

THE MANAGEMENT OF *EX SITU* GRAPEVINE GERMPLASM COLLECTIONS

Carmen Florentina Popescu ^{1*}, Adriana Bădulescu ¹, Diana Ștefănescu ², Gerald Călin ²

¹ National Research and Development Institute for Biotechnology in Horticulture Stefanesti-Arges, Sos. Bucuresti-Pitesti, no. 37, Stefanesti, CP 117715, County Arges, Romania

² Museum for Viticulture and Fruit Growing Golesti, Str. Banul Radu Golescu, no. 1, Stefanesti, CP 117715, County Arges, Romania

Abstract

This paper deals with the main issues related to the management of grapevine germplasm collections, meaning specific decisions for: obtaining plant material (accessions), establishing the germplasm collection with this material, applying the specific and adequate technology to ensure survival and preservation of plants, characterization and evaluation of the accessions to identify each individual and determine their usefulness, information and documentation (activities of recording), and finally, germplasm utilization. All these decisions are explained and exemplified by concrete results from grapevine germplasm collection owned by the NRDIBH Stefanesti. A complete characterization of each accession by ampelographic descriptors and molecular methods is essential to evaluate the genetic diversity of Vitis genus and to identify the traits for resistance to biotic and abiotic stresses or for oenologic and marketing importance. Well organized activities, with complete documentation and identification the proper performance indicators are essential for making decisions. The final results are: efficient use of preserved plant material (accessories) in breeding activities, providing the basic material for establishing new vineyards, maintenance and protection of endangered varieties.

Keywords: ampelographic descriptors, documentation, genetic resources, molecular markers, Vitis sp.

1. INTRODUCTION

In any research activity, the need to use a correct and fully characterized biological material requires the existence of germplasm collections in which plant species are preserved safely. The primary objective of conserving genetic resources in germplasm collections is to preserve the genetic diversity of plant species / genera / populations, which may subsequently be available for scientific and economic activities. The importance given by the Romanian state to the conservation, sustainable use of biodiversity and capitalizing the genetic diversity of plant species, as well as the alignment with the legislative requirements in Europe and throughout the world, is demonstrated by national rules aiming to *conserve germplasm resources in order to preserve the national and global genetic patrimony* (Decision no. 27 of January 1994) put into force and updated.

Collaboration at international level to maintain and reevaluate plant genetic resources is a necessity because: a) not all the countries have sufficiently large and adequate capacities to maintain all genetic resources possible to collect; b) regional and international collaborations for the preservation and use of genetic resources ensure the rationalization of the methods for collecting, capitalizing the vegetal material, and maintenance of this material in optimal conditions to express

their traits (the morphological, anatomical, physiological, biochemical and phenological characteristics of plants and their organs); c) ensure international knowledge of genetic resources belonging to a species or genus existing in national / regional collections.

The existence of plant genetic resources at national and international germplasm collections implies an adequate and effective management.

The grapevine, as one of the most important crop, has a very long historic evolution and strongly linked with human society. Today, this crop is represented by a wide range of varieties, diversely expressed at morphological and molecular levels. For grapevine, like any other plant species, the germplasm collections need specific procedures of management for: establishing the collection, collecting plant materials, characterization, evaluation, maintenance and conservation of the accessions into collection, documentation for each accession and distribution of grapevine varieties. This paper presents the main aspects to follow for an *ex situ* grapevine germplasm collection, in accordance to the international rules established by "Vitis Working Group of the European Cooperative Programme for Plant Genetic Resources (ECPGR)" (Maggioni *et al.*, 2013) and "Genebank Standards for Plant Genetic Resources for Food and Agriculture" developed by "Food and Agriculture Organization" (2014). Most information required for managing a grapevine germplasm collection are documented by relevant notes about *ex situ* collection established at the National Research and Development Institute for Biotechnology in Horticulture Ștefănești-Argeș (NRDIBH).

