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Ecological characterization of wild grapevine habitats focused on arbuscular mycorrhizal symbiosis

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Summary

The European wild grapevine, *Vitis vinifera* L. ssp. *sylvestris* (Gmelin) Hegi, is cited as a dioecious relative of cultivated vines, so it can play an important role as phytogenetic resource. There is a lack on the knowledge about the susceptibility of wild grapevine to arbuscular mycorrhizal (AM) association. In consequence, the aim of the present work is to confirm the presence of arbuscular mycorrhizal fungi (AM) in the wild grapevine rhizosphere spread in different kinds of soils from 18 wild populations from Spain and France. On the other hand, the accompanying flora, the edaphic characteristics and the presence of parasitic organisms on vines were also determined. The spore density of mycorrhizal fungi in the rhizosphere of the selected plants was relatively low. However, the diversity of mycorrhizal fungi was quite high. The taxonomic diversity of AM observed is 56 taxa, 15 of which were identified to species and 41 to genus. Some morphotypes do not correspond to any of the species described up to now. Results indicate the important quality and ecological value in the sites studied and, in consequence, the necessity of their preservation.

Key words: Spain and France; botanical supporters; parasitic species; soil.

Introduction

The Eurasian wild grapevine, *Vitis vinifera* L. ssp. *sylvestris* (Gmelin) Hegi, is cited as an autochthonous and dioecious relative of cultivated vines (Vavilov 1926). Its relic populations spread out mainly along gallery forests from Portugal to the Hindu Kush mountain-range (Arnold 2002), within the approximate area limited by parallels 50 (Rhine valley, Germany) and 30 (Ourika valley, Morocco) (Ocete *et al.* 2007). Their main habitats are gallery forests situated along rivers and creeks growing on fluvisols (Arnold 2002).

When powdery and downy mildews arrived in Europe along the XIX century, both fungal diseases caused a heavy impact on vineyards and also on wild populations. Probably, Phylloxera, *Daktulosphaira vitifoliae* (Fitch) infestation provoked a minor damage, because the kind of soils of wild grapevine habitats is not suitable for this North American homopteran (Ocete *et al*. 2011). The negative effect caused by cited parasitic organisms on wild grapevine populations was multiplied by human activities, mainly forest exploitations and public works, like road construction and river management led to direct eradications of wild grapevine populations throughout Europe (Arnold 2002).

Due to the absence of human selection, this subespecies can play an important role as phytogenetic resource for viticulture (Grassi *et al.* 2003). These efforts would result in the alleviation of the genetic erosion suffered by the grapevine after millennia of selective culture (Ramishvili 2001). Most varieties of *Vitis vinifera* L. are susceptible to different pests and diseases such as *Tetranychus urticae, Colomerus vitis, Calipitrimerus vitis*, downy and powdery mildews.

Arbuscular mycorrhizal (AM) are known to colonize the roots of the above 90 % of land plants (Tsimilli-Michael *et al.* 2000), including grapevines (Likar *et al.* 2013), that are even fully dependent on AM (Menge *et al.* 1983). AM has played a key role in plant evolution on Earth as well as on the development and maintenance of the structure and diversity of terrestrial ecosystems. This symbiosis normally increases plant growth (Linderman and Davis 2001) and abiotic stress tolerance (Schreiner *et al.* 2007). The knowledge about susceptibility and effectiveness of wild grapevine to AM-association is not documented although considered very important due to the described reasons. In consequence, the aim of the present work is to confirm the presence of arbuscular mycorrhizal fungi (AM) in the wild grapevine rhizosphere spread in different locations of Spain and France, as well as an initial taxonomic approach to the diversity of AM found.

