ENVIRONMENTAL, SANITARY AND AMPELOGRAPHIC CHARACTERIZATION OF WILD GRAPEVINE IN WESTERN PYRENÉES (SPAIN, FRANCE)

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Abstract

Aims: The aim of this paper was to locate and describe the wild grapevine as a phytogenetic resource in Western Pyrenees, including Navarre, Aragon (Spain) and the department of Pyrénées atlantiques (France).

Methods and results: An ampelographic description of wild grapevine populations was made as well as a study of their oenological potential and an evaluation of their sanitary condition. The ampelographic results confirmed the dioecious nature of all the specimens. The microvinification results of two wine samples showed low alcohol content, high acidity (low pH) and high intensity of colour. The sanitary study revealed that the main parasites of the vines were eriophyids (mites), powdery and downy mildews (fungus), with aerial organs being most susceptible (leaves, bunches). No symptoms of phylloxera, nematodes or root destroying fungi were detected. The paper is complemented by a list of the main accompanying botanical species.

Conclusion: This phytogenetic resource is well represented in the area of study, exhibiting a good sanitary status.

Significance and impact of the study: This paper could provide the basis for future genetic studies addressing the possible links between the wild grapevines and the cultivated varieties of the region. Wild grapevines could also be used in future breeding programs.

Key words: ampelography, ecology, oenology, parasites, *Vitis vinifera* L. subspecies *sylvestris* (Gmelin) Hegi

Résumé

Objectifs : Le but de ce travail a été de localiser et de décrire la vigne sauvage comme ressource phytogénétique dans les Pyrénées occidentales, y compris la Navarre et l'Aragon (Espagne) et dans les Pyrénées atlantiques (France).

Méthodes et résultats : Nous avons fait une description ampélographique et étudié le potentiel œnologique et évaluer les conditions sanitaires. Les résultats confirment la nature dioïque de tous les spécimens. Les résultats de la microvinification des deux échantillons de vins étudiés montrent une faible teneur en alcool, une acidité élevée et une grande intensité de couleur. L'étude sanitaire a révélé que les principaux parasites des vignes étaient des organismes aériens comme les ériophydes, le mildiou et l'oïdium. Nous n'avons pas détecté de symptômes de phylloxéra, de nématodes ou de champignons destructeurs de la racine. L'article a été complété par une liste des principales espèces botaniques.

Conclusion : Cette ressource phytogénétique est bien représentée dans l'aire de l'étude et montre un bon état sanitaire.

Signification et impact de l'étude : Cet article pourrait servir de base à des études génétiques pour rechercher des possibilités de liens entre les vignes sauvages et les cépages de la région. Les vignes sauvages pourraient être utilisées dans des programmes futurs de culture.

Mots-clés : ampélographie, écologie, œnologie, ravageurs et maladies, *Vitis vinifera* L. subsp. *sylvestris* (Gmelin) Hegi

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INTRODUCTION

Since the Neolithic period, man has guided the process of obtaining the thousands of grapevine varieties now existing. The raw material selected during this process of domestication was the hermaphrodite specimens, which appear in wild grapevine populations composed mainly of dioecious vines (SCOSSIROLI, 1988 ; McGOVERN, 2003).

The wild grapevine, *Vitis vinifera* L. subspecies *sylvestris* (Gmelin) Hegi, has frequently been a component of ecosystems in Central and Southern Europe, mainly in riverbank forests (ARNOLD, 2002). Up to the second half of the 19th century, the use of different products obtained from these plants was very frequent (OCETE *et al.*, 2007). At that time, the arrival of the American downy and powdery mildews, which cause serious diseases in vineyards, began to wreck havoc on the most sensitive specimens, particularly in those sites where the environmental conditions were ideal for their development. Nowadays, symptoms caused by both of these fungal parasites appear in wild populations far from vineyards (GALLARDO, 2005).

As far as animal parasites are concerned, phylloxera must have a very limited impact on wild specimens, as this homopteran cannot survive in swampy and/or sandy soils, as is the case of wild grapevine habitats. By contrast, this insect forced the reconstruction of European vineyards with phylloxera-resistant rootstocks. This was considered as a homeopathic measure for vineyard : the original American vines had introduced the homopteran into Europe, but their use as rootstocks was a remedy against phylloxera attack on cultivar roots (OCETE *et al.*, 2006). Several of the New World species used as rootstocks in Europe grew in riverside forests. Some of these species were very invasive and gradually displaced the European vines from their ecological niches (TERPÓ, 1974 ; LAGUNA, 2003 ; ARRIGO and ARNOLD, 2007), due to their greater resistance to the cited fungi.

In addition to what has already been described in the previous paragraph, other anthropic actions must also be taken into account, such as the canalization of rivers, the construction of dams, lumber and horticultural developments, the clearance of river banks, and the improvement of road networks, among others. Such actions have converted this subspecies of *Vitis vinifera* into a threatened phytogenetic resource (IUCN, 1997).

