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Genetic diversity of wild and cultivated grapevine accessions from southeast Turkey

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Wild grapevine genetic diversity in southeast Turkey has not been documented to date. In the present work, in order to clarify the relationships between wild and cultivated grape accessions from southeastern Turkey, 22 nuclear and three chloroplast microsatellite loci were used on 21 wild grapevine *Vitis vinifera L.* ssp. *sylvestris* (*Gmelin*) and 13 cultivated grapevine *Vitis vinifera* ssp. *sativa* accessions. The number of alleles per SSR locus ranged from 4 (VVIn16) to 20 (VVIv67) and the mean allele number per locus was 10.09. Expected locus heterozygosity ranged from 0.586 (locus VVIb01) to 0.898 (locus (VVIv67)). The three cpSSR molecular markers presented variation in size both in cultivars and in wild Turkish accessions. Two size variants were detected for cpSSR3 (106 and 107 bp) for cpSSR5 (104 and 105 bp), and for cpSSR10 (115 and 116 bp). The six alleles in wild grapevines fell into three haplotypes B, C and D. A genetic structure according to accessions taxonomic status (wild or cultivated) was revealed by UPGMA analysis. This highlighted a clear separation between domesticated and wild accessions in Turkish germplasm. The results pointed out the need to further collect and characterize this wild and cultivated grapevine germplasm.

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Grape is unique, not only as a major global horticultural crop but also because of its ancient historical connections with human culture development. McGovern (2003) suggested that human beings encountered wild grapes for the first time in the upland regions of eastern Turkey. Indeed, seeds of domesticated grapes dated circa 8000 BP were found in Georgia and in Turkey (This et al. 2006), while the oldest wild grape (*Vitis vinifera* ssp. *sylvestris*) seeds known (dated 8400 years BP) were excavated in Turkey on the slope of Euphrates side valley (Hauptmann 1997; Pasternak 1998).

Wild grapevines (*Vitis vinifera* L. ssp. *sylvestris*) are heavily threatened in their natural habitats and high priority is given to the collection and preservation of this germplasm (Forneck et al. 2003). Indeed, the preservation of wild populations of *V. v.* ssp. *sylvestris* is considered essential for the maintenance of genetic variability and the resistance to genetic erosion (Cunha et al. 2009).

Turkey is an important center of origin both for cultivated *Vitis vinifera* ssp. *sativa* and wild *Vitis vinifera* ssp. *sylvestris* (Arroyo Garcia et al. 2006). Correspondingly, Turkey is rich in wild grapevines and grape cultivars (approx. 1200 accessions) which offers to grape breeders a valuable gene pool from where to extract genes of interest (Uzun and Bayir 2010). More specifically, Anatolia has long been linked with grapevine, especially in its eastern and southeastern regions to which earlier authors commonly ascribe the origin of viticulture and wine making (Ağaoğlu and Çelik 1987; Ağaoğlu et al. 1998). With this long-standing history, southeast Anatolia can boast both significant wild grapevine populations and a rich panel of local cultivars (Karataş et al. 2007). Analysing genetic diversity and relationships between wild (*Vitis vinifera* ssp. *sylvestris*) and cultivated (*Vitis vinifera* ssp. *sativa*) populations in this unique grapevine diversity 'hotspot' could help us understand the process of grapevine domestication.

SSR markers were useful as a complementary tool to traditional ampelography for cultivar identification. Wild grape populations have recently been studied using molecular markers (De Mattia et al. 2008; Bodor et al. 2010; Garcia Muñoz et al. 2011; Laucou et al. 2011; Ergül et al. 2011; De Andres et al. 2012).

Southeastern Turkish wild grape *V. v.* ssp. *sylvestris* populations and their relationship with cultivated grape genotypes have however not been studied yet and the present work aims to analyse genetic relationships between

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wild and cultivated grape accessions in this area of particular significance in grapevine domestication history.

