

Identification of disease resistance-linked alleles in *Vitis vinifera* germplasm

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Abstract. Introgression of genetic resistance to fungal diseases from American and Asian *Vitis* species traditionally pursued in grape breeding programs, although facilitated by molecular tools, has an impact on wine quality that still slows down development of competitive varieties. A contribution to the genetic improvement of grapevines for resistance to pathogens may come from unexplored genetic resources of the Eurasian *Vitis vinifera* L. In the present study, a hundred grapevine accessions from Georgia, Armenia and Azerbaijan were genotyped with SSR markers linked to QTLs for resistance to downy and powdery mildew, and with 21 SSR markers widely used for genetic diversity and relationship analysis. Looking at population genetic structure, Armenian and Azerbaijani accessions fell within the same cluster and were included among the Central Asian grape varieties of a homogeneous dataset, while Georgian accessions formed a separate group. Pattern of SSR alleles flanking the locus Ren1 and associated with resistance to *Erysiphe necator* in 'Kishmish vatkana', 'Dzhandzhal kara' and other Central Asian cultivars were found in three varieties from the Azerbaijani population that reached very high scores when assessed for PM resistance.

1. Introduction

Present day cultivars of the European species *Vitis vinifera* L. are susceptible to downy (DM) and powdery (PM) mildew, two fungal diseases introduced to Europe from North America during the second half of the XIX century. However, some grapevine populations that have contributed to the origin of modern varieties seem to have evolved specific resistances after the arrival of the pathogens (the oomycete *Plasmopara viticola* and the ascomycete *Erysiphe necator* respectively) over a period of sexual reproduction in natural conditions outside cultivated areas. Recent findings also suggest that PM may have existed in the Old World for longer than currently assumed, since several grape species native to Central Asia and China are known to express PM resistance [1].

Even though Russian grapevine breeders had already used *V. vinifera* germplasm of Central Asian origin as a source of resistance to PM [2], only the identification of the Ren1 locus of resistance to *Erysiphe necator* in the 'Kishmish vatkana' grapevine cultivar [3] has recently

brought attention to the Eurasian genetic resources of *V. vinifera* for current resistance breeding programs.

Low levels of susceptibility to diseases in field and controlled conditions were reported for several grapevine accessions coming from South Caucasus, which also show desirable fruit traits and a certain degree of tolerance to environmental stresses [4, 5]. To investigate the genetic basis of the most interesting phenotypes among this germplasm, several accessions were screened in the present study with molecular markers linked to Ren1 locus and to several genetic loci of partial resistance to DM (Rpv) and PM (Run, Ren) formerly identified in American and Asian *Vitis* species.

2. Materials and methods

A collection of a hundred accessions of *V. vinifera* ssp. *sativa* and ssp. *sylvestris* from Georgia, Armenia and Azerbaijan established at DiSAA (University of Milan, ITA 426) within the Cost Action FA1003 [6] was genotyped at 21 neutral SSR loci, including the standard set of microsatellites widely used for grapevine genetic identification. The list of molecular markers and

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conditions of analysis were previously reported [7]. Population genetic structure and main diversity statistics were investigated with STRUCTURE v2.3.4 and GenAlex v6.502 software, including homologous genetic data of the grapevine germplasm from Central Asia and Israel as described by [8, 9].

Non-redundant grapevine genotypes from Georgia, Armenia and Azerbaijan were further analyzed at 26 marker loci (24 SSR, 1 CAPS and 1 SCAR) linked to several QTLs for DM and/or PM resistance according to published protocols, as summarized in Table 1. The QTLs Rpv1/Run1, Rpv3, Rpv10, Rpv12 and Ren3 were identified for resistance to *P. viticola* (Rpv) and *E. necator* (Run, Ren) in North American and Asian *Vitis* species. Molecular markers linked to the Ren1 genomic region were detected in the Central Asian grapevine cultivars 'Kishmish vatkana' and 'Dzhandal kara' (Table 1).