In France, there are several national laws for the protection of wild grapevine : Arrêté January 20th 1982 modified in August 31st 1995. Regional laws include Arrêté June 28th 1993 for Alsace and Arrêté February 8th 1988 for Champagne-Ardennes, for example. In Spain, where grapevine cultivation is of great social and economic importance, it is quite the opposite as there is a complete absence of specific legislation for the preservation of wild grapevine. In spite of warnings in this respect, national and regional Spanish authorities have shown little interest in further development. Nevertheless, it would be highly recommended to find an appropriate protecting body, not only from a merely conservationist point of view, but also because certain wild specimens could be used to improve cultivated vines, to produce new interesting varieties within the framework of the « integrated production » philosophy, to increase agro diversity as well as to restore riverbank forests (OCETE *et al.*, 2007).

In order to mitigate this constant destructive process, the conservation ex situ of wild specimens has been adopted in traditional germplasm banks such as in Finca El Encín (IMIDRA, Community of Madrid), Rancho de la Merced (Andalusia), Finca Guadajira and Cultural Santa Ana (Extremadura), and that corresponding to the Fruit Production Station in Zalla (Diputación Foral in Vizcaya, Basque Country). Another collection of Andalusian material is conserved in vitro at the Institute for Natural Resources and Agro biology in Seville (CSIC). In France, there are at least three collections of wild grapevines maintained at the French National Institute for Agricultural Research (INRA) of Montpellier, Bordeaux and Colmar.

The first reference to the presence of wild grapevine within the geographical area of Western Pyrenees, where this project has been undertaken, was reported in Navarre by MARTÍNEZ DE TODA *et al.* (1991). Other publications already exist on the ampelography, distribution and ecology of the wild grapevine near the Pyrenees (LEVADOUX, 1956 ; OCETE and PÉREZ, 1995 ; OCETE *et al.* 1999, 2008 ; LACOMBE *et al.*, 2004) and its relationship with the beginning of viticulture (MARINVAL, 1997 ; TERRAL *et al.*, 2010). This paper attempts to show the present situation of this phytogenetic resource in the area of study, as a basis for future deeper multidisciplinary investigations, and advocate for its conservation.

MATERIALS AND METHODS

1. Study areas

The prospection of wild vines on the Spanish side was undertaken in the basins of the Salazar, Irati and Eska Rivers in the regions of Navarre and Aragón in 2007. River erosion has excavated narrow gorges called foces, which cross the Leyre Mountains composed of marine-originated material dating from between the late Mesozoic and the Palaeozoic eras. In France, observations were made in the Bearnais fluvial network between the Pierre Saint Martin pass and the wine growing region of

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Figure 1 - Location of the studied area.

Jurançon, in the proximity of the city of Pau, and in the area between the town of Oloron Sainte-Marie in the Béarnais and the Port de Larrau pass in the Pyrénées atlantiques (figure 1).

Global Positioning System (GPS) techniques were used to get the geographical coordinates of all studied sites. Geodesic heights were calculated by means of Google Earth (earth.google.com). Data were introduced in a table to obtain the level distribution profile of the vines in the sampled basin of each river and to calculate the surface area of the sites.

2. Ampelographic observations

The flower type of the vines was observed with the aim of determining their dioecious (male or female *versus* hermaphrodite) character, and the descriptors laid down by OIV (2007) were evaluated on 50 plants of each sex. The number of seeds/berry was counted on a sample of 100 fruits harvested at the Salazar, Irati and Eska Rivers. At the same time, a morphometric examination of the seeds was performed according to LOGOTHETIS´ (1970; 1974) methodology.

3. Sanitary evaluation

Symptoms caused by phytophagous arthropods and pathogenic agents were investigated on the shoots (height of less than 3 m) of all the specimens. In addition, the fine roots were examined to a depth of 40 cm for symptoms of infection by phylloxera, nematodes and root destroying fungi. In the last case, the number of plant tested varied between 6 (in the smallest population group) and 20 (in the rest of the populations).

4. Surrounding flora

The determination of the main species of the surrounding flora was based on the botanical keys of AIZPURU *et al.* (2003). That corresponding to gastropods was established according to the taxonomic criteria of LARRAZ (1982).

5. Oenological analysis

Harvest was undertaken at Salazar (Sample A) and Eska (Sample B) Rivers on October 11th, 2007. The berries were de-stemmed by hand and microvinification was carried out with the ripest fruits. Fermentation was performed with autochthonous yeasts and a 10-day maceration in an isothermal room at a temperature of 20ºC, without the addition of potassic met bisulphate and with two daily stirrings. The analytical methods used are indicated in the regulations of the EEC (1990) on wines.

RESULTS

1. Location of the populations

The landscape of the Pyrenees area sampled is one of the best conserved in Western Europe, as it was possible to sample almost 493 putative wild vines. The geographical location of populations at each site (Salazar River, Irati River, Areta River, Eska River and Basque Country) and their respective number of individuals is shown in table 1. The number of vines includes developed specimens (trunks larger than 20 cm in diameter) as well as medium-sized ones and small plants (at least two years old).