MATERIAL AND METHODS

Plant material

In total 34 samples were analyzed on this study with 21 wild grapevines samples and 11 cultivated grape accessions collected from three different locations (Diyarbakır, Elazığ, Siirt) in southeast Turkey (Fig. 1, Table 1); Cabernet Sauvignon and Merlot were used as reference (outgroup) cultivars. In this study, the wild populations collected were usually located along river banks in both hilly areas and on the sides of valleys, natural expanses which have not been markedly altered for a long time and remote from agricultural and residential areas. All wild samples were ampelographically characterized on the collecting sites (Değirmenci Karataş et al. 2014) and further grown in greenhouse conditions. From these samples shoot tips were collected later for DNA analyses.

DNA isolation and PCR amplification

DNA was extracted from spring young leaves as described by Laucou et al. (2011). Microsatellite analyses were performed on 22 microsatellite markers (nSSRs) well distributed across the 19 grape chromosomes (Doligez et al. 2006) as previously described (Lacombe et al. 2007), two of the VMC series (VMC1b11, VMC4f3; Vitis Microsatellite Consortium, (Adam-Blondon et al. 2004)), nine of the VVI series (VVIb01, VVIn16, VVIh54, VVIn73, VVIp31, VVIp60, VVIv37, VVIv67, VVIq52, (Merdinoğlu et al. 2005)), eight of the VVMD series (VVMD5, VVMD7, VVMD21, VVMD24, VVMD25, VVMD28, VVMD27, VVMD32 (Bowers et al. 1996, 1999)), VVS2 (Thomas and Scott 1993; Thomas et al. 1994), VrZAG62 and VrZAG79 (Sefc et al. 1999). We also used the three chloroplast loci (ccmp3, ccmp5 and ccmp10 (Powell et al. 1995)) found to be polymorphic in *Vitis vinifera* samples (Arroyo-Garcia et al. 2002).

Amplifications were performed using a TC412 (Techne) thermocycler as described by Laucou et al. (2011). Reactions were performed on a mixture (20 µl final volume) containing 10 ng μl^{-1} genomic DNA, 2 µl buffer 10× (Qiagen), 200 µM deoxynucleoside triphosphate, 2.5 mM $MgCl_2$, 0.08 µl Taq polymerase (Qiagen), 0.32 pM of unlabelled primer and a variable quantity of the labelled primer depending on the marker. Amplification conditions included an initial denaturation step of 4 min at 95°C followed by 35 cycles of 1 min at 94°C, 1 min at 56°C for all loci (except for locus VMC1b11 and VMC4f3 at 60°C), 2 min at 72°C, with a final extension step (6 min at 72°C). Multiplexes were planned with a maximum of three colours, taking into account the size of the amplified fragments and up to seven markers per sequencing run were mixed as previously described by Di Vecchi Staraz (2007) and Lacombe et al. (2007).

Fig. 1. Collection sites of *Vitis vinifera* ssp. *sylvestris* from Eastern Turkey.

Data analysis

The genetic analysis 'IDENTITY' 1.0 program (Wagner and Sefc 1999) according to Paetkau et al. (1995) was used to calculate allele frequency and number, expected and observed heterozygosity, estimated frequency of null alleles, and probability of identity per locus. Genetic dissimilarity was determined with the 'MICROSAT' program, ver. 1.5 (Minch et al. 1995) using proportion of shared alleles, which was calculated using ps (option 1 – (ps)) as described by Bowcock et al. (1994). The results were then converted to a similarity matrix and a dendrogram was constructed with UPGMA (unweighted pair-group method with arithmetic mean, Sneath and Sokal 1973), using the NTSYS-pc software (Numerical Taxonomy and Multivariate Analysis System, ver. 2.0, Rohlf 1988).

GENETIX 4.02 computer package (Belkhir 1999) was used to calculate gene diversity (He) (Nei 1973) and observed heterozygosity (Ho) per population.

For cpSSR analysis, allelic and haplotypic frequencies within each population were directly estimated as the percentage of individuals sharing the same allele or haplotype in each sample of cultivated and wild grapevines. Gene diversity (He) was calculated as in Weir (1996), where n equals the number of alleles and pi equals the frequency of the allele in the population. Haplotype diversity (Hd) was calculated in the same manner as gene diversity, with n and pi referring to haplotypes.

RESULTS

Genetic diversity of the Turkish grape accessions was measured for nuclear microsatellites by estimating the average number of observed alleles per locus (Na), observed heterozygosity (Ho) and estimated heterozyosity (He).

