

ECPGR Activity Grant Scheme – Phase X - Sixth Call

Activity Report

Increasing the efficiency of conservation of *Vitis sylvestris* genetic resources in Europe

(Sylvestris)

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Front cover photograph: Group photograph on Mount Lekani, Greece, while visiting the occurring *V. sylvestris* natural populations. Courtesy of G. Merkouropoulos (ELGO-DIMITRA).

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INTRODUCTION

Vitis vinifera spp. *sylvestris* Gmel. (hereafter: *V. sylvestris*), the wild grapevine, is the ancestor of the cultivated one; in recent times, it has attracted immense research interest in terms of location of natural populations occurring in Europe, their classical and molecular ampelographic description, and evaluation of the end product, wine. A previous ECPGR Activity entitled 'Increasing the efficiency of conservation of wild grapevine genetic resources in Europe' (InWiGrape), defined the basic guiding principles for the identification and conservation of *V. sylvestris* (Zdunic et al., 2017, Guiding principles for identification, evaluation and conservation of *Vitis vinifera* L. subsp. sylvestris. *Vitis* 56, 127–131. DOI: 10.5073/vitis.2017.56.127-131). As a result, a map showing the localized natural populations of *V. sylvestris* was developed (Figure 1).

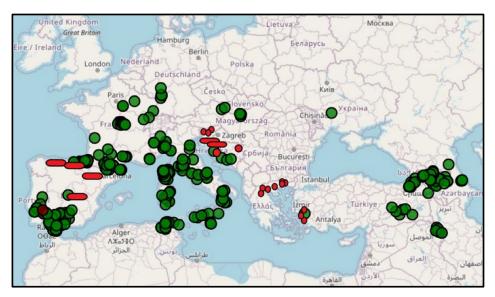


Figure 1. Localization of the V. sylvestris natural populations. Newly identified sites are indicated by red circles or ovals (for multiple sites in a region). [Original figure from the "The European Vitis Database" – http://www.eu-vitis.de/index.php]

The idea to continue the common efforts in this field emerged during the workshop 'Sylvestris' held at the XIII International Symposium on Grapevine Breeding and Genetics in Landau, Germany (2022). There it was suggested to propose a continuation of the InWiGrape Activity focusing on attracting country partners that had not participated in InWiGrape, and to discover *V. sylvestris* natural populations in their territory; in parallel, an update on the InWiGrape results would also be possible. ECPGR provided the perfect platform to host this activity again thanks to an existing group of experienced people who had successfully cooperated in the recent past. Their expertise and know-how could also guide and demonstrate procedures to newcomers.

The proposal for the 'Sylvestris' Activity was submitted to the ECPGR Phase X Sixth Call for Proposals launched in November 2022, and accepted by the ECPGR Executive Committee in December 2022. Eventually, 32 partners from 16 European countries participated in the Sylvestris Activity (Table 1). As was first pointed out, special care was set on attracting partners from countries that had not participated in the previous wild grapevine activity. Such countries were: Georgia, Greece, Italy, Romania, Slovenia, and Türkiye. Noteworthy to mention is that (four) new institutional members were registered in the ECPGR *Vitis* Working Group in order to participate in Sylvestris.