3. Results and discussion

Based on allelic profiles at neutral SSR loci, the collection of grapevines from South Caucasus was finally composed of 17 different varieties of Armenian origin, 35 varieties of Azerbaijani origin, and 7 putative *V. vinifera* ssp. *sylvestris* individuals and 18 varieties from Georgia.

When compared with other grapevine germplasm included in the genetic dataset, Georgian and Israeli genetic resources showed the highest allelic richness, both populations also containing a large proportion of private alleles (Figure 1). Analysis with STRUCTURE indicated K=3 and K=5 as the most likely number of clusters in the genetic structure of the germplasm. At K=3 all Armenian and Azerbaijani accessions fell within the same cluster with a percentage of assignment higher than 80%, and were included among the accessions of Tajikistan, Uzbekistan and Kyrgyzstan regions of Central Asia described by [8]. Georgian and Israeli germplasm formed two separate groups (Figure 2). At K=5 Georgian accessions revealed two clusters roughly corresponding to wild and cultivated genotypes while the cluster of Armenian and Azerbaijani accessions was separated from the group of Central Asia (data not shown).

Within the population from Azerbaijan, four unique multilocus genotypes corresponding to accessions 12AZ3, 12AZ16, 12AZ23 and 12AZ41 showed identical marker profile to accessions HOP L04-19 Rish Baba, 2271Mtp2 Baian chirei, 2842Mtp1 Ag Kiourdach p.e. and DVIT0310 Barmak Isium included in the large germplasm evaluated at Ren1-linked markers by [1]. The last study provided evidence that PM resistance was present in other cultivated *V. vinifera* of Central Asia besides 'Kishmish vatkana' and 'Dzhandzhal kara' and also in individuals of *V. vinifera* ssp. *sylvestris*, suggesting that additional germplasm may share a Ren1-like local haplotype.

Disease evaluation under natural and controlled conditions has indicated phenotypes of reduced susceptibility to DM and/or PM within the South

Caucasus germplasm included in our survey, with Armenian and Azerbaijani accessions worthy of note for resistance to PM and accessions from Georgia less susceptible to DM [4, 5].

Patterns of SSR alleles of the expected size in both regions flanking the locus Ren1 were actually found in three varieties of the Azerbaijani population (Table 2) that reached very high scores when assessed for PM resistance on leaves (OIV descriptor code 455). In addition, another 15 accessions scored from 7 to 9, including three putative *V. vinifera* ssp. *sylvestris* individuals, deserving further attention, as they showed only some of the Ren1-linked alleles compared to the 'Kishmish vatkana' and 'Dzhandzhal kara' haplotype (a few examples are shown in Table 2).

Conversely, no allelic sizes linked to Run1 and Ren3 resistance loci were detected in the South Caucasus populations. Similarly, no accessions showed DM resistance-linked alleles on both sides of the targeted Rpv QTLs, which would have suggested a hybrid origin of the accessions, though not supported by preliminary phylogenetic analyses. Nevertheless, some SSR alleles associated with resistance at Rpv1 and Rpv3 loci (in *V. rotundifolia* and *V. rupestris*, respectively) and with resistance at Rpv10 and Rpv12 loci in *V. amurensis* were detected in the germplasm on one side or the other of the QTLs (Table 3).

Table 1. DM and PM resistance markers applied in this study.

