ECPGR Activity Grant Scheme – Second Call, 2015

Activity Report

Increasing the efficiency of conservation of wild grapevine genetic resources in Europe

(InWiGrape)

1 January 2016 – 31 December 2016

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May 2017

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1. INTRODUCTION

The importance of preserving grape genetic resources in Europe was emphasized through several previous initiatives and projects within the *Vitis* research community. Within the EU project GENRES081 (1997-2002), primary and secondary OIV descriptors for morphological description and evaluation of agronomic traits were selected. The importance of preserving old and neglected varieties has been emphasized, while SSR markers were recommended as a complementary method for identification (Maul and This 2008). Later, in the framework of the EU project GRAPEGEN06 (2007-2010), a specific work package (WP4) for the genetic resources of wild grapevine was introduced (Maul et al. 2012). Efforts to preserve wild grapevine continued in the COST FA1003 Activity (2010-2013), which resulted in a series of publications about European wild grapevine genetic resources and collaboration among different research groups (Failla 2015). A perspective platform for wild grapevine management was given by Ocete and collaborators who stated that the Eurasian wild grapevine preservation requires adoption of legal measures to be implemented in formal state legislation (Ocete et al. 2015).

Following these previous works on wild grapevine preservation and evaluation, the *Vitis* Working Group of the ECPGR initiated the "InWiGrape Activity", to harmonize protocols referring to genetic resources of wild grapevine (*Vitis vinifera* subsp. *sylvestris*). We proposed a set of indicators that will assist in identification, preservation and study of genetic resources of wild grapevine, including a minimum set of descriptors for phenotyping and genotyping, as well as vulnerability indicators of populations. To get a clear picture about still existing *sylvestris* populations in Europe, the InWiGrape Activity partners collected bibliography on wild grapevine and produced the current distribution map of wild grapevine.

The InWiGrape Activity proposal, including list of partners, is available from the InWiGrape webpage (http://www.ecpgr.cgiar.org/working-groups/vitis/inwigrape/).

2. MATERIALS AND METHODS / APPROACH

Thirteen institutional partners from eleven European countries participated in the InWiGrape Activity funded under the second call of the ECPGR Activity Grant Scheme. Literature on grapevine genetic resources was available through the European *Vitis* Database[1] generated during previous projects. For characterization and evaluation of wild grapevine 25 characteristics have been proposed: 23 descriptors from the OIV descriptor list for grapevine varieties and *Vitis* species (OIV 2009), and 2 characteristics (colours of leaves in autumn and length of seed beak compared with whole seed length) which are not included in the OIV descriptor list.

[1] http://www.eu-vitis.de/index.php

2.1. InWiGrape partners meeting

During a meeting held in Split, Croatia, in July 2016 (www.ecpgr.cgiar.org/working-groups/vitis/inwigrape), Activity partners discussed several important aspects for the conservation of wild grapevine in order to jointly propose a set of indicators that will help in preservation and study of genetic resources of wild grapevine. The following tasks were considered:

- Task 1: compilation of bibliography/available information on habitats and wild grapevine research,
- Task 2: identification of subsp. *sylvestris* individuals in the wild,
- Task 3: *in situ* and *ex situ* conservation and characterization of agro-biological traits.

3. RESULTS

3.1. Compilation of bibliography/available information on habitats and wild grapevine research

The bibliography about wild grapevine available from usual bibliographic databases (Web of Science, Scopus, etc.) was collected and made available online on the InWiGrape webpage.[2] A first section includes 155 publications examined, studying different aspects, methods and results with a focus on *sylvestris*. The most common aspect was the identification and study of genetic diversity of wild grapevine populations through Simple Sequence Repeats (SSR) markers. A second section records 60 publications produced outside of the traditional academic channels, unpublished articles or materials published in local journals. Such material, although less available to the wider academic community, may provide necessary information, evidence or locations of the wild grapevine and therefore could be very important for preservation. In a short term the 155 articles will be made retrievable from the European *Vitis* Database. An import module will be created for addition of further bibliography.