2. MATERIALS AND METHODS

The data presented in this paper are based on, and in accordance with the requirements of international documents elaborated by:

- IBPGR (International Board for Plant Genetic Resources) set up in 1974;
- OIV (International Organization of Vine and Wine) dealing with the technical and scientific aspects of viticulture and winemaking;
- MADR (Ministry of Agriculture and Rural Development) as the national, central public authority responsible for the conservation and sustainable management of soils and of plant and animal genetic resources, and which has the role of developing, implementing and monitoring policies and strategies in this field;
- Cost Action FA1003 "GRAPENET – East-West Collaboration for Grapevine Diversity Exploration and Mobilization of Adaptive Traits for Breeding" (Working Group 1), international project which was finalized by drawing up "Three protocols for germplasm sustainable conservation".

3. RESULTS AND DISCUSSIONS

A. General principles

- The collection of grapevine germplasm consists of the genetically representative material of the *Vitis* genus, which is kept / maintained safely; the genetic integrity of this material is preserved from one generation to the next one by vegetative propagation or by *in vitro* multiplication.
- A collection of germplasm may include: a) old varieties removed from the cultivated assortment; b) traditional varieties, with restricted crop area; c) cultivated varieties and new creations obtained through breeding techniques; d) genotypes, lines, mutants and elites selected in the breeding cycles; e) varieties and wild species closely related to the cultivated ones.
- The fundamental objective of a germplasm collection is to capture the maximum amount of genetic variation in the smallest number of samples and individuals. The culture conditions for the

ex situ germplasm collection must ensure the genetic uniformity and integrity of the biological material from the point of view of authenticity, health status and minimum number of individuals (Marshall and Brown, 1975).

- An *ex situ* collection maintains a vegetal material outside its area of origin, under appropriate conditions to prevent changing of its genetic constitution. *Ex situ* conservation should be considered as a complex process designed to protect endangered plant varieties and maintain them in areas other than those of origin, but as far as possible similar to them.

- Maintaining plant material in the *ex situ* germplasm collection should be considered as a complementary conservation measure and not an alternative to *in situ* conservation. Maintaining a broad range of genetic variants at species, population, individual and allelic level is essential because genetic diversity is the basis for adaptation to the living environment, as well as to future environmental changes.

- Conservation of genetic resources requires a realistic and integrated approach involving theoretical and practical knowledge on: a) the ecology of the species introduced into collection; b) genetic diversity within the group(s) kept in the collection; (c) appropriate methods for the conservation of species/genus in *ex situ* collections.

- The management of *ex situ* collections must ensure: 1. the integrity of the preserved plant material; 2. identifying duplicates or incorrectly registered accessions, for the purpose of maintaining strictly necessary and properly authenticated genetic material; 3. the subsequent regeneration of this material for the purpose of maintaining a minimum number of plants of the same variety within the collection; 4. regeneration of plant material to restore populations in the wilderness, or to supplement populations that are on the brink of extinction; 5. the use of genotypes present in the collection as genetic reference material for the research activities; 6. harvesting of plant material for exchanges with other germplasm collections in the country or from other countries, as well as initial propagating plant material of horticultural, agricultural or pharmaceutical interest; 7. availability for educational and teaching activities as proof of genetic variability within species/genus.

B. The main components of managing an *ex situ* germplasm collection includes specific procedures for: collecting and establishing the collection, characterization and conservation of the accessions and efficient use of plant material.

a) Obtaining genetic material for the germplasm collection

- Any collection of germplasm must have documents attesting the necessity of setting up the collection, its location, the accessions held and all the information underlying the documentation of the existing genetic material in the collection.

- The responsibility to have a germplasm collection lay down in the law of organization and function for the research unit. This document specifies the species that are kept into collection and can be exploited, as well as legal considerations regarding the plant genetic resources in the national and international legislative context. *E.g.* NRDIBH Ștefănești was established and operates on the basis of GD 2113/2004 and, according to Art. 3, A, r, of this law, carries out research and development activities in the field of conservation for grapevine germplasm. According to its own policy, NRDIBH Ștefănești holds genetic material from *Vitis sp.* (*Vitis vinifera* L. *subsp. vinifera* L., and *Vitis vinifera* L. *subsp. sylvestris* (Gmelin) Hegi), as well as interspecific hybrids. Existing and purchased plant material is used to extend the collections for the original material G0 and G1, as well as, to set up the *ex situ* collection.