As occurs in the different habitats of wild vines in other sites, occasionally some cultivated vines grow in a

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Table 1 - Location (with respective number of individuals) of wild grapevine populations.

Site	P	N	GPS coordinates	Additional information
Salazar River	A	6	42° 50´ 20,2´´/1° 6´4,5´´	Vicinity of Ibilzieta
	B	47	42°49´58,7´´/1°6´1,5´´	Surroundings of Sarriés
	C	39	42°48´41´´/1°4´57,7´´ to 42°48´42,5´´/1° 4´51,1´´	Jabros River (tributary of the Salazar River)
	D	57	42° 48´30,6´´/1°5´45´´ to 42°48´23,6´´/1°5´46,3´´; 42°45´12,7´´/1°6´5,1´´; 42°43´15,4´´/1°7´19,7´´	Vecinity of Güesa
	E	53	42°42´57,4´´/1°7´9´´ to 42°41´51,8´´/1°10´21,5´´; 42°42´4,9´´/1°10´3,3´´; 42°43´0,8´´/1°9´24´´	Between Bigüézal rest area and Aspurz
	F	27	42°41´49,3´´/1°10´26,2´´ to 2°41´49,7´´/1°10´46´´	Foz de Arbayún
Irati River	A	17	42°53´31´´/1°18´10,5´´ to 42°53´22´´/1°18´21´´	Between Oroz-Betelu and Itoiz dam
	B	15	42°51´48,9´´/1°22´38´´ to 42°51´57´´/1°22´40,7´´	Urrobi River
	C	21	42°38´4,8´´/1°18´7,1´´ to 42°37´31,2´´/1°18´8,9´´	Foz de Lumbier
Areta River	A	18	42°43´21,8´´/1°23´25,8´´ to 42°46´28,2´´/1°16´51,8´´	Surroundings of Sansoaín
	B	19	42°46´30´´/1°14´45,8´´ to 42°46´34,2´´/1°14´33,1´´	Close to the hermitage of Santa Fe
	C	13	42° 50´7,6´´/1° 14´12,4´´	Near Elcóaz
Eska River	A	23	42°49´53´´/0°56´49,6´´ to 42°49´46,7´´/0°56´54,0´´; 42°49´42,4´´/0°56´46,6´´	Between Isaba and Roncal
	B	41	42°43´9,6"/1°0´19,1´´ to 42°40´6´´/1°0´21,1´´	Foz of Burgui
	C	26	42°39´20,6´´/1°0´56,1´´; 42°39´0,9´´/1°0´41,1´´	Close to Salvatierra de Escá
	D	35	42°47´22,9´´/1°0´47,8´´; 42°46´55,2´´/1°0´35,6´´; 42°46´23´´/1°0´27,5´´; 42°45´53,4´´/1°0´36´´	Close to Vidángoz
French Basque Country	A	19	43°6´7,8´´/0°48´25,4´´ to 43°6´10´´/0°48´22,5´´	Between Lanne en Bateus and Montury
	B	17	43°3´33,7´´/0°52´36,3´´ to 43°3´28,9´´/0°52´36,3´´	Between Tardets and Larrau, on the Saison River

P = Population; N = Number of vines examined

wild state and even more frequently rootstocks of American origin (mainly *V. rupestris*) and hybrids (Richter 110 to be precise). On the outskirts of Tardets, some rootstocks growing in a wild state were found among the riverbank vegetation. One even larger population was found at the 4 km point on this road, on the outskirts of Licq-Atherey.

Figure 2 shows that the slopes of the river basins are very regular, so the level distribution of vines was also very homogeneous along the Salazar, Biniés, Eska and Areta basins. These sites are disseminated within an area of 81.225 hectares with a perimeter of 110 km.

2. Main accompanying botanical species

The main supporters of wild grapevines were: *Acer campestre, Acer monspessulanum, Acer opalus, Arbutus unedo, Buxus sempervirens, Corylus avellana, Crataegus monogyna, Ficus carica, Frangula alnus, Fraxinus excelsior, Fraxinus ornus, Tamus communis, Pinus sylvestris, Populus nigra, Prunus spinosa, Quercus coccifera, Quercus ilex, Quercus robur, Rhamnus alaternus, Robinia pseudoacacia, Rosa sempervirens, Rubus ulmifolius, Ruscus aculeatus, Salix atrocinerea, Salix elaeagnos, Sambucus nigra, Sedum album, Sorbus*

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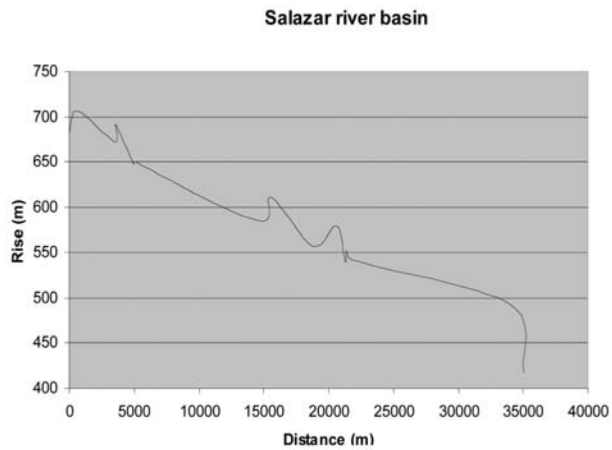

Figure 2a - Profiles of the Salazar River Basin.

