

Enhancing quality and quantity of *Vitis* genetic resources in AEGIS

(AEG-VIT-IS)

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Image. From left to right: 1) Drawing of Melonera (prime name "Rayada Melonera") described for Andalucía/Spain by Simón de Rojas Clemente y Rubio in 1807; 2) Photo of Corropio (prime name "Rayada Melonera") cultivated in Alentejo/Portugal. Source (1 and 2): Maul, Erika & Toepfer, Reinhard. (2015). *Vitis International Variety Catalogue (V IVC): A cultivar database referenced by genetic profiles and morphology*. BIO Web of Conferences. 5. 01009. 10.1051/bioconf/20150501009; 3) Albaranzeuli variegato. Source: AKINAS. 2017. Uve di Sardegna

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INTRODUCTION

Background

AEGIS is an integrated system for plant genetic resources for food and agriculture in Europe, conserving genetically unique accessions that are important for Europe and making them readily available and easily accessible for breeding and research. During the ECPGR workshop held in Madrid at the end of 2018, aimed at assessing procedures to strengthen AEGIS, the slow progress of AEGIS was noted based on the low number of accessions flagged in EURISCO as part of the “European Collection”. The European Collection should substantially increase its size, the quality standards are expected to be agreed upon for most crops and the blueprint of a quality monitoring system should be tested.

Currently, the number of total European accessions is 59,684 (56,730 when this activity started), but only 156 belong to the genus *Vitis* (145 before this activity), flagged for AEGIS by 5 countries (8 from Albania, 90 from Croatia, 3 from the Czech Republic, 36 from Germany, 13 from Italy and 6 new accessions from Slovenia). A joint action of the ECPGR *Vitis* Working Group was deemed necessary to identify and raise the number of unique *Vitis* genotypes AEGIS flagged in EURISCO with harmonized and quality criteria based on the existing European *Vitis* Database (EVDB). A total of 24,039 prime names from *Vitis* accessions mostly conserved in European repositories are cited in the *Vitis* International Variety Catalogue (VIVC). Misnaming in the worldwide grapevine collections is estimated to be between 5% and 10%. Historically, many grapevines identical cultivars were named differently, or distinct grapevines were known under the same or a similar name. There are still unsolved identities, and the difficulties caused by synonyms and homonyms are unexpectedly high.

Expected outcomes according to ECPGR objectives

The efficient conservation and access to unique germplasm in Europe through AEGIS and the European Collection is clearly addressed in ECPGR objective 1 in the current phase X (2019–2023). Some activities within objective 1 are fully or partly under the responsibility of Crop Working Groups (i.e. 1.2.2 “Verification of the European Collection by crops in terms of representation of the *ex situ* PGR diversity”, 1.6.2. “Standards: agree on crop-specific genebank standards” and 1.8.2. “ECPGR-mediated characterization, evaluation and/or phenotyping/genotyping of AEGIS accessions”) and provide relevant information for other activities as 1.2.1: “Identification of new European Accessions for inclusion into AEGIS”. Expected outcomes in this activity are:

- (1) Increase the number of *Vitis* candidates to AEGIS by identifying and selecting unique genotypes and then propose them to national coordinators for inclusion into AEGIS.
- (2) Improve the quality system by implementing operational genebank manuals in a higher number of *Vitis* repositories.
- (3) Enhance the preservation of AEGIS accessions through the identification and implementation of safety duplicates, particularly of the most threatened genotypes.
- (4) Make AEGIS better known and more accessible to potential members.

Partners contribution

Partners preserving public grapevine collections with unique genotypes and located in countries with important wine-growing regions showed interest in carrying out the proposed Activity.

Partners with long experience in recovering and managing *Vitis* genetic resources offered their expertise to establish common guidelines for AEGIS development in *Vitis*.

The role of every partner in the Activity is to share ampelographic and molecular data (in particular data acquired during previous European projects GenRes01, GrapeGen06, Cost FA1003) to search unique genotypes and clarify unsolved identities. All partners will contribute to elaborate a list of safety

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duplicates in their repositories to make AEGIS Quality System (AQUAS) operational, and they will review and update the standards for the conservation of *Vitis* accessions.