Material and Methods

The main supporters of the vines from the accompanying vegetation were determined using botanical keys. Samples of soil of 1kg were collected in 18 populations of wild grapevine situated in river bank forests located along the territories of Basque country (Spain and France), Castilla y León, La Rioja, Extremadura and Andalucía. Each sample contained edaphic material from the surface up to a maxi-

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mum of 40 cm of depth. All of them contained abundant fragments of roots of wild grapevines. Rhizosphere soil was used for isolation and multiplication (enrichment) of AM fungi associated to the target plants. AM spores were isolated from 50 g of rhizosphere soil (Sieverding 1991). AM identification is based on ontogenic and the morphology of the spores, spore formation and the structure of their wall. Size, shape, pigmentation, ornamentation, and supporting hyphae form of occlusion are the main criteria in order to group the spores for the identification of the different species and the use of molecular biology as a complementary tool (Brundrett *et al.* 1994, Oehl *et al.* 2006).

Results and Discussion

The locations and main ecological characteristics of the different places are shown in Tab. 1. The main supporters are typical plant species from river-bank forests. Its scarce diversity depends on the latitude of the population considered. However, the main parasitic species are very homogenous. The main native mite species causing infestation on vines are the erineum strain of *Colomerus vitis* (Pagenstecher) and *Calepitrimerus vitis* (Nalepa) (Acari, Eriophyidae) as it was cited by Ocete *et al.* (2011). The presence of this last species is higher in populations situated in the North of Spain and South of France (Basque country). On the other hand, the presence of symptoms caused by imported downy and powdery mildews are very frequent, on the majority of the wild grapevines observed from the diverse geographical areas sampled.

The AM species determined are shown in Tab. 2. The total number of identified taxa of AM corresponding to 56, 15 of them were determined up to species level and 41 up to genus, according to biodiversity estimated by Ohel *et al.* (2006). According to results obtained in this study, it is necessary to remark the wide representation of the main genus indicated in this last reference. It would be consid-

Table 1

Location and main ecological characteristics in wild grapevine populations sampled.

Name	Province	Intervals of longitude and latitude	Soil pH	Main botanical supporters
El Chorreadero	Cádiz	36º49'26''N – 36º49'17''N 5º29'55''W – 5º29'35''W	8.2	*Fraxinus angustifolia*, *Nerium oleander*, *Salix sp.*
Pantano de los Hurones	Cádiz	36º42'43'' N – 36º42'28'' N 5º33'47''W – 5º33'51'' W	7.9	*Fraxinus angustifolia*, *Nerium oleander, Populus nigra*
El Bosque	Cádiz	36º44'38'' N – 36º44'36'' N 5º30'13''W – 5º30'14'' W	7.9	*Alnus glutinosa, Fraxinus angustifolia*, *Nerium oleander*
Saratxo	Álava	43º 1' 30,4'' N 3º 0´ 33,2'' W	7.9	*Corylus avellana*¸*Populus tremula*, *Salix triandra*
Peña Angulo	Burgos	43º2'28,4'' N 3º11'26,1'' W	9.4	*Corylus avellana*¸ *Populus tremula*, *Crataegus monogyna*
Cadagua river	Vizcaya	43º 14´25''N 2º59´50''W	8.7	*Acer campestre*,*Corylus avellana*, *Tilia cordata*
La Concha	Vizcaya	43º 13´30''N 3º21´39''W	9.1	*Acer campestre*, *Acer monspessulanus*, *Corylus avellana*
Casalarreina	La Rioja	42º 32' 28.2'' N 2º 54' 52.5'' W	7.6	*Corylus avellana*¸ *Crataegus monogyna, Populus nigra*
Peñaladros	Burgos	43º3'45,1'' N 3º8'59,5'' W	8.2	*Corylus avellana*, *Crataegus monogyna, Tilia cordata*
Saint Jean de Luz	Lapurdi (France)	43º 24´34''N 1º37´23''W	7.6	*Corylus avellana, Fraxinus latifolia*,
Sobrón	Alava	42º45´36''N 3º05´38'' W	8.0	*Arbutus unedo*, *Corylus avellana*, *Quercus ilex*,
Valle de Mena	Burgos	43º9'33,3'' N 3º13'38,7'' W	7.6	*Corylus avellana*¸ *Crataegus monogyna, Populus nigra*
La Minilla	Sevilla	37º39'34'' N – 37º40'7'' N 6º9'25'' W - 6º10'9'' W	7.3	*Alnus glutinosa, Fraxinus angustifolia, Nerium oleander*
Río Agrio	Sevilla	37º 30' 47.5'' N 6º 13' 24.6'' W	7.2	*Fraxinus angustifolia*, *Ficus carica, Populus nigra*
Casas del Monte M1	Cáceres	40º 13'15.58'' N 5º 58'42.6'' W	6.2	*Fraxinus angustifolia*, *Quercus suber*, *Populus nigra*,
Casas del Monte M2	Cáceres	40º13'15.58'' N 5º58'42.6'' W	5.6	*Fraxinus angustifolia*, *Populus nigra*
Zarza-Granadilla	Cáceres	40º12'31.12'' N 6º0'10.53'' W	5.5	*Fraxinus angustifolia*, *Ficus carica*, *Populus nigra*
El Acebrón	Huelva	37º8'32'' N 6º32'46'' W	5.4	*Ficus carica*, *Pistacia lentiscus, Quercus suber*