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Table 1: Participant countries and institutional partners							
Partner ID No.	Country	Participants	Institute	Participation in the in-person meeting (in grey tone)	Contribution of each participant country		
1	Albania	Frida Çarka	Institute of Plant Genetic Resources, Agricultural University of Tirana				
2	Armenia	Kristine Margaryan	Institute of Molecular Biology NAS RA				
3	a	Edi Maletić	University of Zagreb, Faculty of Agriculture		105 individuals: Paklenica (34), Imotski (21),		
4	Croatia	Goran Zdunic	Institute for Adriatic Crops and Karst Reclamation		Lukovdol (20), Psunj (28), Gizdavac (2).		
5	Cyprus	Savvas Savvides	Agricultural Research Institute				
6		Thierry Lacombe					
7	France	Valérie Laucou	Institut Agro Montpellier & INRAE, Montpellier				
8	Georgia	David Maghradze	Scientific Research Center of Agriculture				
9		Shengeli Kikilashvili	Caucasus International University (CIU)				
10	G	Erika Maul	Julius Kühn-Institut (JKI) - Federal Research				
11	Germany	Franco Röckel	Centre for Cultivated Plants Institute for Grapevine Breeding Geilweilerhof				
12	Greece	Georgios Merkouropoulos	Hellenic Agricultural Organization DIMITRA (ELGO-DIMITRA), Institute of Olive tree,				
13		Dimitrios Taskos	Subtropical crops, and Viticulture, Department of Vitis		About 150 individuals at 8		
14		Evangelia Avramidou	Hellenic Agricultural Organization DIMITRA (ELGO-DIMITRA), Institute of Mesditerannean Forest Ecosystems		sites in northern Greece		
15	Italy	Gabriella De Lorenzis					
16		Osvaldo Failla	Università degli studi di Milano				
17		Maria Antonietta Palombi	CREA – Research Centre for Viticulture and Enology				
18	North Macedonia	Klime Beleski	Institute of Agriculture, Ss. Cyril and Methodius University in Skopje				
19	Portugal	Jorge Cunha	National Institute for Agrarian and Veterinarian Research, I.P. (INIAV)				
20		Lucia Cintia Colibaba	University of Life Sciences Iasi (IULS)				
21	Romania	Anamaria Mirabela Dumitru	National Research and Development Lrstitute for Biotechnology in Horticulture, \$tefanesti -				
22		Andreea Elena Manolescu	Arges				
23	- Serbia	Dragoslav Ivanišević	University of Novi Sad, Faculty of Agriculture Institute for Fruit Growing & Viticulture		One population in the		
24		Dragan Nikolić	University of Belgrade, Faculty of Agriculture		National Park Tara (Rača, Rastište)		
25	Slovenia	Anastazija Jež Krebelj			New individual		
26		Katja Šuklje Antalick	Agricultural Institute of Slovenia		populations: Vinje 1, Vinje 2, Vinje 11, Vinje 15.		
27		Stanko Vršič	UC of viticulture and enology				
28		Andrej Perko	University of Maribor, Faculty of Agriculture and Life Sciences				
29	- Spain	Gregorio Muňoz Organero	IMIDRA		508 individuals at 101 sites		
30		Javier Tello	Instituto de Ciencias de la Vid y del Vino (ICVV)				
31	Türkiye	Metin Kesgin			Populations at: İzmir- Gumuldur/Menderes,		
32		Selcuk Karabat	Manisa Viticulture Research Institute		Kusadasi-Merkez / Yenikoy Manisa Spil Mountain,.		

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EXPERIMENTAL WORK: LOCALIZATION AND MICROSATELLITE ANALYSIS OF VITIS SYLVESTRIS SAMPLES FROM NATURAL POPULATIONS

During the preparation of the Sylvestris Activity Proposal, the following experimental targets were defined:

- 1. Localize natural populations of *V. sylvestris* (especially in the countries that participated for the first time)
- 2. Analyze a large number of *V. sylvestris* samples collected from all participating countries, at least, on 20 microsatellite loci, to gather substantial material for a common scientific publication.

An additional third target was to update the existing InWiGrape bibliographic list of scientific publications on *V. sylvestris*.

To assist the newly participating countries in analyzing their sample contributions, Greece and Germany volunteered to perform the molecular work (on ten microsatellite loci, each). The contribution of each participant country is shown in Table 1. Due to practical issues, the Türkiye partner did not contribute samples for the molecular analysis. However, natural populations have been identified over the past three years in the areas of *Manisa-Center* and *İzmir-Menderes-Gumuldur, Aydin* and *Kuşadasi* and they will be analyzed in the future (personal communication on 28/11/2024).

Upon the completion of the experimental work, the German partner (Dr Franco Röckel) dealt with nearly 3,000 unique genetic profiles of *V. sylvestris* samples (collected from bibliography plus 1,460 new ones) from 20 countries, and after "data curation and alleles' sizes standardization according to reference varieties and allelic frequencies distribution patterns, this dataset was used to explore the genetic diversity and population structure of *V. sylvestris* in a broad context. Preliminary results identified a series of clusters of genetically related samples, which mirrored their region of origin and reflected a major geographic substructuring within the explored sites. Additionally, a core collection of *V. sylvestris* genetic resources for targeted preservation across national borders was designed. Altogether, this work provided a global update of the current status of this endangered subspecies, and relevant data for its proper management and conservation" (Franco Röckel communication to the group).

