botta R, Robelakis-Angelakis KA, Ibanez J, Pejic I, Wagner HW, Glossl J, Steinkller H (2000). Microsatellite variability in grapevine cultivars from different European regions and evolution of assignment testing to assess the geographical origin of cultivars. *Theor Appl Genet.* 100: 498-505.

Silvestroni O, Dipietro D, Interieri C, Vignani R, Fillipetti I, Del Casino C, Scali M, Cresti M (1997). Detection of genetic diversity among clones of cv. Fortana *(Vitis vinifera* L.) by microsatellite DNA polymorphism analysis. *Vitis*, 36: 147-150.

Slatkin M (1995). A measure of sample subdivision based on microsatellite allele frequencies. *Genetics*, 139: 457-462.

Tafazoli E, Hekmati J, Firozeh P (1989). *Grapevine*. Shiraz University Press (in Farsi).

This P, Cuisset C, Boursiquot JM (1997). Development of stable RAPD markers for the identification of grapevine rootstocks and the analysis of genetic relationships. *Am J Enol Vitic.* 48: 492-501.

This P, Jung A, Boccacci P, Borrego J, Botta R, Constantini L, Crespan M, Dangl GS, Eisenheld C, Ferreira-Monterio F, Grando S, Ibanez J, Lacombe T, Laucou V, Magalhaes R, Meredith CP, Milani N, Peterlounger E, Renger F, Zulini L, Maul E (2004). Development of a standard set of microsatellite reference alleles for identification of grape cultivars. *Theor Appl Genet.* 109:1448-58.

Thomas MR, Scott NS (1993). Microsatellite repeats in grapevine reveal DNA polymorphism when analyzed as sequence-tagged sites. *Theor Appl Genet.* 68: 985-990.

Thomas MR, Cain P, Scott NS (1994). DNA typing of grapevines: A universal methodology and database for describing cultivars and evaluating genetic relatedness. *Plant Mol Biol.* 25: 939-949.

Ulanovsky S, Gogorecna Y, Martinez Detoda F, Ortiz JM (2002). Use of molecular markers in detection of synonymies and homonymies in grapevine (*Vitis vinifera* L.). *Sci Hortic.* 92: 241-254.

Vroh BI, Harvenge IL, Chandelier A, Mergaei G, Jardin DU (1996). Improved RAPD amplification of recalcitrant plant DNA by the use of activated charcoal during DNA extraction. *Plant Breeding.* 115: 205-206.

Weising K, Winter P, Huttel B, Kahl G (1998). Microsatellite markers for molecular breeding. *J Crop Prod.* 1: 113-143.

Yeh FC, Boyle TJB (1997). Sample genetic analysis of co-dominant and dominant markers and quantitative traits. *Belgian J Bot.* 129: 157.

Zohari D (1995). The domestication of grapevine *Vitis vinifera* L. In the near East In: McGovern PE, Fleming SJ, Katz SH(eds). *The origin and ancient history of wine*. Gordon and Breach Publisher. PP: 31-43.