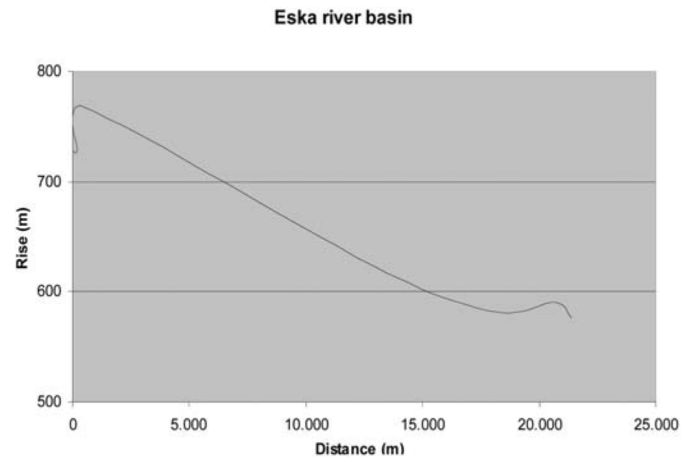

Figure 2b - Profiles of the Eska River Basin.

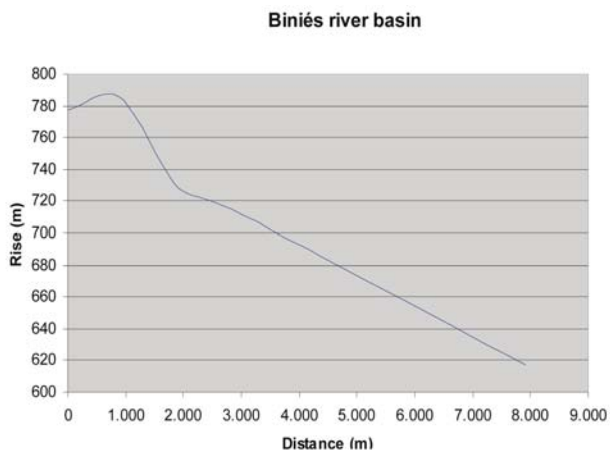

Figure 2c - Profiles of the Biniés River Basin.

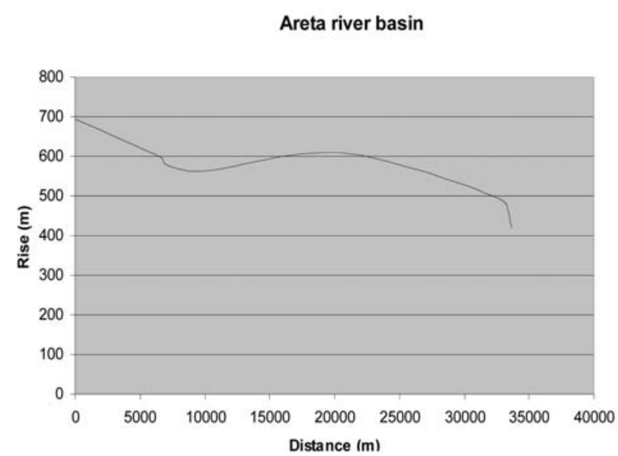

Figure 2d - Profiles of the Areta River Basin.

aucuparia, Tilia cordata, Ulmus minor and *Viburnum tinus*.

The main climbers were: *Bryonia cretica* ssp. *dioica*, *Clematis vitalba*, *Hedera helix* and *Lonicera periclymenum*.

3. Other frequent annual and perennial species

a) Pteridophyta: *Equisetum telmateia, Lycopodiella inundata, Osmunda regalis, Phyllitis scolopendrium* and *Pteridium aquilinum*.

b) Angiospermae: *Arctostaphylos uva-ursi, Atriplex hortensis, Daboecia cantabrica, Calluna vulgaris, Cichorium intybus, Erica cinerea, Erica vagans, Fumaria muralis, Helleborus foetidus, Helleborus viridis, Lathyrus pratensis, Montia fontana, Urtica dioica, and Urtica urens*.

4. Ampelographic description

The overall description of the specimens that were examined is compiled in table 2. The indexes of the descriptors were very similar to those belonging to other populations from the Basque Country in Spain and France (OCETE *et al.*, 2004; 2008).