Partners from Albania, Austria, Croatia, Cyprus, the Czech Republic, France, Georgia, Germany, Greece, Italy, Latvia, Montenegro, North Macedonia, Portugal, Serbia, Slovenia, Spain and the United Kingdom have participated in this Activity.

MATERIAL AND METHODS/APPROACH

The following tasks were implemented:

1. Establishing common rules for selecting candidate genotypes within each repository.

2. Searching unique genotypes candidates for AEGIS.

Each partner created a list of candidates in their own repository, considering ampelographic, molecular and phenotypical minimal descriptors selected within the framework of previous EU projects (GenRes081, GrapeGen06, COST FA1003) based on the European *Vitis* Database.

3. Selection and clarification of unsolved identities.

In order to identify the Most Appropriate Accessions (MAA) to be AEGIS-flagged in EURISCO, the candidate genotypes have been checked for their varietal identity. Accessions of unsolved identity will be analyzed, sharing characterization data from all partners and examining information in the literature to classify accessions as true-to-type, synonyms or misnames. Within the scope of the EC-funded project GrapeGen06 (WP5) and the COST Action FA1003 (WP1), procedures to identify questionable accessions were developed. Genetic profiles encompass the 9 GrapeGen06 SSR markers: VVS2, VVMD5, VVMD7, VVMD25, VVMD27, VVMD28, VVMD32, VrZag62, VrZAG79.

An Excel sheet with questionable genotypes was prepared by each country. Alleles were coded in a common file and then a search for matching genetic profiles was conducted.

4. Safety duplications in repositories

Comparison of information among all partners will allow the detection of safety duplicates. A list of current duplicates will be elaborated and locations in other repositories for candidate accessions not duplicated will be proposed. Minor varieties or accessions at a higher risk of extinction will be marked as a priority. To preserve intra-varietal diversity, synonyms of rare, threatened varieties will be preserved into AEGIS instead of being discarded.

5. Review of standards and implementation of operational manuals.

Vitis-specific genebank standards have been reviewed, updated and integrated to facilitate the implementation of operational genebank manuals in *Vitis* repositories. Several standards have been suggested so far (FAO field genebank standards, standards for the conservation of *Vitis* accessions, three protocols for germplasm sustainable conservation and a template for the preparation of operational genebank manuals), but only five countries (including two *Vitis* repositories from Switzerland and the Czech Republic) have implemented operational manuals.

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RESULTS

Guidelines for selecting *VITIS* AEGIS candidates

Requirements advisable for *Vitis* candidates:

- a) Name validated
- b) It is important to provide identification of the material as true-to-type, using the VIVC number when it exists, as well as known synonyms.
- c) Legal status
- d) Priority should be given to minor varieties and neglected, endangered and non-registered material because they need special support to be conserved. Each country should pay particular attention to the national and autochthonous plant material.
- e) Safety duplicates. It is recommended to have the safety duplicates stored in a repository from another European country, when possible. It is also advisable to preserve different clones.
- f) Biological category. Among the different categories: landrace (traditional) and wild material should be prioritized over breeding (new) material.
- g) Passport data. In addition to the FAO/Biodiversity Multicrop passport descriptors, specific descriptors for grapevine are published in the European *Vitis* Database and are recommended to be taken into account.
- h) Ampelographic and molecular characteristics. At least a minimal list of morphological characters (GenRes081) and the nine molecular markers of GRAPEGEN06 (VVS2, VVMD5, VVMD7, VVMD25, VVMD27, VVMD28, VVMD32, VrZag62, VrZAG79) should be used. The selected primary descriptors (OIV001, 004, 016, 051, 067, 068, 070, 076, 079, 081-2, 084, 087, 223 and 225) show a good discriminating power between varieties. Pictures of main organs are also suggested to be considered. Agronomical, oenological and other characters are important characters for use in breeding programmes, research, wine or fruit production, and others. Their use should be evaluated in the medium/long term.
- i) Sanitary status for main viruses. It is difficult to have all the plant material virus free in the repositories, and the sanitary status in the field also can change over time. However, it is recommended to know the sanitary status of the material to avoid spreading viruses when multiplying the material.