Genetic diversity within the collection of 34 grapeaccessions were assessed by 22 nuclear and three chloroplast SSR markers. We detected a total of 61 alleles at the 22 nSSRs loci analyzed. The number of alleles per SSR locus ranged from 4 (VVIn16) to 20 (VVIv67) and the mean

Table 1. *List of grapevine accessions analyzed in this study.*

N	Population	Location (province)	Location (town)	Main use – genotype
1	C1	Diyarbakır	Çüngüş1	Wild (*V. v.* ssp. *sylvestris*)
2	C2	Diyarbakır	Çüngüş2	Wild (*V. v*. ssp. *sylvestris*)
3	C3	Diyarbakır	Çüngüş3	Wild (*V. v.* ssp. *sylvestris*)
4	C4	Diyarbakır	Çüngüş4	Wild (*V. v.* ssp. *sylvestris*)
5	D1	Diyarbakır	Boğazkere	Wine (*V. v.* ssp. *sativa*)
6	D2	Diyarbakır	Tahannebi	Table (*V. v.* ssp. *sativa*)
7	D4	Diyarbakır	Abdullah	Table (*V. v.* ssp. *sativa*)
8	D6	Diyarbakır	Hatunparmağı	Table (*V. v.* ssp. *sativa*)
9	E1	Elaziğ	Öküzgözü	Table (*V. v.* ssp. *sativa*)
10	E2	Elaziğ	Silfoni	Table (*V. v.* ssp. *sativa*)
11	E3	Elaziğ	Besni	Table (*V. v.* ssp. *sativa*)
12	E4	Elazığ	Kespir	Table (*V. v.* ssp. *sativa*)
13	E5	Elazığ	Kirmizi Silfoni	Table (*V. v.* ssp. *sativa*)
14	E6	Elazığ	Agin	Table (*V. v.* ssp. *sativa*)
15	E7	Elazığ	Kohnu	Table (*V. v.* ssp. *sativa*)
16	ER1	Diyarbakır	Ergani1	Wild (*V. v.* ssp. *sylvestris*)
17	ER2	Diyarbakır	Ergani2	Wild (*V. v.* ssp. *sylvestris*)
18	K1	Diyarbakır	Kulp1	Wild (*V. v.* ssp. *sylvestris*)
19	L1	Diyarbakır	Lice1	Wild (*V. v*. ssp. *sylvestris*)
20	L3	Diyarbakır	Lice3	Wild (*V. v.* ssp. *sylvestris*)
21	L4	Diyarbakır	Lice4	Wild (*V. v.* ssp. *sylvestris*)
22	M1	Elazığ	Maden 1	Wild (*V. v.* ssp. *sylvestris*)
23	M2	Elazığ	Maden 2	Wild (*V. v.* ssp. *sylvestris*)
24	M3	Elazığ	Maden 3	Wild (*V. v.* ssp. *sylvestris*)
25	M4	Elazığ	Maden 4	Wild (*V. v. ssp. sylvestris*)
26	M5	Elazığ	Maden 5	Wild (*V. v.* ssp. *sylvestris*)
27	M7	Elazığ	Maden 7	Wild (*V. v.* ssp. *sylvestris*)
28	M8	Elazığ	Maden 8	Wild (*V. v.* ssp. *sylvestris*)
29	M9	Elazığ	Maden 9	Wild (*V. v*. ssp. *sylvestris*)
30	S1	Siirt	Pervari1	Wild (*V. v.* ssp. *sylvestris*)
31	S2	Siirt	Pervari2	Wild (*V. v.* ssp. *sylvestris*)
32	S3	Siirt	Pervari3	Wild (*V. v.* ssp. *sylvestris*)
33	CS	International	Cabernet-Sauvignon	Wine (*V. v.* ssp. *sativa*)
34	M	International	Merlot	Wine (*V. v*. ssp. *sativa*)

allele number per locus was 10.09 (Table 2). Expected heterozygosity ranged from 0.586 (VVIb01) to 0.898 (VVIv67). The lowest observed heterozygosity (0.545) was detected at the VVMD21 locus and the highest one (0.906) at the VVMD28 locus. Probability of identity values ranged from 0.030 (VVIv67) to 0.801 (VVIh54).