- For the collection, the plant material of *Vitis* species can be purchased by: a) collecting from different natural areas; b) exchanges of material with other collections of germplasm; c) multiplication from individual plants, initially authenticated and already existing in its own germplasm collections; d) acquisition from other units, researchers, breeders, or individuals.
- When purchasing new samples of plant material, the curator (the person in charge for managing the collection) assigns a unique number to that variety. This unique number represents an information-data for each accession introduced into collection and it is permanent. The curator must check a number of issues, such as: identity of plants, their state of health, and existence of information on how the material was obtained, and whether there are legal restrictions on the use of the purchased genetic material.

Required documents: the law of establishment and function the research unit, a brief description of the mandate of the unit based on which the germplasm collection was organized, own germplasm purchase policy, the representative documents for acquisitions of plant material, the agreements at national or international level, and the species / genus for which these agreements have been concluded, the strategy of missions for collecting plant material.

b) Terms for the establishment the *ex situ* grapevine germplasm collection. When setting up a collection of germplasm, a few rules must be accomplished to ensure minimum conditions for preserving the genetic diversity:

- The agro-ecological conditions (climate, soil) for the location of the *ex situ* collection should be as close as possible to the area of origin of the material introduced into the collection.
- The land on which the collection is located must be a legally guaranteed property.
- The location must be at minimum risk for natural disasters, unfortunate occurrences, or human damage.
- The germplasm collection area must be easily accessible to personnel, supervisors and equipment for maintenance and application of plant protection treatments.
- When establishing a collection, shall be taken into account the genetic diversity of the varieties to be introduced into the collection (those already existing in the area, and those possibly purchased from similar collection holdings).
- Depending on the available area, the number of accessions (varieties, clones, intra- or interspecific hybrids, and species) to be introduced into the germplasm collection and a number of plants from each genotype will be determined.

Required documents: document with legal possession of the land, its geographic position and map of the area for germplasm collection.

c) Conditions for the establishment the *ex situ* grapevine germplasm collection

- Depending on the available area, there are established: the number of accessions, number of plants from each genotype, and is estimated the final number of accessions possible to be preserved.
- In a germplasm collection must be ensured the viability of at least 4 plants of each accession. With the exception of varieties of rootstocks, only grafted plants on the same rootstock will be planted in the collection; the rootstock should be suitable for the soil and to ensure the expression of ampelographic characters for all / most genotypes.
- When setting up the collection, are compulsory to fulfil the health conditions: soil free of dangerous pests and planting of only virus-free plants, according to national legislation and Directive 2005/43 / EC.

- A collection of grapevine germplasm must contain the reference varieties, such as: 'Bicane', 'Cabernet Sauvignon', 'Chardonnay', 'Chasselas blanc', 'Merlot', 'Pinot noir', 'Riesling weiss', 'Rkatziteli', 'Sauvignon'.
- A suitable and convenient cutting system has to be applied. Most often, the Guyot system with a single / double cord is fitted.
- The planting distance must provide sufficient light and promote optimum vegetative development of each plant. Generally, the recommended planting distance is 0.8-1 m between plants, and 2 m between rows.

Table 1.a. Quantity of plant material own by NRDIBH / Type of collection / Preservation procedures for the grapevine genetic resources

Item	Collection type	Characteristics and components	Accessions (no)
G0	Initial plant material for multiplication	Germplasm collection in greenhouse, under controlled conditions / plants grown in individual pots / four (4) plants of each accession	323
G1	Varieties and clones obtained through genetic improvement at the NRDIBH	Germplasm collection in greenhouse, under controlled conditions / plants grown in individual pots / thirty (30) plants of each genotype	21
<i>Ex situ</i>	Grapevine Collection	Collection in the field with: - autochthonous varieties, grown all over in vineyards, international recognized; - autochthonous varieties, major local cultivars, of local importance, but extensively grown; - autochthonous varieties, minor local cultivars, of local importance, fairly used; - autochthonous varieties, local neglected cultivars, at risk of extinction; - wild species related with grown ones; - varieties obtained by genetic improvements and grown on large areas; - areas 0,8 ha; - twelve (12) plants of each genotype	171

Table 1.b. The ex situ collection established between 2016 and 2018, organized to preserve certain type of grapevine varieties

Category / type	Varieties number	Planted in 2017	Planted in 2018
Varieties for white wines	59	42	17
Varieties for flavour wines	6	5	1
Varieties for red wines	31	26	5
Table varieties – seeded	42	28	14
Table varieties – seedless	11	6	5
Varieties and species of rootstocks	11	9	2
Populations of <i>Vitis sylvestris</i>	11	8	3
TOTAL	171	124	47

Required documents: the centralized table with the existing accessions in the collection (e.g. table 1.a and 1.b) and a map with the location of each accession.

d) Storing and conserving germplasm – the objective is to maintain the material viable and genetically intact by controlling growing conditions of the plants. All applied methods should preserve the accessions to the highest standard over long periods of time.