3.2. Distribution map of wild grapevine

The distribution map of wild grapevine locations was generated on the basis of available GPS coordinates extracted from referred scientific publications and from unpublished data held by partners. A specific geographical module was built and implemented in the European *Vitis* Database by Partner 6 (Julius Kühn-Institut, Germany). The distribution map is available for public access (http://www.eu-vitis.de/index.php; menu Public access > Vitis sylvestris locations) and staying open for continuous updating.

[2] InWiGrape Bibliography (Excel file, www.ecpgr.cgiar.org/working-groups/vitis/inwigrape)

4. RECOMMENDATIONS

4.1. Identification of subsp. *sylvestris* individuals in the wild

Before starting molecular characterization by applying SSR markers, it is necessary to perform morphological evaluation to confirm trueness to type. Morphological identification should be carried out for every individual according to the international format of descriptors for grapevine (OIV descriptors). Very often intruder plants can be found among the wild grapevine individuals in a population. With the utilization of the proposed morphological descriptors it should be possible to discriminate between wild grapevine, cultivated grapevine, hybrids and other *Vitis* species. Table 1 shows recommended OIV descriptors for *in situ* identification of wild grapevine individuals. Morphological identification is recommended to be performed in two steps examining in total 25 characteristics (23 OIV descriptors + 2 characteristics not included in the OIV list). In the first step 10 distinctive OIV descriptors are recommended to determine whether the observed individuals belong truly to *Vitis vinifera* species or not. In the second step 17 characteristics (15 distinctive characteristics + OIV076 and OIV078 already tested in the first step) are recommended to determine whether the observed individuals belong to *vinifera* (synonym *sativa*) or *sylvestris* subspecies. After morphological evaluation, it is recommended to continue with molecular analysis using the nine SSR markers (VVS2, VVMD5, VVMD7, VVMD27, VrZAG62, VrZAG79, VVMD25, VVMD28, VVMD32) agreed in the GENRES081 project as a standard set for grapevine identification (This et al. 2004). Characterization and evaluation based on additional OIV descriptors from the OIV descriptor list (OIV 2009) is recommended to be carried out on accessions deposited in *ex situ* collections (Benito et al. 2017).

4.2. *In situ* preservation

The most efficient way to preserve endangered plant species is to protect their natural habitats and ecosystems. Each country should make efforts to include wild grapevine into their national list of endangered species, following the positive examples of France, Germany and Hungary. ECPGR National Coordinators should possibly support this effort within their respective countries. Researchers working on wild grapevine are in the right position to educate and inform responsible people about the importance of wild grapevine and its preservation. This applies to the people in charge of protected areas, public and private forests, like associations, environmental organizations and similar institutions. In order to prevent losses by fire, cleaning of riversides or other accidents, it is in fact necessary to share information on wild grapevine hot spots with all the potential stakeholders.

For preservation, it is necessary to estimate the degree of sensitivity of each specific population to direct human impact. The following vulnerability indicators (often depending on human activities) should be taken into consideration for *in situ* efficient conservation: distance from roads, distance from villages/towns, number of individuals found destroyed in a certain time, distance from commercial vineyards, traces of viticulture activity in the past, ratio of female and male individuals, genetic pollution by other cultivated grapevines (e.g. *vinifera* cultivars, hybrids, rootstocks) within the population and population size.

