SSR ANALYSIS OF SOME SYNONYMS AND HOMONYMS OF GRAPE CULTIVARS (*VITIS VINIFERA* L.) GROWING IN SOUTHEASTERN TURKEY

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Abstract. The goal of this study was to analyze the genetic diversity of grape cultivars growing in Diyarbakır and Şanlıurfa by performing SSR molecular identification of the synonymous and homonymous genotypes of 21 cultivars growing in these two regions to investigate the homonyms (*Vitis vinifera* L. cvs.). Microsatellite analysis was performed using a minimal standard SSR marker set involving 7 highly polymorphic loci (VVS2, VVMD5, VVMD7, VVMD27, VrZAG47, VrZAG62, and VrZAG62). Mean number of alleles per locus was 7.14 (range, 7-9), with the highest number of alleles detected in VVS2 and the lowest in VVMD7, VVMD27. The expected and observed heterozygosity were 0.70 and 0.69, respectively. The dendrogram indicated 3 distinct groups, each of which involved several subgroups. A total of 5 synonyms and 10 homonyms were identified for the 21 genotypes.
Keywords: *Vitis vinifera L., grape cultivars, DNA extraction, synonym, homonym, dendrogram*

Introduction

Diyarbakır and Şanlıurfa provinces are located in Southeastern Turkey where viticulture is widely performed. Grapevine is a well-known perennial garden plant well adapted to these regions. Some preliminary surveys and genomic studies have revealed more than 70 grape cultivars growing in these two regions. Moreover, these two regions not only are well-known for viticulture but also are among the regions with the highest grape production in Turkey. Nevertheless, there is little or no information regarding the exact cultivar-based vineyard capacity of these regions.

The use of different regional names for grape cultivars results in major problems and confusion regarding the correspondence between these names and those used in studies and those used in each phase of production. However, these problems and confusion can be eliminated by the administration of molecular markers of polymorphism.

The aim of this study was to analyze the genetic diversity of grape cultivars growing in Diyarbakır and Şanlıurfa by performing molecular identification of the genotypes of cultivars growing in these two regions and to investigate the homonyms used for the 21 distinct cultivars (*Vitis vinifera* L. cvs.) previously identified in these regions using the SSR technique. Simple Sequence Repeats (SSR) is a well-known molecular marker which has been commonly used for the identification of cultivars and the determination of synonyms and homonyms of grape genotypes (Thomas and Scott, 1993; Bowers et al., 1996; Meredith et al., 1996; Sefc et al., 1997, 1999; Meredith, 2001; Riaz et al., 2002; Fatahi et al., 2003; Grando et al., 2003; Hvarleva et al., 2004; Riaz et al., 2004; Adam-Blondon et al., 2004; Zulini et al., 2005; Costantini et al., 2005; Di Vecchi Staraz et al., 2006, 2009; Şelli et al., 2007; Bodor et al., 2010; Cipriani et al., 2010; Ocete et al., 2011; De Andres et al., 2011; Garcia-Munoz et al., 2012; Emanuelli et al., 2013; Alifragkis et al., 2015; Maletic et al., 2015; Biagini et al., 2016; Li et al., 2017; Zequim Maia et al., 2018; Van Heerden et al., 2018).

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Materials

Molecular analysis was conducted on 21 grapevine genotypes including 9 cultivars collected from Diyarbakir Province and its districts (D), 8 cultivars collected from Şanlıurfa Province and its districts (Ş) and 4 genotype samples of the cultivars that had been previously transplanted to the Tekirdağ National Germplasm Repository Vineyard (TD, TŞ) (*Figure 1*).

Figure 1. *Provinces where the grapevine genotypes collected from Turkey*

One-year-old seedlings with 3-5 buds were collected from each genotype and were planted in polyethylene tubes filled with a 2:2:1 mixture of perlite, turf, and powder and then germinated in greenhouse conditions until the buds were rooted. DNA extraction was performed with the fresh leaves of these buds, as described by (Lodhi et al., 1994).