- The conservation conditions (in the greenhouse or in the field) should ensure plant development and optimal growth microenvironment.
- Plants grown in greenhouse, or in the field, should be vigorous and in sufficient numbers to represent the genetic variability of the genus.
- The exact site where each accession was planted should be recorded on a map; the accessions must be identified both in the field, and as plants.
- The plants, as possible, should be protected to prevent pest attacks and diseases.
- Specific activities and adequate treatments should be applied to assure plants viability, their normal development and good growth.
- Regeneration and propagation protocols will be applied to assure the required number of plants from each accession, and to distribute plant material if necessary. Only healthy, true-to-type and vigorous plants should be used to obtain new individuals.

Required documents: annual maintenance and treatment plan, record of work performed and applied treatment dosages.

e) Germplasm characterization – if optimal conditions are ensured, the germplasm has to be characterized and evaluated. Aiming to be used, all accessions preserved into germplasm collection have to be correctly identified, characterized and the usefulness traits specified.

According to Upadhyaya *et al* (2008), the germplasm characterization means the recording of distinctly identifiable characteristics, which are heritable. For grapevine, the standards for characterization and identification were clear defined by Maghradze *et al* (2015) and comprise:

- morphologically aspects according to the 'OIV Descriptor list for grape varieties and *Vitis* species' (2009) with 48 descriptors and applying the standardized methodology reported by Rustioni *et al.* (2014);
- Molecular markers using at least the nine SSR-markers (VVS2, VVMD5, VVMD7, VVMD25, VVMD27, VVMD28, VVMD32, VrZAG62, VrZAG79) recommended by Maul *et al* (2015) and using the details available on the EVDB.

The ampelographic description should be compared with the description from reference literature to obtain a preliminary confirmation of the authenticity of the studied accessions. The molecular analyses results and comparing the obtained data with those already presented in the international databases allows the correct identification of each accession (detection of unique varieties, synonyms, homonyms, accessions incorrectly identified by means of ampelographic descriptors, redundant germplasm).

The complete characterization with standardized descriptors and a set of at least nine SSR markers will allow true-to-type identification. According to Maul *et al* (2012), the identity of an accession requires information and knowledge of two independent methods: molecular data and the morphologic description. Confirmations of identity have to be done by combining: a) ampelographic description on reference materials (accessions from other old collections); b) information on reference description from old documents; c) SSR-marker data sources; d) molecular data and morphologic description on own accessions.

There could be three different situations:

- identical or very similar accession / variety names and identical fingerprints: in this case plant material is true-to-type;
- identical or very similar accession / variety names and different fingerprints (homonymy): will be necessary to clarify which accession is the true-to-type genotype, which accession is the homonyms, and which are misnomers;

- different accession / variety names and identical fingerprints (synonymy): in this case are identified the true synonyms and the misnomers.

Required documents: the file of each variety, which contains: the registration number of the accession in the collection, the VIVC number, the synonymies, the colour of berry skin, use, the ampelographic description (with at least 48 descriptors), the molecular analysis (with 9 molecular markers) and representative photographs (young shoot tip, young leaf, mature leaf, bunch).

f) **Documentation.** Complete characterization and correct identification have to be kept in the informative file. All data about grapevine collection, the accessions descriptions and their possible use are facilitated by an information access system. The own computerized information system and the international databases are valuable tools for dissemination information about germplasm collections and for improvement the efficiency use of the component accessions.

The documentation includes:

- passport and collecting data – with information regarding the place of collection, or possible origin of plant material, data about environmental characteristics of the site of collection, and reference literature. For all accessions should be a file in accordance with the FAO/IPGRI multi-crop passport descriptors. All files are stored and changes updated (Maghradze *et al.*, 2015);
- characterization data with complete description of the accessions at all developmental stages.