Locus	Associated marker	Allele size linked to resistance (bp)	Ref.
Rpv1/Run1	VMC1g3.2	118	[10,11,12]
	VVIM11	292	
	VMC8g9	156	
	VMC4f3.1	188	
	CAPS GLP1-12	650, 200	
Rpv3	UDV305	299	[13,14]
	VMC7f2	210	
	UDV737	296	
Rpv10	GF09-44	230	[15]
	GF09-46	410	
	GF09-47	296	
	GF09-48	355	
	GF09-52	390	
Rpv12	UDV343	161	[16]
	UDV345	220	
	UDV350	308	
	UDV360	208	
	UDV370	198	
Ren1	UDV124	214	[3,17]
	SC8-0071-014	142	
	SC47-18	248	
	VMC9h4	282	
	VMC4e10	258	
Ren3	ScORA7-760	760	[18]

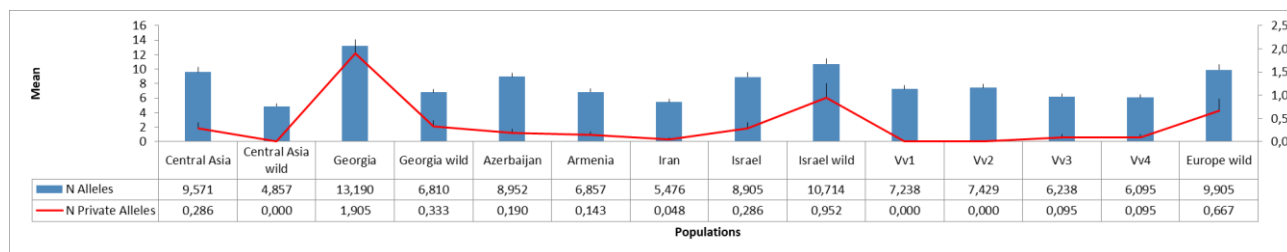


Fig. 1. Mean number of alleles per locus and private alleles for each population of the South Caucasus and the homologous dataset (Vv1, Vv2, Vv3 e Vv4 refer to ancestral subpopulations of cultivated grapevine detected by [7] and consistent with Negru’s grouping).

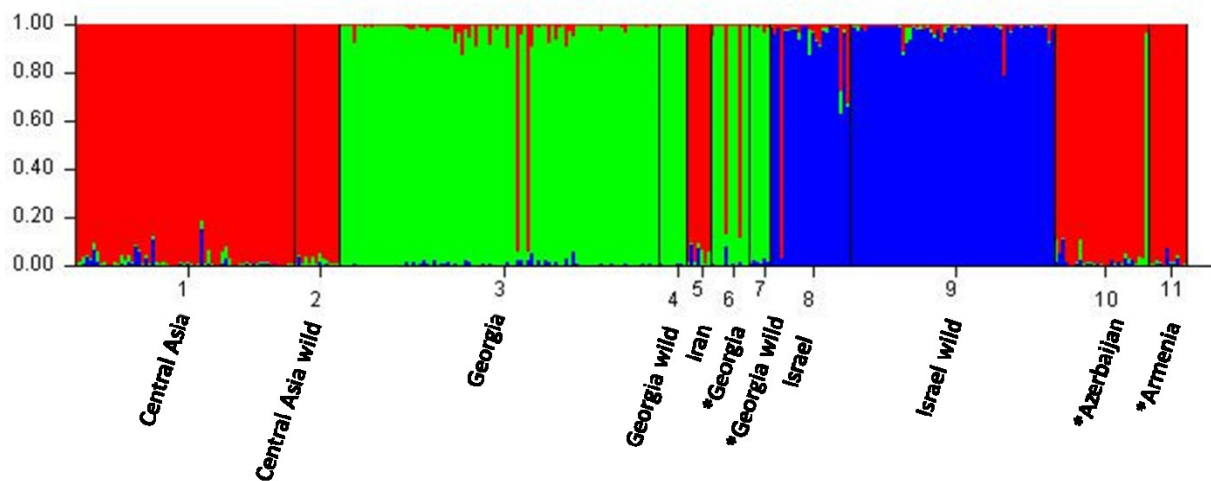


Fig. 2. Genetic population structure for K=3. Each unique SSR genotype is shown as a vertical line portioned into segments representing the estimated coefficients of membership proportion in the three genetic clusters inferred with STRUCTURE. Regional populations also investigated at resistance loci (examples in Table 2 and Table 3) are indicated by an asterisk (*).