Methods

In order to allow a comparison among internationally grown homonymous varieties, a minimal standard SSR marker set was employed (This et al., 2004), which includes 7 highly polymorphic loci as follows: VVS2, VVMD5, VVMD7, VVMD27, VrZAG47, VrZAG62, VrZAG79 (*Table 1*).

DNA amplification was carried out using GeneAmp PCR System 9700 with EU-Applied Biossystems, followed by PCR optimization for each cultivar. PCR amplification was achieved using a reaction volume of 20 µl containing 5 µl of DNA (10 ng/µl), 2 µl of 10X Buffer (Qiagen), 1.2 µl of Mg Cl2 (Qiagen), 0.6 µl of dNTP (10mM), 1 µl of primer (25 µM) 1,1 µl of primer 2 (25 µM), 0.2 µl of GoldTaq (0.5 U), and 9 of µl distilled water. Touchdown PCR was carried out using the following cycling conditions: 95°C for 10 min, 94°C for 30 s, and 52°C for VVS2, VVMD5, and VVMD7, 58°C for VVMD27, 55°C for VrZAG47, and 62°C for VrZAG62 and VrZAG79 for 30 s each, based on the rate of primer annealing, with a decrease of 0.2°C/cycle. After completing 25 cycles, additional 15 cycles were administered with a reduction of 5°C from the primer annealing temperature, finally followed by holding at 72°C for 40 min.

To understand whether amplification occurs for each fragment in each locus, at least 10 fragments typifying each locus were placed on agarose gel. After viewing the amplification, the amplified fragments were subjected to sequencing using ABI Prism

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3730 automated DNA sequencer with GeneScan™ 500 LIZ™ dye Size Standard. The outcomes were analyzed, visualized, and processed using GeneMapper v 3.7 software. The number of alleles in each locus was calculated based on the peak levels.

Table 1. *Primers used for the study*

Primer	5´-3´	Base sequences of primers		Reference
VVS2	F	VIC-CAG CCC GTA AAT GTA TCC ATC	Vic	Thomas and Scott (1993)
	R	AAA TTC AAA ATT CTA ATT CAA CTG G		
VVMD5	F	6-FAM-CTA GAG CTA CGC CAA TCC AA	Fam	Bowers et al. (1996, 1999)
	R	TAT ACC AAA AAT CAT ATT CCT AAA		
VVMD7	F	NED-AGA GTT GCG GAG AAC AGG AT	Ned	
	R	CGA ACC TTC ACA CGC TTG AT		
VVMD27	F	NED-GTA CCA GAT CTG AAT ACA TCC GTA AGT	Ned	
	R	ACG GGT ATA GAG CAA ACG GTG T		
VrZAG47	F	VIC-GGTCTGAATACATCCGTAAGTATAT	Vic	Sefc et al. (1999)
	R	ACGGTGTGCTCTCATTGTCATTGAC		
VrZAG62	F	6-FAM-GGT GAA ATG GGC ACC GAA CAC ACG C	Fam	
	R	CCA TGT CTC TCC TCA GCT TCT CAG C		
VrZAG79	F	6-FAM-AGA TTG TGG AGG AGG GAA CAA ACC G	Fam	
	R	TGC CCC CAT TTT CAA ACT CCC TTC C		

Genetic similarity between the 21 genotype samples characterized by 7 loci was analyzing using the Microsat software (Minch et al., 1995) and the genetic parameters (number of alleles per locus, allele frequency, expected heterozygosity, observed heterozygosity, parentage, null allele frequency, and probability of identity) were analyzed using the IDENTITY 1.0 software (Wagner and Sefc, 1999). The dendrograms were formed and viewed using the NTSys software (version 2.02g, Exeter Software, Setauket, NY). Clustering analysis was carried out using the UPGMA method (Unweighted Pair-Group Method using Arithmetic means).