The aims of documentations are: a) to centralize information about preserved grapevine accessions and their potential use; b) facilitate breeders' selection of valuable accessions for certain features or for crop improvement.

Required documents: storing information (that mentioned previously); computerized systems to record information in databases; publications, dissemination and knowledge-sharing to promote utilization of the conserved germplasm; analysis, drawing conclusions and management decisions.

g) **Germplasm utilization.** The plant material conserved into a germplasm collection represents a valuable source of diversity from a certain species / genus which are available first of all for researchers in public or private sectors. Plant material from a germplasm collection can be used:

- Directly, as genetic reference material for all research activities, in exchanges with other germplasm collections in our country or in other countries, as propagating material for species of horticultural, agricultural or pharmaceutical interest, as initial plant material for regeneration and restoring populations in the wilderness, or to supplement populations that are on the brink of extinction.
- Grapevine germplasm collections could be a natural platform for educational and teaching activities, with good opportunities for getting practical skills in evaluation the ampelographic descriptors used in the description of the preserved accessions.
- All germplasm should be distributed in compliance with national laws and relevant international treaties and conventions. In this case, the samples should be accompanied by all relevant documents required by the donor and the recipient country. Are required a minimum information including: accession identification and key passport data with ampelographic description.

Required documents: agreements, partnerships, list of plant material used for multiplication, exchanges and distributions.

4. CONCLUSIONS

The management of an *ex situ* germplasm collection is a complex activity, of national importance, with long-term perspective and of great responsibility for the next generation.

All the basic concepts, tools and methods used to establish, maintenance and reevaluate a germplasm collection are necessary to understand and estimate the operational costs. Each research unit having the mandate to hold a plant collection have to consider the following: a) short-term output costs for collection of plant materials and germplasm use (ensuring availability for exchange plant material, providing authorized information about each accession in germplasm distribution); b) long-term output costs for covering genetic variability preservation of the conserved genus, or species, maintaining genetic integrity and ensuring security of plant material in collection. All costs depends on: biological characteristics of the conserved crop (annual, biannual, or multiannual, on own roots / grafted individuals and number of stored accessions), methods used for conservation (field / greenhouse / *in vitro* / low temperature / cryopreservation techniques), institutional capacities (facilities / infrastructure and staff characteristics as number and skills), and environmental conditions for germplasm location.

Very well documented activities (recording, organizing, analyzing) are essential for knowledge about preserved plant material and making decisions on its management. The more we know about grapevine germplasm, the more its value and importance increase.

Characterization together with regeneration demand the highest investment, and these costs depend on the number of accessions and the diversity preserved into *ex situ* collection. Molecular characterization represents, for the moment, the most efficient, rapid, accurate and sensitive way for identification of the accessions, but with high costs. Using correct and complete characterized accessions from the beginning, saving time and labours for their growth, mean in the end to save money. Using molecular methods is possible the identifying of duplications (which are not introduced into collection, or are removed from the collection), and also, certain economically important traits are identified. Thus, although molecular methods (as complementary ones to the conventional methods of description) are expensive, the high degrees of sensitivity, specificity and accurately bring guarantee in authenticity of the accessions and, in the end, result in a more efficient use of germplasm.

Efficient way to maintain and capitalize an *ex situ* collection imposes identifying performance indicators for assure the genetic integrity, security, longevity and availability of the accessions and applying the most efficient decisions.

All activities and decisions in managing the germplasm collections have to take into consideration the national and international rules aiming to maximize efficient use of preserved plant material. A good management of germplasm collection represents a proof of respecting the natural resources, environment, local traditions and culture.

5. ACKNOWLEDGEMENTS

This paper is the result of a collaborative project Sectorial 2 PS – *Research in supporting the development and protection of national patrimony for genetic material from plant crops and animal livestock, traditional and important economically*, aiming to promote knowledge and consensus in using plant genetic resources. As source of information were used documents from the germplasm collection established through ADER 3.1.3. Project.