The SSR-based dendrogram showing the genetic relationships among Turkish wild and cultivated grapevine accessions is shown in Fig. 2. Southern Turkish grape accessions clustered in four major groups.

The dendrogram revealed four groups labelled G1, G2, G3 and G4. Group 1, included most of Elazığ accessions and only two of Diyarbakır wild accessions, D2 (Tahannebi-standard variety which is grown in Diyarbakır and Elazığ) and S3 (wild sample from Siirt city, very close to Elazığ). It can be noted that wild accession C1 originated from Çüngüş town of Diyarbakır, is also very close to Elazığ city. Most of Diyarbakır accessions were included in group 2 in which local standard grape cultivars were grouped with wild samples. Only three grape cultivars, E4 (local Elazığ sample), M7 (wild sample of Maden town in Elazığ) and Besni (E3 standard cultivar) were classified in this group. The Öküzgözü grape variety, one of the best wine cultivar in Turkey, located in Elazığ, clustered in group 1. Similarily, the Boğazkere grape variety, also one of the best wine cultivar of Diyarbakır city, clustered in group 2. The rest of the wild accessions studied grouped in group 3 and 4. Reference cultivars Cabernet Sauvignon and Merlot presented a subgroup of the group 4.

The closest genetic relationship was observed between the two genotypes D1(standard cultivar 'Boğazkere') -ER2 (*V. v. sylvestris*) (0.875), followed by E5 (local cultivar) - M9 (*V. v. sylvestris*) (0.861) and D6 (standard cultivar 'Hatunparmağı') - M7 (*V. v. sylvestris*) (0.750). Our microsatellite-based dendrogram revealed a clear separation between domesticated and wild accessions in

Table 2. *Genetic parameters for SSR loci in Turkish accessions.*

Locus	Allele size range (bp)	N	He	Ho	F	PI
VMC1b11	165–196	13	0.826	0.757	0.037	0.086
VMC4f3	162–203	15	0.869	0.748	0.068	0.049
VVIb01	290–312	5	0.586	0.781	−0.123	0.336
VVIh54	139–179	13	0.839	0.848	−0.005	0.801
VVIn16	147–155	4	0.650	0.594	0.034	0.269
VVIn73	256–269	6	0.719	0.594	0.481	0.219
VVIp31	172–190	10	0.859	0.848	0.005	0.065
VVIp60	303–330	11	0.749	0.580	0.560	0.139
VVIq52	71–83	6	0.708	0.676	0.018	0.245
VVIv37	145–177	12	0.847	0.636	0.114	0.062
VVIv67	329–397	20	0.898	0.818	0.042	0.030
VVMD21	241–255	8	0.639	0.545	0.057	0.220
VVMD24	204–218	8	0.804	0.818	−0.007	0.111
VVMD25	238–254	6	0.770	0.848	−0.044	0.164
VVMD27	172–191	10	0.816	0.824	−0.004	0.108
VVMD28	216–280	15	0.873	0.906	−0.017	0.051
VVMD32	239–271	8	0.829	0.866	−0.020	0.091
VVMD5	223–244	11	0.834	0.719	0.063	0.082
VVMD7	233–255	9	0.817	0.818	−0.0005	0.102
VVS2	122–153	12	0.876	0.906	−0.016	0.052
VrZAG62	188–204	8	0.817	0.710	0.059	0.107
VrZAG79	238–270	12	0.802	0.706	0.053	0.117
Mean		10.090	0.792	0.752	0.062	0.159

N: Number of alleles, He: Expected heterozygosity, Ho: Observed heterozygosity, PI: Probability of identity, F: Frequency of null alleles

Turkish germplasm (Fig. 2). Except for a few grape accessions, the similarity index value was generally below 0.500. Therefore, for each sample collected from different locations in nature, it can be said that wild vines have acquired a distinct genotype.

Genetic variability within the samples studied: observed and expected heterozygosity (Ho and He), mean number of alleles (MNA), averaged over loci, are presented in Table 3. Gene diversities (He) were high both in wild and cultivated populations. The highest value was obtained for the wild sample (He = 0.7914) and the lowest variation was observed in the cultivated grape germplasm (He = 0.7119). The mean number of alleles per population (MNA) ranged from 8.8 (wild) to 5.6 (cultivated).