Table 2

List of classified species according to location and soil pH

Alkaline ■ Slightly alkaline ■ Neutral ■ Acid ■

	El Chorreadero	Pantano de Hurones	El Bosque	Saratxo	Peña Angulo	Cadagua River	La Concha	Casalarreina	Peñalabros	San Jean de Luz	Sobrón	Valle de Mena	La Minilla	Agrio river	Casas del Monte (M 1)	Casas del Monte (M2)	Zarza Granadilla (M 13)	El Acebrón	Total per each species
Acaulospora sp. 1																■	■		1
Acaulospora sp. 2						■													1
Acaulospora sp. 3									■										1
Acaulospora sp. 4													■						1
Ambispora sp.	■					■													2
Claroideoglomus claroideum	■			■				■	■			■							5
Claroideoglomus etunicatum				■				■											2
Claroideoglomus sp. 1									■										1
Claroideoglomus sp. 2									■										1
Diversispora sp	■																		1
Entrophospora infrecuents											■								1
Non described species		■																	1
Funneliformis coronatus																	■		1
Funneliformis mosseae		■															■		2
Funneliformis sp. 1											■								1
Funneliformis sp. 2		■																	1
Funneliformis sp. 3													■						1
Gigaspora margarita																■			1
Glomoide 1									■										1
Glomoide 2									■										1
Glomoide 3												■							1
Glomoide 4										■									1
Glomoide 5										■									1
Glomus badium	■			■	■	■		■	■		■	■							8
Glomus intraradices		■								■									2
Glomus macrocarpum				■															1
Glomus magnicaule type				■							■								2
Glomus microagregatum							■												1
Glomus rubiforme	■	■							■				■			■	■		6
Glomus sp. 1	■																		1
Glomus sp. 2	■																		1
Glomus sp. 3	■																		1
Glomus sp. 4	■																		1
Glomus sp. 5		■				■													2
Glomus sp. 6		■																	1
Glomus sp. 7							■												1
Glomus sp?. 8 iregulare type			■																1
Glomus sp. 9									■										1
Glomus sp. 10															■			■	2
Glomus sp. 11														■					1
Glomus sp. 12				■															1
Glomus sp. 13								■	■										2
Glomus sp. 15									■										1
Glomus sp. 16									■										1
Glomus sp. 18									■										1
Paraglomus ocultum									■										1
Scutellospora sp. 1																■			1
Scutellospora sp. 2									■										1
Septoglomus constrictum				■				■											2
Septoglomus sp.											■								1
Claroideum type						■	■								■				3
Constrictum 1 type						■													1
Etunicatum type					■														1
Intraradices type									■						■	■			3
Ocultum type							■												1
Tricispora nevadensis																■		■	2
Total species per location	9	7	1	7	2	6	4	5	16	3	5	3	3	1	3	6	3	2	56

ered an index of the high quality of the ecological values of the considered wild grapevine populations.