IN-PERSON MEETING: KAVALA (DIKILI TASH), GREECE

A 2-day in-person meeting was held on 10-11 October 2023 in the city of Kavala, northern Greece. A total of 29 partners (25 institutional ones plus two accompanying persons and two local academics: Dr Nikolaos Kontoudakis of the Democritus University of Thrace, and Mrs Nikoleta Symeonidou of the Kalampaki Foreign Language Center) participated in the meeting.

The first day of the meeting was held in the Archaeological Museum of Kavala in cooperation with the Ephorate of Antiquities of Kavala (Ministry of Culture), attracting members of the very active local archaeological community who delivered three short lectures emphasizing the history of the region and especially the nearby neolithic settlement of Dikili Tash, which is, so far, the oldest site in Europe showing evidence of grapevine cultivation and wine production, using *V. sylvestris* berries. These lectures were followed by 14 presentations by the Sylvestris Activity participating countries/partners (Figure 2 and Table 1).

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During the second day of the meeting, participants went on an excursion to visit the natural populations of *V. sylvestris* that had been discovered in the mountains surrounding Dikili Tash: Mount Lekani and Mount Pangaion. In total, three natural populations were visited: two populations on Mount Lekani and one population on Mount Pangaion.

On the way from Mount Lekani to Mount Pangaion, the group stopped to visit the neolithic settlement of Dikili Tash. The Head of the Ephorate of Antiquities of Serres, Dr Dimitria Malamidou, gave an outdoor tour of the settlement before introducing the group to the local storage room where all the 6,300-year-old archaeological findings are stored (Figures 2 and 3). It is noted that both the neolithic settlement and the storage room are not yet available to the public: they were opened only for the highly specialized ECPGR Sylvestris scientists. In the afternoon, the group visited the *V. sylvestris* natural populations on Mount Pangaion and the excursion was completed by visiting the nearby Monastery of Eikosifoinissa (Figure 4), established in 400 BCE.

CONCLUSIONS

Upon the completion of the ECPGR Sylvestris Activity the following tasks have been completed (as scheduled):

- 1. Multiple natural *V. sylvestris* populations have been localized in the countries that participated for the first time in such activities several individuals or small populations have been localized around the Aegean Sea (northern part of Greece, western coast of Türkiye). The older country members have localized additional populations, especially in the Balkans (eastern coast of the Adriatic Sea and the northern and eastern part of Iberia) (Figure 1 and Table 1).
- 2. Molecular analysis has been performed on 20 microsatellite loci and the outcome will be presented in a forthcoming joint publication. Preliminary results have already been presented at the 45th World Congress of Vine and Wine (October 2024) by Franco Röckel¹ (Röckel *et al.*, 2024).
- 3. An update of the InWiGrape V. sylvestris publication list has been performed (available here).
- 4. Six member countries that did not participate in InWiGrape, did participate in Sylvestris. In addition, four new members have become part of the ECPGR *Vitis* Working Group.

¹ F Röckel, K Margaryan, G Merkouropoulos, V Laucou, G De Lorenzis, J Tello, G Zdunić, M De Andrés, F Baeta, J Cunha, O Failla, MS Grando, J Ibáñez, T Lacombe, L Marinov, G Muñoz, G Pellissetti, G. S Savvides, A Schneider, D Taskos, I Uzun, D Ivanišević, E Maletic, A Perko, MT Rabanus-Wallace, S Vrisic, R Töpfer, E Maul (2024) A population genetic study of Vitis vinifera L. subsp. sylvestris Gmelin based on 3.000 individuals from 20 countries. 45th World Congress of Vine and Wine. DOI: https://digital.csic.es/handle/10261/379119

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Figure 2: Photograph of the outdoor tour in the Dikili Tash neolithic settlement.



Figure 3: Photograph of the indoor tour in the Dikili Tash storage rooms.



Figure 4: Photograph of our last visit: the Monastery of Eikosifoinissa on Mount Pangaion.