In the population group 4A, we found two specimens with predominantly male flowers as well as another male specimen with long bunches which were over 16 cm in length.

All the berries were red and showed an uneven development between the ripening and the maturation periods, both in size, usually less than 1 cm in diameter, and in pigmentation. It is notable that the volume of pulp was much smaller than that of the cultivated varieties of the region, such as Garnacha in the Navarre region and Tannat, Cabernet franc, Petit and Gros Manseng, Courbu, Camaralet, Lauzet, etc. in the Irouleguy and Jurançon regions. Therefore, the extracted amount of must was low (about 17 % of berry weight) and highly coloured by the anthocyanins of grape skins (see the vinification results, table 4).

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Table 2 - Ampelographic description of female and male individuals.

	Code OIV (IPGRI)	Descriptors	Female	Male
Vegetative characters	1 (6.1.1)	Young shoot: opening of the shoot tip	5 (fully open)	5 (fully open)
	3 (6.1.2)	Young shoot: intensity of anthocyanin coloration of tip	3 (high) - 5 (medium)	3 (high) - 5 (medium)
	4 (6.1.3)	Young shoot: density of prostrate hairs on tip	3 (low)	3 (low)
	8 (6.1.7)	Shoot: colour of the ventral side of internodes	1 (green) - 2 (green and red striped)	1 (green) 2 (green and red striped)
	51 (6.1.16)	Young leaf: colour of upper surface	3 (with bronze spots) 4 (copper-reddish)	3 (with bronze spots) 4 (copper-reddish)
	65 (6.1.21)	Mature leaf: size of blade	3 (small)	5 (medium)
	67 (6.1.22)	Mature leaf: shape of blade	2 (wedge-shaped)	3 (pentagonal)
	68 (6.1.23)	Mature leaf: number of lobes	1 (entire leaf)	2 (three) - 3 (five)
	76 (6.1.27)	Mature leaf: shape of teeth	1 (both sides concave) - 2 (both sides straight)	1 (both sides concave) 2 (both sides straight)
	77	Mature leaf: size of teeth in relation to blade size	3 (small) - 5 (medium)	3 (small) - 5 (medium)
	78 (6.1.29)	Mature leaf: length of teeth compared with their width at the end of the base	3 (short) - 5 (medium)	3 (short) - 5 (medium)
	79 (6.1.30)	Mature leaf: degree of oppening of petiole sinus	1 (very wide open) 3 (half open)	1 (very wide open)
	84 (6.1.35)	Mature leaf: density of prostrate hairs between veins in lower side of blade	5 (medium) - 7 (high)	5 (medium) - 7 (high)
	85 (6.1.36)	Mature leaf: density of erect hairs between veins	3 (low)	3 (low)
	86 (6.1.37)	Mature leaf: density of prostrate hairs on main veins	3 (low)	3 (low)
	87 (6.1.38)	Mature leaf: density of erect hairs on main veins	0 (absent) - 1 (very low)	0 (absent) - 1 (very low)
	65 (6.1.21)	Mature leaf: size of blade	1 (very small) - 3 (small)	5 (medium)
Inflorescence and fruit characters	151 (6.2.1)	Flower: sexual organs	4 (reflexed stamens and fully developed gynoecium)	1 (fully developed stamens and no gynoecium) - 2 (fully developed stamens and reduced gynoecium)
	202 (7.1.5)	Bunch: size	1 (very short) - 3 (short)	-
	204 (6.2.3)	Bunch: density	1 (very loose) - 3 (loose)	-
	206 (6.2.4)	Bunch: lenght of peduncle	5 (medium) - 7 (long)	-
	220 (6.2.5)	Berry: size	1 (very small)	-
	223 (6.2.6)	Berry shape	2 (globose)	-
	224 (6.2.7)	Berry: presence of seeds	3 (well developed)	-
	225 (6.2.8)	Berry: skin colour	6 (blue-black)	-
	231 (6.2.9)	Berry: intensity of flesh anthocyanin coloration	5 (medium)	-
	236 (6.2.12)	Berry: particular flavour	1 (none)	-

Note: in the case of the male description, only differential characters are remarked

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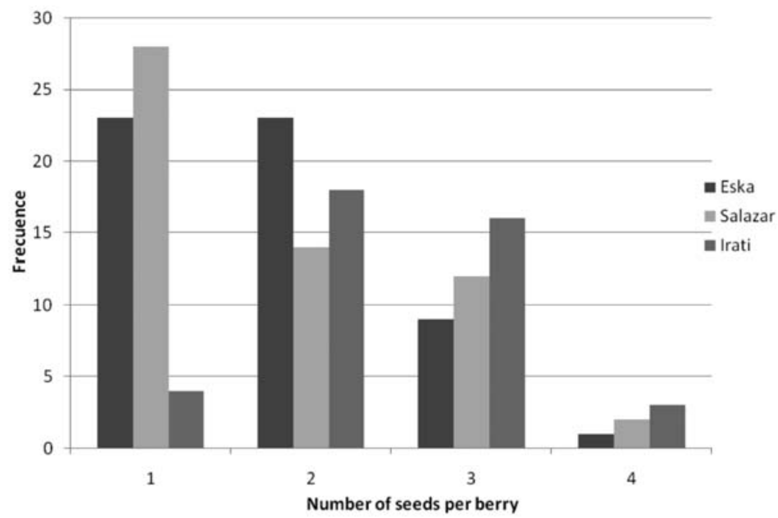

Figure 3 - Frequency of the number of seeds per berry in wild grapevine populations.