Result and Discussion

Data regarding the molecular analysis of the 21 genotype samples characterized by 7 loci were presented in *Table 2* in the form of peak levels and the number of alleles and basepair per locus.

The SSR analysis indicated a total of 50 alleles for the 21 genotype samples obtained from Diyarbakır, Şanlıurfa, and the Tekirdağ National Germplasm Repository Vineyard. *Table 3* presents the number of alleles and the expected and observed heterozygosity for these genotypes. Mean number of alleles per locus was 7.14, which was reported to be 9.6, by Borrego et al. (2001) who analyzed 406 accessions characterized by 8 microsatellite markers, 11.4 by Fatahi et al. (2003) who analyzed 62 genotypes characterized by 9 microsatellite markers, 8.1 by Hvarleva et al. (2004) who analyzed 74 accessions characterized by 9 microsatellite markers, 9.1 by Akkak et al. (2005) who analyzed 60 local cultivars characterized by 12 microsatellite markers, 11.9 by Vouillamoz et al. (2006), who analyzed 116 accessions characterized by 12 microsatellite markers. Of these studies, the ones that identified higher mean numbers of alleles compared to that of our study were carried out with higher numbers of genotypes that also showed greater variation compared to those in our study. However, the studies conducted by Crespan and Milani (2001), Dangl et al. (2001), Hvarleva et al. (2004) and Costantini

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et al. (2005) were carried out with lower numbers of genotypes and therefore detected lower mean numbers of alleles compared to that of our study (*Table 3*). On the contrary, although Fatahi et al. (2003) and Akkak et al. (2005) analyzed a smaller variety of genotype samples, the two studies identified greater mean numbers of alleles compared to that of our study.

Table 2. *Allele sizes of the genotypes characterized by 7 microsatellite loci*

No	Genotype	VVS2		VVMD5		VVMD7		VVMD27		VrZAG47		VrZAG62		VrZAG79	
1	*D* Abderi	131	133	230	232	240	244	181	181	159	159	186	190	246	246
2	*TD*Abderi	131	133	230	232	240	244	181	181	159	159	186	190	246	246
3	*Ş* Avderi	139	149	228	234	244	244	191	191	169	169	190	202	254	254
4	*D* Hatunparmağı beyaz	139	143	232	242	236	244	191	191	169	169	186	202	244	248
5	*D* Hatunparmağı siyah	121	121	232	242	236	252	185	191	163	169	202	202	244	246
6	*Ş* Hatunparmağı siyah	131	149	230	232	230	244	191	191	169	169	194	202	244	254
7	*Ş* Hatunparmağı beyaz	131	133	222	230	244	246	191	191	169	169	190	202	240	248
8	*D* Kızılbanki	131	141	232	236	240	246	181	191	159	169	188	188	244	246
9	*Ş* Kızılbanki	131	141	222	232	240	246	181	191	159	169	188	188	244	254
10	*D* Şire mazrumi	131	131	232	232	244	246	191	191	155	169	198	202	244	246
11	*D* Şire şirelik	131	131	232	232	244	246	177	191	155	169	198	202	244	244
12	*Ş* Şire	133	139	222	234	244	246	177	191	155	169	194	198	246	256
13	*D* Tahannebi	131	131	232	232	244	246	177	191	155	169	198	202	244	246
14	*TD* Tahannebi	131	155	230	234	244	244	191	191	169	169	190	202	254	254
15	*Ş* Tahannebi	131	155	230	234	244	244	191	191	169	169	190	202	254	254
16	*TD* Siyahüzüm	133	155	222	232	244	246	181	191	159	169	190	198	244	246
17	*Ş* Siyahüzüm	133	139	222	234	244	246	191	191	155	169	194	198	246	246
18	*D* Şarabi	149	155	232	234	244	246	183	191	161	169	190	198	248	256
19	*Ş* Şarabi	141	149	230	242	230	246	175	191	153	169	202	202	240	244
20	*D* Zerik	133	153	230	232	244	244	191	191	157	169	190	198	248	254
21	*TŞ* Zerik	131	143	228	228	244	246	191	191	155	155	190	202	246	248