6. REFERENCES

- Marshall, D.R., Brown, A.H.D. (1975). Optimum sampling strategies in genetic conservation. In Frankel OH and Hawkes JG, eds., *Crop genetic resources for today and tomorrow* (pp. 53-80). Cambridge University Press. Cambridge, UK
- FAO. (2014). Genebank Standards for Plant Genetic Resources for Food and Agriculture. Food Agric. Org. United Nations, Rome. (<http://www.fao.org/agriculture/crops/thematic-siemap/theme/seeds-pgr/gbs/en/>)

- Maggioni, L., Engels, J., Maul, E., Ortiz, J., Lipman, E. (2013). [Report of a Working Group on Vitis](#). In European Cooperative Programme for Plant Genetic Resources (ECPGR), Bioversity International, Rome (Italy). (42 pp.) Second meeting, 18-20 September 2012, Siebeldingen, Germany
- Upadhyaya, H.D., Gowda, C.L.L., Sastry, D.V.S.S.R. (2008). Management of germplasm and their use by mini core and molecular approaches. *Capacity building for risk management systems On genetic resources*, 35-70.
- Maul, E., Sudharma, K. N., Kecke, S., Marx, G., Müller, C., Audeguin, L., Boselli, M., Boursiquot, J. M., Bucchetti, B., Cabello, F., Carraro, R., Crespan, M., De Andres, M. T., Eiras Dias, J., Ekhvalia, J., Gaforio, L., Gardiman, M., Grando, S., Gyropoulos, D., Jandurova, O., Kiss, E., Kontic, J., Kozma, P., Lacombe, T., Laucou, V., Legrand, D., Maghradze, D., Marinoli, D., Maletic, E., Moreira, F., Muñoz-Organero, G., Nakhutsrishvili, G., Pejic, I., Peterlunger, E., Pitsoli, D., Pospisilova, D., Preiner, D., Raimondi, S., Regner, F., Savin, G., Savvides, S., Schneider, C., Simon, S., Staraz, M., Zulini, L., Bacilieri, R., This, P. (2012). The European *Vitis* Database (www.eu-vitis.de) – a technical innovation through an online uploading and interactive modification system. *Vitis*, 51, 79-85.
- Maul, E., Töpfer, R., Carka, F., Cornea, V., Crespan, M., Dallakyan, M., De Andrés Dominguez, T., De Lorenzis, G., Dejeu, L., Goryslavets, S., Grando, S., Hovannisyan, N., Hudcovicova, M., Hvarleva, T., Ibáñez, J., Kiss, E., Kocsis, L., Lacombe, T., Laucou, V., Maghradze, D., Maleti, E., Melyan, G., Mihaljević, M. Z., Muñoz-Organero, G., Musayev, M., Nebish, A., Popescu, C. F., Regner, F., Risovanna, V., Ruisa, S., Salimov, V., Savin, G., Schneider, A., Stajner, N., Ujmajuridze, L., Failla, O. (2015). Identification and characterization of grapevine genetic resources maintained in Eastern European Collections. *Vitis*, 54 (Special Issue), 5–12.
- Maghradze, D., Maletic, E., Maul, E., Faltus, M., Failla, O. (2015). Field genebank standards for grapevines (*Vitis vinifera* L.). *Vitis*, 54 (Special issue), 273-279.
- OIV. (2009). OIV Descriptor List for Grapevine Varieties and *Vitis* species, 2nd ed. O I V (Off. Int. Vigne Vin). Paris, France.
- Rustioni, L., Maghradze, D., Popescu, C.F., Cola, G., Abashidze, E., Aroutiounian, R., Brazão, J., Coletti, S., Cornea, V., Dejeu, L., Dinu, D., Eiras Dias, J.E., Fiori, S., Goryslavets, S., Ibáñez, J., Kocsis, L., Lorenzini, F., Maletić, E., Mamasakhlisashvili, L., Margaryan, K., Mdinaradze, I., Memetova, E., Montemayor, M.I., Muñoz-Organero, G., Nemeth, G., Nikolaou, N., Raimondi, S., Risovanna, V., Sakaveli, F., Savin, G., Savvides, S., Schneider, A., Schwander, F., Spring, J.L., Pastore, G., Preiner, D., Ujmajuridze, L., Zioziou, E., Maul, E., Bacilieri, R., Failla, O. (2014). First results of the European grapevine collections' collaborative network: validation of a standard eno-carpological phenotyping method. *Vitis*, 53 (4), 219-226.