The list of AEGIS candidates was prepared by some countries and some of them had accessions included in the European Collection before this Activity:

- Albania: 8 accessions (included in AEGIS)
- Croatia: 90 accessions (included in AEGIS)
- Czech Republic: 3 accessions (included in AEGIS)
- Germany: 36 accessions (included in AEGIS), 25 new candidates.
- Italy: 13 accessions (included in AEGIS)
- Portugal: 47 AEGIS candidates.
- Slovenia: 6 genotypes (included in AEGIS), 3 new candidates.

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Searching for unique genotypes and safety duplication

All partners are committed to updating the *Vitis* International Variety Catalogue (VIVC). This catalogue allows locating duplicates in other genebanks and consequently proceed with safety duplication actions. Some examples:

1. Prime Variety Name: Jarrosuelto. Preserved in only one Spanish genebank at the beginning of the Activity. It needs urgent safety duplication in other genebanks (at present, it is conserved in two Spanish collections).
2. Prime Variety Name: Rayada Melonera. Preserved in eight genebanks from Argentina, Brazil, Spain (two holding institutions), France and Portugal (three holding institutions).
3. Prime Variety Name: Saperavi. Preserved in 30 genebanks from Austria, Azerbaijan, Bulgaria, Czech Republic, China, Germany, France, Georgia, Italy, Japan, Moldova, Montenegro, Romania, Russia, Serbia, Spain, Ukraine and USA.

A plan of safety duplication was discussed during the Activity. Most accessions preserved in each genebank have at least one duplicate in another location within the same country. It is recommended to duplicate the accessions at risk of extinction in other countries, for example, 1.

Safety duplication actions and identification of unique genotypes do not end within the Activity period.

Operational Manuals

All partners received the indications and know the procedure to prepare the operational genebank manuals according to the ECPGR template. It was suggested to all members to prepare the manual, including descriptions of the current routine genebank management procedures and practices in each institution holding *ex situ* collections.

The three protocols for germplasm sustainable conservation of *Vitis* should also be considered to improve the genebank management operations:

- Field genebank standards for grapevines (*Vitis vinifera* L.)
- *Vitis* spp. operational field genebank collections manual
- Phytosanitary rules for grapevine (*Vitis vinifera* L.) propagation material introduction into EU for germplasm conservation and scientific purposes.

These protocols are part of the results of the COST Action FA1003 (2015) and were formally endorsed by the *Vitis* WG.

Each partner will develop the document in the short or medium term. As a first result, the operational genebank manual of the Julius Kühn-Institute Geilweilerhof (Germany) was finalized and published on the ECPGR website in February 2021. It is the first operational manual of a *Vitis* genebank in AEGIS.

This is the first step to ensure transparency of operation among the AEGIS partners and start identifying quality management aspects that may need improvement, as recommended by the AEGIS Quality System (AQUAS) to assure an appropriate quality of the activities in AEGIS.

Unsolved identities

In grapevine collections, the assessment of trueness to type is a long-lasting challenge. It is crucial to determine the true identity of misnomers (i.e. material with a name that is incorrectly applied) and identify synonymies and homonymies.

Vitis vinifera accessions that are supposed to be traditional varieties of the country deserve the highest priority towards a correct identification. Other questionable *Vitis vinifera* accessions from other countries were also considered.

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A total of 284 accessions from 11 countries were studied: Albania (15), Austria (20), Croatia (7), France (30), Germany (21), Italy (49), Latvia (13), Montenegro (53), Portugal (50), Slovenia (5) and Spain (21). Molecular profiles were compared with the databases in the largest European repositories (FRA139, ESP080, DEU098, ITA360, and ITA388). More than 50 genotypes match with other genotypes in a different country. Not all matches were found, most likely because some genotypes are of local importance. A high number of genetic profiles sent by AEG-VIT-IS participants were published before. For that reason, many varieties are already present in the *Vitis* International Variety Catalogue (VIVC). Currently, the study of these results is in progress and is expected to be published during 2022. Then, a list of unique and non-duplicated genotypes will be disclosed.