D: Diyarbakır Province; *T:* Tekirdag Province (National Germplasm Repository Vineyard); *Ş:* Sanlıurfa Province; *TD:* Tekirdag National Germplasm Repository (studied cultivars from Diyarbakır); *TŞ:* National Germplasm Repository (studied cultivars from Sanliurfa)

Microsatellite markers used in our study, the most polymorphic marker was VVS2 (9 alleles) and the lowest were VVMD7 and VVMD27 (7 alleles) (*Table 3*). Similarly, previous studies also indicated that VVS2 the most polymorphic marker, followed by VVMD5 and VVMD27 (9 alleles), and VVMD7, VrZAG62, and VrZAG79 (7 alleles) (López et al., 1999; Borrego et al., 2001; Lefort and Roubelakis-Angelakis, 2001; Fatahi et al., 2003; Martín et al., 2003; Núñez et al., 2004). In our study, the expected heterozygosity per locus ranged from 0.47 and 0.80, with lowest expected heterozygosity detected in VVMD27 and the highest in VVS2. However, no significant difference was found between these two values, implicating that the cultivars analyzed in our study were highly heterozygous. In contrast, the observed heterozygosity detected in our study was lower than those reported in the literature (Sefc et al., 2000; Dangl et al., 2001; Fatahi et al., 2003; Aradhya et al., 2003; Costantini et al., 2005; Vouillamoz et al., 2006).

Clustering analysis was performed based on the proportion of shared alleles between the cultivars collected from Diyarbakır and Şanlıurfa. The dendrogram indicated 3

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distinct groups, each of which involved several subgroups. The dendrogram demonstrated that the genotypes of the cultivars obtained from these two regions were not completely dissimilar but showed close relationship with each other (*Figure 2*).

Table 3. *Number of alleles per locus, He, Ho, and PI*

Marker	Number of alleles	He	Ho	PI
VVS2	9	0.80	0.82	0.78
VVMD5	7	0.78	0.82	0.75
VVMD7	6	0.64	0.82	0.59
VVMD27	6	0.47	0.41	0.44
VrZAG47	8	0.65	0.55	0.61
VrZAG62	7	0.79	0.77	0.77
VrZAG79	7	0.79	0.64	0.76
Mean	**7.14**	**0.70**	**0.69**	**0.67**