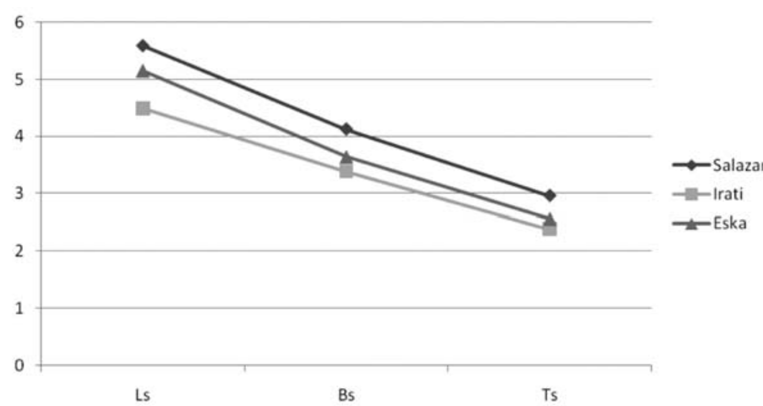

Figure 4 - Representation of Logothetis´ Index.

The frequencies of the number of seeds per berry in grapevine populations from Salazar, Irati and Eska are set out in figure 3.

The values of Logothetis'index (1970; 1974) are presented in figure 4. As can be seen in all three populations, the angle formed by the straight lines obtained by representing length, width and thickness is approximately 180º (to be exact, 178º in the Salazar and Eska Rivers and 179º in the Irati River). Therefore, the three populations may be considered as pure wild vines, according to this author's criteria.

5. Incidence of phytophagous species and pathogens

The incidence of arthropod and fungal pests is compiled in table 3 with the number of vines with symptoms of mites (*Colomerus vitis*, *Calepitrimerus vitis*) and fungus (*Erysiphe necator*, *Plasmopara viticola*). Mites only produced symptoms on leaves, while fungal diseases affected both leaves and bunches. No symptoms of phylloxera infections were detected.

It is interesting to note that vineyards, often located very near the wild populations (sometimes less than 80 m away in the case of Spain), were attacked by phylloxera at the beginning of the XXth century. The Wine Cooperative in Lumbier holds evidence of this: a subsoiler constructed by the famous Spanish company of Castillo y Orbañanos of Haro (La Rioja) and used in the reconstruction of vineyards with phylloxera-resistant rootstocks during the 1930s.

No symptoms of infestation or infection attributable to phylloxera, nematodes or mycelium of *Armillaria* root rot were detected due to edaphological conditions, as indicated by OCETE *et al.* (2007) in Andalucía.

The percentage of vines infested by the erineum strain of *Colomerus vitis* (Pagenstecher) (Acari, Eriophyoidae) varied between 27 % (Irati River) and 91 % (Eska River).

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Table 3 - Incidence of pests and diseases (number of plants with symptoms).

Population size	*Colomerus vitis* (leaf)	*Calepitrimerus vitis* (leaf)	*Erysiphe necator* (leaf)	*Erysiphe necator* (bunch)	*Plasmopara viticola* (leaf)	*Plasmopara viticola* (bunch)
1 A (6)	2	0	2	0	6	0
1 B (47)	15	4	3	0	26	0
1 C (39)	12	8	7	3	26	6
1 D (57)	24	6	20	8	32	7
1 E (53)	26	5	17	2	38	6
1 F (27)	12	3	10	2	22	3
2 A (17)	9	0	5	0	16	5
2 B (15)	4	0	6	0	9	0
2 C (21)	17	9	11	6	17	8
3 A (18)	8	3	6	2	12	7
3 B (19)	6	0	5	1	15	6
3 C (13)	7	3	5	2	7	4
4 A (23)	11	6	9	4	13	6
4 B (41)	14	8	16	9	23	9
4 C (26)	9	6	4	6	23	5
4 D (35)	32	16	9	15	27	26
5 A (19)	8	3	9	2	13	2
5 B (17)	6	4	7	3	8	1

The number of erinea/leaf was usually low, not exceeding 15 %. The percentage of vines infested by the other monophagous eriophyid, *Calepitrimerus vitis* (Nalepa), varied between 0 % (Salazar and Irati Rivers) and 46 % (Eska River).

With reference to insects, the only presence worth of mention was pre-imaginal tetigonal orthopterans feeding on the leaves of some vines, causing the apparition of corrosion marks and perforations on the affected limbs. The percentage of affected plants was lower than 5 %.

