Genetic Diversity in Portuguese native *Vitis vinifera* L. ssp. *vinifera* and ssp. *sylvestris*

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Abstract: To assess the different origins of Portuguese grapevine varieties, we used six nuclear and four chloroplastidal microsatellites as molecular markers, in order to compare the genetic structure of native wild-vines with native grapevine varieties. Both native subspecies have a great diversity, and a high interrelationship across the six nuclear microsatellites. Although identical numbers of alleles were found in each population, their distribution was different in the *vinifera* and *sylvestris* subspecies. Portuguese wild-vines have only chlorotypes A and B; A being the most frequent. The fifty-seven analysed Portuguese varieties have chlorotypes A, B, C, and D. The most frequent was the chlorotype A (75%), followed by D (21%). The results obtained reinforced the idea of Western Europe as having been one of the domestication centres for the grapevine, with contributions from the Eastern European gene pool. The observed genetic structure is a starting point from which to clarify the high number of native cultivars found in Portugal, and reinforces their probable origin in the Iberian Peninsula.

Keywords: domestication centre; genetic structure; Portuguese *vinifera* and *sylvestris* subspecies; vine

The plausible origin of Portuguese native grapevine varieties (*Vitis vinifera* ssp. *vinifera*) is from the domestication of local wild vines (*V. vinifera* ssp. *sylvestris*), the propagation of natural crosses among ancient introduced Eastern *vinifera* varieties with local *sylvestris*, or from directed crosses among all of the existing gene pool. Looking for the different origins of old native Portuguese varieties, we compared the genetic structure of wild vines with native grapevine varieties using nuclear and chloroplastidial microsatellites as molecular markers.

Young leaves were collected from fifty-three plants of four different wild vine populations (Cunha *et al.* 2009) and from fifty-seven grapevine varieties of the Portuguese National Ampelographic Collection (PRT051). Different morphological characteristics were the methodology used for selecting wild vines in the field. DNA from all samples was extracted, following the protocol of Thomas *et al.* (1993). Primer sequences and DNA analysis were carried out, following the protocols described by Almadanim *et al.* (2007) and Cunha *et al.* (2009). GENALEX software (Peakall & Smouse 2006) was used to calculate the number of alleles per locus (Na), the alleles' frequencies, and the percentage that were homozygous for each nuclear microsatellite locus.

In both subspecies a total of sixty-eight alleles were observed across the six nuclear markers. In spite of identical overall alleles, its distribution was different in *vinifera* and *sylvestris* subspecies (Table 1). From the total alleles found, 56% are present in the two subspecies; revealing a high

Table 1. Identified alleles and their frequencies in wild vines (fS); Portuguese native grapevine varieties (fV) across 6 microsatellite loci (VVMD 5, VVMD 7, VVMD 27, VR Zag 62, VR Zag 79, and VVS 2); Id are given in base pairs

n	VVMD 5			VVMD 7			VVMD 27			VR Zag 62			VR Zag 79			VVS 2		
	Id	fS	fV	Id	fS	fV	Id	fS	fV	Id	fS	fV	Id	fS	fV	Id	fS	fV
1	222	0.019	0.114	233	0.085	a	175	a	0.009	184	0.009	a	237	a	0.009	135	0.075	0.158
2	226	0.066	0.228	235	0.547	0.465	179	0.028	0.132	186	0.009	0.096	239	0.151	a	137	0.009	0.018
3	228	0.104	0.035	239	a	0.132	181	0.057	0.377	188	0.038	0.421	241	0.094	a	139	0.009	0.061
4	230	0.038	a	243	0.057	0.035	183	0.009	0.061	192	0.009	0.026	243	a	0.026	141	0.038	a
5	232	0.623	0.105	245	0.075	0.114	185	0.113	0.105	194	0.368	0.211	245	0.019	0.096	143	0.151	0.009
6	234	0.019	0.061	247	a	0.009	187	0.170	a	196	0.132	0.053	247	0.104	0.439	145	0.123	0.219
7	236	0.075	0.158	249	a	0.070	189	0.481	0.228	200	a	0.061	249	0.066	a	147	a	0.140
8	238	0.028	0.158	251	0.019	a	191	0.123	0.009	202	a	0.009	251	0.547	0.316	149	a	0.018
9	240	0.009	0.140	253	0.019	0.132	193	0.019	a	204	0.434	0.123	253	0.009	a	151	0.057	0.018
10	250	0.019	a	257	0.009	a	195	a	0.079				257	a	0.053	153	0.349	0.281
11				259	0.179	0.044							259	0.009	0.044	155	a	0.009
12				261	0.009	a							261	a	0.018	157	0.009	0.009
13																159	0.170	0.053
14																161	0.009	a
15																173	a	0.009

n – number of alleles found; Id – identified alleles; a – undetected alleles; fS – ssp. *sylvestris*; fV – ssp. *vinifera*

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relationship between them. Seven alleles in wild vines and five in grapevine varieties have a frequency above 30%. Twenty-two percent of both subspecies have unique alleles.

The number of homozygous alleles by locus in grapevine varieties is low, going from 5.26% (VVMD5) to 33% (VRZag79), with an average of 16.6%. In wild-vines these percentages are higher, ranging from 26.4% (VVS2) to 49.05% (VVMD27), with an average of 37.7%. Higher values of homozygous alleles in wild-vines are in conformity with the founder effect of a small number of individuals originating from an isolated population. Together with habitat change caused by humans, the European wild vine habitat was severely reduced in the 19th century due to the abundant occurrences of disease and pests (downy mildew, powdery mildew, and *Phylloxera vastatrix*) originating from America. True European wild vine habitat is confined to flood plain forests along rivers.

Portuguese wild vines only have chlorotypes A and B; chlorotype A is the most frequent (66%), in accordance with that expected for the Iberian Peninsula (Arroyo-Garcia *et al.* 2006). The native Portuguese varieties analysed have chlorotypes A, B, C, and D. The most frequent is chlorotype A (75%), followed by D (21%). Chlorotypes B and C are each present in one native grapevine variety (1.7%). Luzidio is the only grapevine variety with chlorotype B, and 83% of its nuclear alleles are common with chlorotype B wild vines. While the grapevine variety Manteudo is the only one with chlorotype C, it has a unique nuclear allele VR Zag 79-*237*. Chlorotype D grapevine varieties also have unique nuclear alleles, specifically Bastardo (VVMD27-*175*), Manteudo (VR Zag 62-*202*), and Tamarez (VVS2-*155*). The presence of exclusive nuclear alleles in native Portuguese grapevine varieties with chlorotypes A and D suggests that it was inherited from diverse ancestor groups. On the other hand, the high frequency of common nuclear alleles in both groups reveals inbreeding among the two groups.

The results obtained reinforced the idea of Western Europe as one of the domestication centres for grapevines (Grassi *et al.* 2003, Arroyo-Garcia *et al.* 2006), with contributions from the Eastern gene pool (Imazio *et al.* 2006). The observed genetic structure is a starting point to clarify the high number of native cultivars from Portugal, and it reinforces their probable origins on the Iberian Peninsula. These varieties contribute to the distinct identity of Portuguese wines.

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