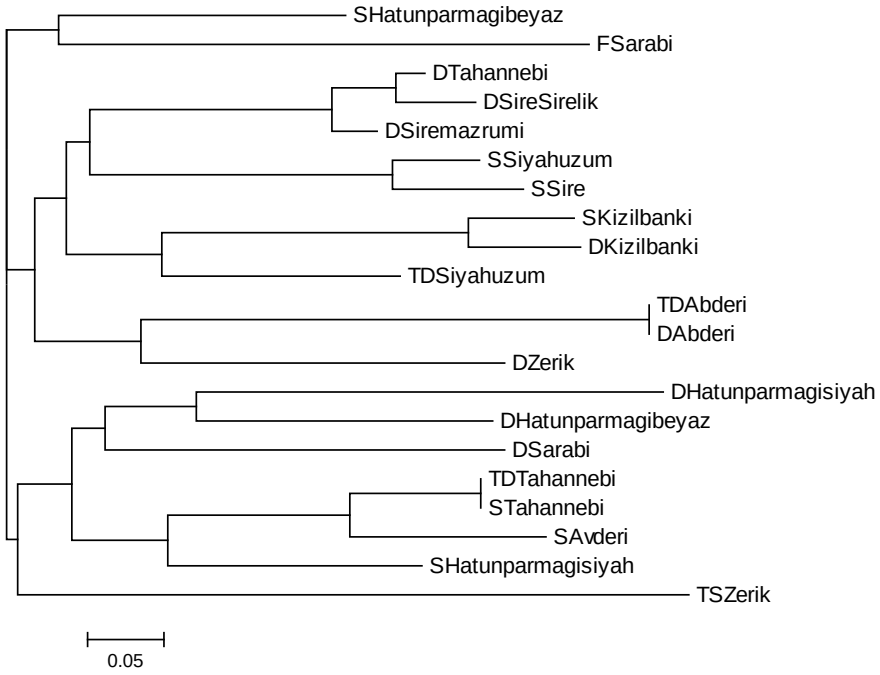

Figure 2. *Dendogram of 21 grape cultivars (Diyarbakır-Şanlıurfa) based on similarity index from SSR data*

The dendrogram indicated that only 6 out of the 17 cultivars sampled from Diyarbakır and Şanlıurfa were classified as homonyms or as cultivars with highly similar names (i.e. clustered in the same group on the dendrogram). However, 2 out of the 4 cultivars sampled from the Tekirdağ National Germplasm Repository Vineyard were found to have dissimilar names (i.e. not clustered in the same group on the dendrogram). The dendrogram also revealed that some of the cultivars with the same name were genetically not identical, which suggests that some of the grape cultivars growing in these regions might develop different genetic traits over time due to the different ecological conditions they grow in. Accordingly, the overwhelming presence of homonyms used for grape

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genotypes is a major problem for the viticulture in Turkey. The extent of this problem is well elucidated in the present study.

The molecular analysis indicated that 5 synonyms and 10 homonyms were identified for the 21 genotypes analyzed with 7 microsatellite markers based on the proportion of shared alleles among the genotypes (*Table 3*). Nevertheless, no clear information was available as to which homonym represented the real name of each genotype.

Table 4. *Synonyms and homonyms identified for the genotype samples obtained from Diyarbakır and Şanlıurfa*

Synonyms
D Tahannebi, *D* Şire şirelik
Ş Siyahüzüm, *Ş* Şire
D Hatunparmağı siyah, *D* Hatunparmağı beyaz
Homonyms
Ş Hatunparmağı beyaz, *Ş* Hatunparmağı beyaz (*D* Hatunparmağı siyah-*D* Hatunparmağı beyaz)
D Şire mazrumi, *Ş* Şire, *D* Şire şirelik
Ş Avderi (*D* Abderi- *TD* Abderi)
D Tahannebi (*TD* Tahannebi-*Ş* Tahannebi)
D Şarabi, *Ş* Şarabi
TD Siyahüzüm, *Ş* Siyahüzüm
D Zerik, *TŞ* Zerik

The microsatellite analysis revealed a total of 13 distinct cultivars for the 21 genotype samples. This finding implicates that the gene sources of the cultivars growing in these two regions should be protected. Moreover, it was also revealed that the 7 loci used in our study were highly appropriate for genetic analysis of grape cultivars and the identification of synonyms and homonyms.

Conclusion

A high level of allelic polymorphism was found between the cultivars that were expected to have dissimilar names and between the cultivars that were expected to have similar names. In our study it was observed that the level of detected polymorphism is highly can be influenced by the source materials. This differentiation of nomenclature could be attributed to several conditions. First, the cultivars with the same names might have been initially genetically identical but later grown in different ecological conditions for long years and thus might have become genetically dissimilar and this differentiation might have been further intensified through the use of different names for the cultivars transplanted to the Tekirdağ National Germplasm Repository Vineyard. Secondly, this differentiation could be associated with the production of these cultivars for constant vegetative propagation and the somatic mutations induced by environmental factors. Turkey is home to numerous grape cultivars with the same or different names as a result of a long-standing viticulture tradition in Anatolia dating back to 7,000-8,000 years ago. To protect this genetic potential, devising a rational nomenclature and identifying the relationships among these cultivars by using DNA-based markers is highly essential.

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