With regard to molluscs, *Helicella itala* (L.) was the main species of gastropods found feeding on the leaves of wild grapevines. The highest level of infestation was observed in Foz de Arbayún, with 21 % of vines attacked.

The main sanitary problem of wild grapevine populations was downy mildew, Plasmopara viticola (Berkeley and Curtis) Berlese and de Toni, which affected leaves and bunches very irregularly in the different locations, where the percentage of plants with symptoms varied extraordinarily. The percentage of infected leaves fluctuated between 6.4 % (Salazar River) and 52 % (Areta River). The percentage of affected bunches varied between 0 % (Salazar and Irati Rivers) and 43 % (Eska River). In many cases, whole bunches were completely ruined by the effect of the fungi, while others had a few dead berries and for the most part were unaffected. It is remarkable that in population 1A (Salazar River), 100 % of the individuals exhibited symptoms on the foliage whereas no infection was found on the bunches.

During the period of observation, the powdery mildew, *Erysiphe necator* Schwein, had an irregular distribution as indicated before. The percentage of vines with affected leaves varied between 36 % (Salazar River) and 62 % (Irati River). At the same time, the percentage of vines with infected bunches varied between 0 % (Salazar and Irati Rivers) and 3 % (Eska River).

The percentage of plants co-infected with both cryptogams varied from 3 % (Salazar River) to 27 % (Areta River) in the case of leaves and from 0 % (Salazar and Irati Rivers) to 27 % (Eska River) in the case of bunches.

6. Analysis of microvinifications

The raw material used in the two microvinifications corresponds to bunches from the Salazar and Eska Rivers, where there were enough available bunches to harvest from 13 and 11 female vines, respectively. It is necessary to indicate that only about 17 % of the total weight of each berry depends on the must extracted. The oenological analyses are compiled in table 4.

The alcoholic content was low in both samples in comparison to that of wine-producing varieties cultivated in the area. The total acidity of both samples was high, given the medium/high content level of malic acid resulting from the fact that the analysis was completed before the malolactic fermentation. Therefore, and quite logically, pH values were low. Similarly, the results of both volatile acids were low, which may be a consequence of the absence of sulphurous anhydride, having not added potassic met bisulphate during fermentation.

The colour intensity was an indication that both wines were intense and very characteristic red wines. They may be considered average when compared to cultivated red wine varieties. The tones were comparable to a young red

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Table 4 - Analytical data of wine samples produced from wild grapevines.

Parameters	Salazar River	Eska River	Methods
Alcoholic content	9.40 % (v/v)	9.23 % (v/v)	Near-Infrared (NIR)
Total Acidity	5.63 g/l	5.79 g/l	OIV
Volatile Acidity	0.79 g/l	0.85 g/l	Autoanalyzer PCSA
pH	3.25	3.17	pH Meter
Tartaric acid	6.10 g/l	6.35 g/l	Autoanalyzer
Malic acid	3.4 g/l	3.7 g/l	FCSA
Colour intensity	11.2	10.6	OIV
Tones	0.39	0.36	OIV
Total Polyphenols	78.1 mg/l	75.3 mg/l	Ribéreau-Gayon (U.V. 280 nm)
Anthocyanins	317 mg/l	304 mg/l	Ribéreau-Gayon (U.V. 280 nm)
Tannins	2.3 g/l	2.0 g/l	Ribéreau-Gayon (U.V. 280 nm)

wine with acid pH. The concentration of total polyphenols was quite high, that of anthocyanins was medium to high and the concentration of tannins was average.

DISCUSSION

The dioecious character of wild grapevine was confirmed, once again, in the studied area, where no hermaphrodite plants were observed. The vines showed different foliar morphologies, as the rest of European populations described by ARNOLD (2002) from the Iberian Peninsula to the Danube mouth.

The majority of the identified botanical species belong to Mediterranean vegetation, under a clear Atlantic influence. The main supporters were typical of gallery forests of the Cantabrian Basin (AIZPURU *et al.*, 2003) and similar to riparian, Atlantic-« collinean » vegetation (TERRAL *et al.*, 2010).

A remarkable fact is that female grapevine plants usually had smaller leaves than the male ones, which is in contradistinction to Southern Spanish populations (OCETE *et al.*, 2007).

The frequencies of the number of seeds/berry were very similar to those found by GALLARDO (2005) in the case of Western Andalusian gallery forests situated in the Sierra Morena mountain range. They were more rounded and had a shorter beck than those from cultivars (according to the comparison with the Tempranillo cultivar in the last reference).

However, the data of Logothetis´ index indicated that the three populations may be considered as pure wild vines. Those values were very close to those found by OCETE *et al.* (2007) in populations from Andalucía.