A high level of gene diversity was detected in the wild grape population despite its smaller size. This can be correlated with the outbreeding mating system of these dioecious individuals (Grassi et al. 2003).

The three cpSSR molecular markers presented variation in size both for the cultivar sample and in wild accessions (Table 4). Two size variants were detected for cpSSR3 (106 and 107 bp), cpSSR5 (104 and 105 bp), and cpSSR10 (115 and 116 bp). Allelic frequencies for cultivars varied from 0.45 (cpSSR3–107, cpSSR5–104 and cpSSR10–115) to 0.55 (cpSSR3–106, cpSSR5–105 and cpSSR10–116). Allele frequencies in the wild gene pool varied from 0.29 (cpSSR10–116) to 0.71 (cpSSR10–115). The three studied loci presented the same genetic diversity (He) value of 0.495 for cultivar population. Concerning wild accessions,

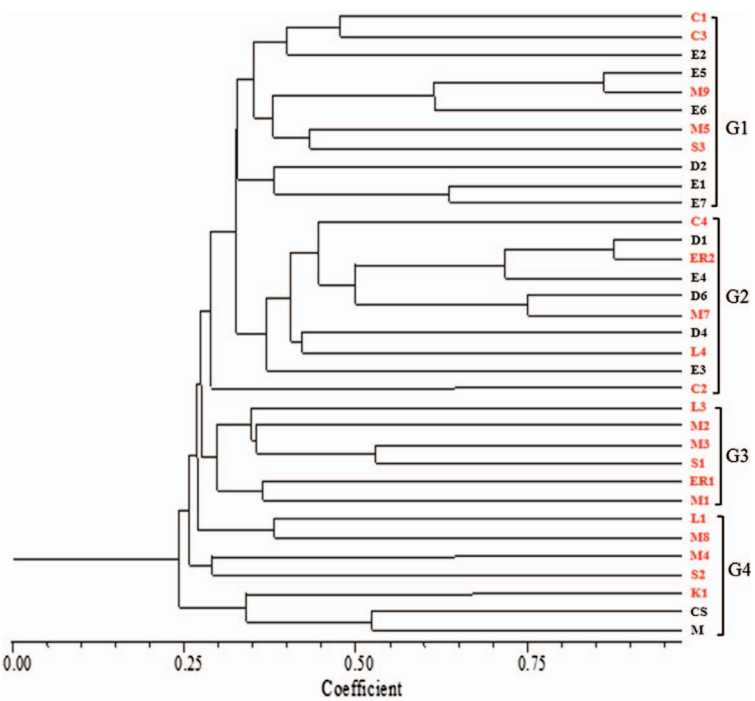

Fig. 2. Genetic relationships among wild (in red) and cultivated Turkish grapevines based on SSR analyses.

Table 3. *Genetic variability within the studied population: observed and expected heterozygosity* (*Ho and He), mean number of alleles (MNA), averaged over loci. Values in brackets are standard deviations.*

Population	H_o	H_e	MNA
Wild sample	0.7773 (± 0.0829)	0.7914 (± 0.0807)	8.8182
Cultivated sample	0.7200 (± 0.0988)	0.7119 (± 0.0948)	5.5909

genetic diversity (He) at these loci ranged from 0.41 (cpSSR10) to 0.5 (cpSSR3, cpSSR5) (Table 4).

The six alleles identified at the three chloroplast microsatellite loci in cultivars gene pool fell within the two haplotypes C and D previously described in the cultivated compartment of grapevine (Arroyo-Garcia et al. 2002, 2006) with haplotype frequencies of 55% and 45% respectively (Table 5). Haplotypic genetic diversity (Hd) for the cultivars was 0.495.

For wild grapevines, the six alleles fell into three haplotypes, B, C and D. Haplotype D was the most frequent (48%) in our sample and with 24% and 28% frequencies respectively for haplotypes B and C (Table 6).