Table 1. Minimal check list of OIV descriptors for morphology evaluation of *sylvestris* individuals in the wild

1st screening: *Vitis vinifera* or other *Vitis* sp.?		**† Expression level for *V. vinifera* L.**
	**OIV001** Young shoot: opening of the shoot tip	Always full open
	**OIV012** Shoot: density of erect hairs on internodes	None or very low
	**OIV016** Shoot: number of consecutive tendrils	Always 2 or less
	**OIV051** Young leaf: color of upper side of blade (4th leaf)	Often green or yellow
	**OIV076** Mature leaf: shape of teeth	Never sharp teeth's (one side concave, one side convex)
	**OIV078** Mature leaf: length of teeth compared with their width	Never very long or very short
	**OIV084** Mature leaf: density of prostrate hairs between main veins on lower side of blade	Rarely none or very low
	**OIV452** Leaf: degree of resistance to *Plasmopara*	Always none or very low
	**OIV455** Leaf: degree of resistance to *Oidium*	Always none or very low
	**OIV461** Degree of tolerance to *Phylloxera* (leaf)	Often high
2nd screening: subspecies *vinifera* (*sativa*) or *sylvestris*?		**† Expression level for *sylvestris***
	**OIV151** Flower: sexual organs	Always dioecious
	**OIV074** Mature leaf: profile of blade in cross section	Often flat or revolute
	**OIV076** Mature leaf: shape of teeth	Often both sides straight
	**OIV078** Mature leaf: length of teeth compared with their width	Often short to medium
	**OIV079** Mature leaf: degree of opening / overlapping of petiole sinus	Always open
	**OIV082** Mature leaf: degree of opening / overlapping of upper lateral sinus	Always open
	**OIV085** Mature leaf: density of erect hairs between the main veins on lower side of blade	Often low
	**OIV087** Mature leaf: density of erect hairs on main veins on lower side of blade	Often low
	***** Colors of leaves in autumn	Always anthocyanin coloration
	**OIV204** Bunch: density	Never dense
	**OIV220** Berry length	Always very short
	**OIV223** Berry: shape	Always round (obloid, globose)
	**OIV225** Berry: color of skin	Always blue black
	**OIV236** Berry: particular flavour	Always none
	**OIV242** Berry: Length of seeds	Often very short
	**OIV243** Berry: Weight of seeds	Always very low
	* Length of seed beak compared with whole seed length	Always short beak

† Expression level for *Vitis vinifera* L. and *sylvestris* estimated as most frequent notation; * characteristics not included in OIV descriptor list

4.3. *Ex situ* preservation

As with other plant genetic resources, particularly with those at risk of extinction, it is necessary to preserve wild grapevine genetic diversity by establishing *ex situ* germplasm collections as a source of material for restoration of plants in the natural habitat, for characterization and other research purposes.

Vegetative (clonal) propagation is preferable because it enables the conservation of the intact genotype of mother plants. Dormant cuttings are preferable material for propagation. As an alternative, green shoots in summer time could also be collected. Generative propagation by seeds is also possible for inclusion into *ex situ* collections. In that case, we recommend checking individuals grown up from seed by an appropriate number of SSR markers, because open pollination entails the possibility of a pollen donor other than *sylvestris*. Propagation from seed is recommended only when vegetative propagation is not possible. Tissue culture can be used for propagation when seeds or cuttings are not adequate (Pence 2010).

After morphological screening *in situ*, the following steps are therefore suggested for the *ex situ* preservation process:

1. Molecular identification – recommended prior setting up *ex situ* collections.
2. Establishing *ex situ* safety duplication sites, to be documented according to Descriptor N. 25 of the FAO/Bioversity Multi-Crop Passport Descriptors V.2.1 (December 2015) – (MCPD) (Alercia et al. 2015). It is recommended to have duplicate collections in botanical gardens or other (public) institutions.
3. The number of plants from each individual should be at least 3.
4. Type of storage (MCPD, descriptor N. 26) – grafting is recommended for ampelographic description. If grafting is not possible during the first year (for example if the diameter of cuttings are too tiny to be grafted), cuttings should be rooted in pots, and grafting made later, when the plants are developed enough. The rootstock to be chosen is up to each collection holder, depending on soil characteristics.
5. If a field collection cannot be established, another type of storage should be chosen (see descriptor N. 26 of the MCPD).

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