AM community composition presents a marked distribution influenced by the pH of the soils. In the cases of *Tricispora nevadensis, Acaulospora sp*. 1, *Gigaspora margarita, Scutellospora sp.* 1 and *Glomus sp*. 10 were distributed in acid soils, as it was cited for the most relevant species, such as *Tricispora nevadensis* and *Gigaspora margarita* (Castillo *et al.* 2006). In the case of other taxa determined, a clear link with the alkaline characteristics of the soils is revealed. On the other hand, *Acaulospora sp.* 4, *Funneliformis sp.* 3 and *Glomus sp.* 11 are found in a slightly alkaline soils, around pH 7 and the *claroideum* type within a pH range from neutral up to alkaline. *Glomus rubiforme*, *and Funneliformis mosseae* and *intraradices* type are present both in acidic soils and alkaline, so pH is not a limiting factor for the presence of such fungi. In the context of the present paper, *Paraglomus ocultum* was observed in alkaline soils, but it is much more abundant in acid soils. These study only shows the most generalist species which had sporulated at the same time. It is necessary to add the limited sample available for taxonomic approach and for multiplication with subsequent trap plant that did not show the expected real potential of biodiversity. This may be due to the nature of the used substrate mixture, inappropriate because it lacked the natural soil from each population.

In the case of the less cited species (at least in the studied locations) is very important to note that some taxa such as *Tricispora nevadensis*, *Gigaspora margarita*, *Glomus*, similar to those from the *magnicaule* type, highlighting the undescribed species belonging to the Pantano de los Hurones and a variety of taxa identified only up to genus level. They could not be studied further because of the low number of spores and degraded state of some specimens in the initial samples. Their multiplying was not possible using trap plants. It could be necessary for to obtain a large number of individuals of the same species that allow obtaining pure cultures able to be used for a more rigorous study.

Regarding the distribution of species is to highlight that *Glomus badium* is the most generalist species, isolated from 8 locations from a total number of 18 and *Glomus rubiforme* represented in 7 locations. *Claroideoglomus claroideum* was present in 5 locations and *Funneliformis mosseae* in 3 locations with alkaline soils. *Glomus* is a common genus in Mediterranean soil (Azcón-Aguilar *et al.* 2003, Ferrol *et al.* 2004 and Alguacil *et al.* 2009).The most diverse populations were Peñaladros, with 16 species and the lowest diversity was found in Agrio River and El Bosque with only one species observed. AM-wild grapevine root association is shown in the Figure. All these results are important in the context of conservation of mycorrhizal diversity as a component of the programmes for propagation and conservation of *Vitis vinifera* L. ssp *sylvestris*. Presumably these fungi play an important role in the survival of these plants in their natural habitats.

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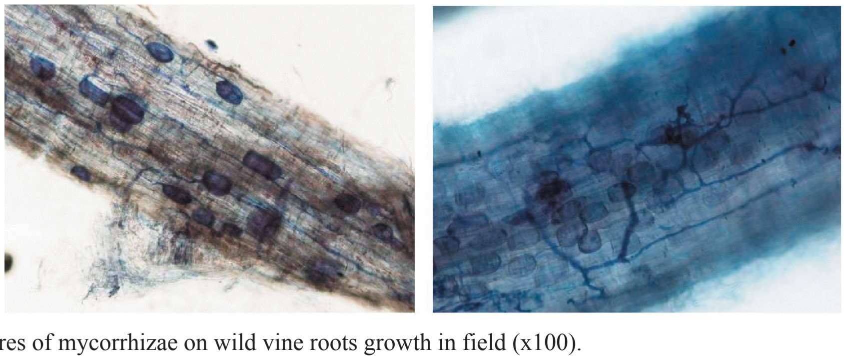

Figure: Typical structures of mycorrhizae on wild vine roots growth in field (x100).

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