DISCUSSION

Our study is the first genetic diversity analysis of the wild grapevines in the southeast region of Turkey. During our research we observed a large number of wild grapevine populations in this area. However, this first study was performed with few genotypes only to provide an example. The most debated subject is: were these wild vines true *Vitis vinifera* ssp. *sylvestris*? To determine whether these wild genotypes are real *Vitis vinifera* ssp. *sylvestris* or not, clear information can be accessed by relationships studies on larger populations, parentage relationships and phylogenetic studies. Wild vines were collected from mountainous areas and riverbanks 50–60 km away from the city center. As, in the area, traditional viticulture is performed without grafting the likelihood of any rootstock genepool introgression is almost nil. During the field prospection, we also found very small berry clusters on some (M3, L2 and K1) sampled individuals. Ampelographic investigation on leaf and flower showed that some of wild samples showed very similar morphologic characters with *Vitis vinifera* ssp. s*ylvestris* (Değirmenci Karataş et al. 2014).

Domesticated plants often have the potential to spontaneously hybridize with their wild relatives that are growing in close proximity (Ellstrand et al. 1999). Such hybridization leads to gene flow "the incorporation of genes into the gene pool of one population from one or more populations" (Futuyma 1998; El Oualkadi 2011). Due to dispersion by birds, cultivated grapevine was able to extend over large territories and often hybridized with native *Vitis sylvestris* plants (Bodor et al. 2010).

Table 4. *Allele size, allelic frequency and genetic diversity* (*He) in the analyzed samples.*

	Cultivars			Wild accessions	
Locus	Allele size	Allelic frequency	He	Allelic frequency	He
CCMP3	106	0.55		0.52	
	107	0.45	0.495	0.48	0.5
CCMP5	104	0.45		0.48	
	105	0.55	0.495	0.52	0.5
CCMP10	115	0.45		0.71	
	116	0.55	0.495	0.29	0.41

Table 5. *Chloroplast haplotypes, frequencies and haplotypic diversity (Hd) in the cultivar samples.*

Sample name	cpSSR3	cpSSR5	cpSSR10	Haplotype	Frequency	Hd
D4	107	104	115	D		
E5	107	104	115	D		
E6	107	104	115	D		
E1	107	104	115	D		
E7	107	104	115	D	0.45	
D1	106	105	116	C		
D2	106	105	116	C		
D6	106	105	116	C		
E2	106	105	116	C		
E3	106	105	116	C		
E4	106	105	116	C	0.55	0.495

Table 6. *Chloroplasts haplotypes, frequencies and haplotypic diversity Hd in the wild accessions.*

Sample name	CCMP3	CCMP5	CCMP10	Haplotype	Frequency	Hd
C1	107	104	115	D		
C2	107	104	115	D		
ER1	107	104	115	D		
K1	107	104	115	D		
L4	107	104	115	D		
M2	107	104	115	D		
M5	107	104	115	D		
M8	107	104	115	D		
M9	107	104	115	D		
S1	107	104	115	D	0.48	
C4	106	105	116	C		
ER2	106	105	116	C		
L3	106	105	116	C		
M7	106	105	116	C		
S2	106	105	116	C		
S3	106	105	116	C	0.28	
M1	106	105	115	B		
M3	106	105	115	B		
M4	106	105	115	B		
C3	106	105	115	B		
L1	106	105	115	B	0.24	0.63

The molecular analysis demonstrate that wild and domesticated Turkish grapevine germplasms are genetically divergent. Wild accessions collected from different locations had different genetic profiles. However, it is important to underline that the results of this phenetic analysis cannot be used to draw conclusions with regard to the degree of kinship between the cultivars since clusters illustrate similarity rather than kinship (Sefc et al. 1999; Pellerone et al. 2001).

Various authors have used 22 SSR loci successfully for relationship studies of wild grape accessions (Bodor et al. 2010; García Muñoz et al. 2011; Laucou et al. 2011). As a result of these analyses, the genetic similarity indexes of wild vine are found to be generally low.

The three cpSSR loci studied here were found to be polymorphic in our sample of cultivated and wild Turkish accessions. This is comparable to results of Arroyo-Garcia et al. (2002), Dzhambazova et al. (2009) and Riahi et al. (2011), although Grassi et al. (2003), Imazio et al. (2006) and Doulaty Baneh et al. (2007), found that only the cpSSR3 and cpSSR10 loci were polymorphic in their conditions.