The absence of phylloxera symptoms in wild grapevines is due to the edaphic conditions of their habitats, where there are several months of flooding every year, since the wild grapevine is sensitive to the homopteran under laboratory conditions (OCETE and LARA, 1994; GALLARDO, 2005). Similarly, this would explain the absence of symptoms caused by root-knot nematodes, such as galls and secondary rootlets (RASKI, 1994), similar to those caused by Meloidogyne.

In spite of the absence of *Armillaria mellea* (Vahl: Fr) Kummer mycelium in the vine roots, the fungi was present in some botanical supporters, mainly those belonging to the species *Populus nigra* in the Salazar and Irati Valleys. This disease is frequent in vineyards from temperate climate areas, where it produces subcortical, whitish plaques (WATLING *et al.*, 1982).

Colomerus vitis is a monophagous species that is widely distributed throughout vineyards of both hemispheres (KEIFER *et al.*, 1982; DENNIL, 1986) and in some populations of wild grapevine in Spain (OCETE and PEREZ, 1995; OCETE *et al.*, 2007).

It is interesting to note the presence of erinea (mite-induced plant hair growth) on the upper leaf surface of some specimens with low level of infestation. Young leaves with damage attributable to the leaf curl strain (FLAHERTY and WILSON, 1996) were only found on seven specimens, as reported in other areas of Navarre (OCETE and PEREZ, 1995).

On the other hand, *Calepitrimerus vitis* has become a pest in vineyards of Europe, United States, South Africa and Australia. In the majority of these regions, the eriophyid is of an occasional nature and of lesser importance (SAZO RODRÍGUEZ *et al.*, 2003; FLAHERTY and WILSON, 2004). The presence of this mite on wild grapevines was first observed on populations of the coast of Guipúzcoa (OCETE *et al.*, 2002), then in Ebro Valley (OCETE *et al.*, 2004) and riverbank forests in Andalucía (OCETE *et al.*, 2007).

The incidence of both cosmopolitan eriophyids (BERNARD *et al.*, 2005) was very variable within each location. The incapacity of both mite species to travel

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great distance and their constant presence on all type of populations would suggest that these two obligatory and monophagous parasites have cohabited on wild grapevines since ancient times.

All the cultivars of *Vitis vinifera* are sensitive to mildew, although there are other species that are relatively resistant to the fungi, such as the American species V. rupestris and *V. rotundifolia* (OCETE *et al.*, 2006).

Sometimes, the shoots belonging to the oldest vines contain black stains corresponding to cleistothecia (powdery mildew disease) (PEARSON and GADOURY, 1987). From the mid-19th century onwards, the species has been found in practically all the vineyards in Europe, Asia Minor and North Africa (LE CANU, 1862). Nowadays, the parasite can be found in the majority of wine producing areas all over the world, even in the tropics (PEARSON and GOHEEN, 1996).

In every case, the level of symptoms caused by this parasitic species varied widely from one vine to another within populations, so complementary studies on parasite tolerance, complemented by genetic ones, would be needed.

Both samples of wine showed a lower alcohol content than the wines obtained from wild grapevines in Andalucía (more than 13 % v/v) (OCETE *et al.*, 2007) and in Sardinia (12,9 % v/v) (LOVICU *et al.*, 2009). They also showed a higher acidity, probably because the Andalusian and Sardinian berries grow under sunny and warmer conditions. Taking into account all the analytical parameter values, both samples of wine could be suitable for ageing in oak casks.

Based on the number of vines examined and observed with binoculars (in the case of inaccessible specimens), the total number of individuals in the different studied areas can be estimated as well over 500. Thus, this Pyrenean region is ideal for carrying out genetic characterization studies, which could be used to estimate the degree of biodiversity of the different sites. This data can be compared to that obtained for other areas by the National Centre of Biotechnology (CSIC) and INIA (ARROYO-GARCÍA *et al.*, 2006). Moreover, the degree of relationship between these wild specimens and the traditional cultivars of Navarre, Aragón, Irouleguy and Jurançon could be studied, as it has been done on the island of Sardinia (LOVICU, 2007). In this way, the pieces of information available could together contribute to solve the puzzle of the process of the polycentric domestication of grapevine.

The wine-producing material selected in natural environments could also be used to test for biological and physical stress, with the aim of having a supply of the best specimens for future use in trials of genetically improved cultivars. This process could be carried out by hybridization with the traditional regional varieties followed by agronomic and oenological evaluation of the hybrids. The trials could even be extended to rootstocks, using both pure American species as well as hybrids obtained from European cultivars, because wild vines show a high resistance to swampy conditions and to high concentrations of active limestone in the absence of sanitary radicular problems (OCETE *et al.*, 2007). Another ecological aspect to be studied concerns mycorrhizal fungi and native yeasts : very little is known about their composition in the natural environment.

Finally, the authors would like to bring to the attention of the environmental authorities the need to preserve this valuable phytogenetic resource.

CONCLUSION

This phytogenetic resource is well represented in the area of study, exhibiting a good sanitary status on both sides of the Pyrenees mountain range.

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