Table 2. Allele data of a subset of grapevine cultivars for SSR markers flanking the Ren1 locus. Allele sizes associated with PM resistance in ‘Kishmish vatkana’ and ‘Dzhandzhal kara’ cultivars are in bold. Degree of resistance to PM is reported according to the OIV-455 scoring (5 = medium, 9 = very high).

Accession name	Origin	OIV 455	Ren1												
			UDV124	SC8-0071-014	SC47-18	VMC9h4	VMC4e10	UDV20							
<i>Kishmish vatkana</i>	control	9	214	-	142	160	232	248	258	282	236	258	142	158	
<i>Dzhandal kara</i>	control	9	214	218	142	176	228	248	276	282	254	258	142	158	
12 AZ 28	Khyndogny N.	Azerbaijan	8,9	194	214	142	172	216	242	270	274	248	252	142	158
12 AZ 32	Madrassa N.	Azerbaijan	9	194	214	142	172	216	240	270	274	252	258	142	158
12 AZ 27	Huseiny	Azerbaijan	9	184	214	160	162	240	248	274	276	252	254	142	158
12 AZ 12	Alykhanly Garagyozy N.	Azerbaijan	8,5	210	218	166	170	228	248	276	282	254	258	142	144
12 AZ 40	Surmeyi N.	Azerbaijan	7,9	210	218	166	170	226	248	276	282	254	258	142	144
12 AZ 30	Khart-Kart	Azerbaijan	9	190	194	160	170	240	248	274	284	-	-	158	160
12 AZ C	N2	Azerbaijan	9	210	218	172	202	236	248	286	286	-	-	142	158
AR 11	Kakhet	Armenia	9	186	214	142	200	236	242	284	298	-	-	132	142

Table 3. Allele data of a subset of Georgian accessions for SSR markers flanking three QTLs for resistance to DM. Disease resistance-linked alleles identified in *Vitis* spp. are in bold.

Accession name	Origin	Rpv3				Rpv12				Rpv10				
		UDV737	VMC7E2	UDV370	UDV350	GF09_46	GF09_47							
<i>V. amurensis</i>	control	-	-	-	-	194	198	308	308	410	420	294	296	
<i>V. rupestris</i>	control	280	296	200	210	-	-	-	-	-	-	-	-	
EST11f	Zhghia Sagviano	Georgia	-	-	200	204	192	194	300	300	-	-	292	296
EST2-8A	Kapistoni Tsitsiliani	Georgia	-	-	202	204	190	192	-	-	410	420	-	-
EST32-2	Borchalo	Georgia	296	296	200	200	190	190	306	308	418	418	294	294
g41	Chitistvala Acharul	Georgia	292	296	200	200	190	192	308	322	406	406	288	292
ESTSK	Saperavi Khashmisi	Georgia	284	284	200	204	190	198	-	-	410	420	294	294
EST.WF10	wild	Georgia	296	296	200	200	192	198	300	322	418	418	294	294
EST.WF110/298	wild	Georgia	-	-	200	204	190	192	300	300	416	416	292	296
L22	Mgalobshvili	Georgia	294	294	206	206	188	196	306	308	402	418	290	294

4. Conclusions

In this preliminary investigation, we exploited available genetic information on fungal disease resistance to characterize a grapevine germplasm collection from South Caucasus [19]. We showed that powdery mildew resistance phenotypes of three cultivated varieties from Azerbaijan not included in previous surveys can be attributed to Ren1-like genotypes. This finding is consistent with a weak genetic structure revealed by neutral SSR markers among regional accessions of Armenian, Azerbaijani and Central Asian origin. Using a marker assisted selection approach, those accessions which may also bring other favorable traits, can directly contribute to resistance gene pyramiding and diversification of plant defense mechanisms by intra-specific cross breeding, perhaps with reduced linkage drag effect on the quality of final wines.

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