Comparable levels of genetic diversity (He) were observed among the studied loci either in cultivated or wild samples. He values for cultivars at loci cpSSR3, cpSSR5 were higher than the genetic diversity observed in Tunisian cultivars (Riahi et al. 2011) who cited a value of 0.21, but were similar to results of Arroyo-Garcia et al. (2002) who reported a 0.49 value for these two loci. However, the level of genetic diversity observed at the cpSSR10 locus is lower than the value recorded for other grapevine cultivars i.e. 0.62 in Riahi et al. (2011) an 0.61 in Arroyo-Garcia et al. (2002).

Concerning wild accessions, genetic diversity (He) at loci cpSSR3 and cpSSR5 was comparable to results of Riahi et al. 2011 for Tunisian wild grapevines (0.40) while level of genetic diversity for cpSSR10 was lower than the 0.65 level recorded in Tunisian wild grapevines (Riahi et al. 2011). Haplotypic genetic diversity (Hd) for cultivars was lower than that observed in Tunisian (0.688, Riahi et al. 2011; 0.74, El Oualkadi et al. 2011), Algerian (0.67, El Oualkadi 2011), Iranian (0.668, Doulaty Baneh et al. 2007), and Morrocan cultivars (0.71, El Oualkadi et al. 2011) as well as in cultivars from Spain and Greece (0.64, Arroyo-Garcia et al. 2002).

The haplotypic genetic diversity was higher in Turkish wild accessions than in cultivars. Comparable levels were recorded in previous reports concerning wild grapevine accessions originating from different areas in the world (Riahi et al. 2011; Grassi et al. 2003). However this diversity level was higher than that observed for Iranian (0.5, Doulaty Baneh et al. 2007), Morrocan (0.32) and French (0.31) wild grapevines (El Oualkadi et al. 2011).

Our results confirm previous studies and highlight that haplotype frequencies of cultivated grapevines seem to depend on the *Vitis vinifera* sample analyzed. A higher haplotypic genetic diversity which reached 0.63 was recorded for wild accessions. Indeed, haplotypes A and B which are absent from our analysis appear most frequently in the analysis of other samples of cultivars. And in the present study, the putatively ancestral chlorotype B was detected at a low frequency of 0.24. Chlorotype B has been suggested to be an ancestral one since it didn't show a marked geographical distribution and was represented both homogeneously and at a low frequency in the Eurasian Region (Arroyo-Garcia et al. 2006). Consequently, a special importance should be given to chlorotype B analysis in future studies in order to better understand grapevine domestication process.

While haplotype D is reportedly present with low frequency in 'ssp. *Sativa*' cultivars, it is present with high frequency in our samples. And its distribution is comparable in both samples. As regards chlorotypes however, chlorotype C is more abundant in the cultivated sample, whereas chlorotype B is totally absent from this sample.

Analyses of chlorotype diversity in *sylvestris* populations showed that central Mediterranean and eastern populations had higher diversity values than western populations (arroyo-Garcia et al. 2006), which, based on phenotypic variation (Gökbayrak and Söylemezoğlu 2010) and in agreement with Negrul in 1938, suggests that the Anatolian peninsula and Transcaucasian regions are indeed the *Vitis vinifera* 'diversity center'.

This et al. (2006) indicated that analysis of wild grapes from eastern countries such as Turkey, Iran or Georgia, the presumed centre of primo-domestication, will be fundamental for understanding the role of *Vitis vinifera* ssp. s*ylvestris* in the domestication process. The genetic distinction observed between wild and domesticated grapevines suggests that wild germplasm could be used as a source of novel alleles (Zecca et al. 2009). Evaluation of the genetic diversity, differentiation and relationship among wild grape specimens from different areas will contribute to a better understanding of the process of grapevine domestication (El Oualkadi et al. 2011).

This work comforts the usefulness of nSSR and cpSSR markers to provide information on genetic diversity and relationship among wild and cultivated grapes. Our genetic data show that southern Turkish wild and cultivated grape germplasms are an important genetic source for grape breeding. Molecular analysis could help understanding the process of grapevine domestication. The results of the present work could also be the basis for future studies about phylogenetic relationships in the *Vitis* genus.

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