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Guiding principles for identification, evaluation and conservation of *Vitis vinifera* L. subsp. *sylvestris*

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Summary

Conservation of grapevine genetic resources is an important and long lasting task. Here, partners of the InWiGrape Activity of the European Cooperative Programme for Plant Genetic Resources have proposed a set of descriptors that will assist in identification, conservation and study of genetic resources of *Vitis vinifera* L. subsp. *sylvestris*. A distribution map of *Vitis vinifera* L. subsp. *sylvestris* populations in Europe was produced, with on-line access through the European *Vitis* Database. The several different aspects of conservation of *Vitis vinifera* L. subsp. *sylvestris* including bibliographical references, identification in the wild, *in situ* and *ex situ* conservation have been discussed. The descriptors and the map will assist different stakeholders, working on biodiversity and ecosystems in more effective conservation of wild grapevine genetic resources.

Introduction

The wild grapevine (*Vitis vinifera* L. subsp. *sylvestris* Gmel.) is a rare and endangered plant subspecies. It is the wild ancestor of cultivated grapevine and it is distributed from Portugal to Turkmenistan and from the banks of the Rhine to the forest of Tunisia (ARNOLD *et al.* 1998). Its preferred habitats are relatively untouched forests with plenty of water nearby. It is a dioecious liana which climbs over

supporting plants in search of better growing conditions. The female plants have very small, loosely clustered blue-black berries with little juice. For wine and grape production, wild grapevine has very limited value today (LEVA-DOUX 1956).

However, the conservation of wild grapevine is very important for several reasons. Populations are on the brink of extinction owing to human activities, such as intensive riverbank and forest management; pathogen spread, which has increased in the last decades, and a demanding reproductive strategy (OCETE *et al.* 2015). Forest communities with wild grapevine usually do not provide favorable conditions for seed germination and natural gene flow between populations (DI VECCHI-STARAZ *et al.* 2009). Many factors constrain wild grapevine regeneration, including: scarcity of light; animal grazing (e.g. deer); snails feeding on the tender plantlets, and the long distance for pollen to be transmitted between plants in such a context. A particular problem for genetic sustainability of wild grapevine is the presence of other *Vitis* species and cultivated grapevine, which are invasive in the natural habitat (ARRIGO and ARNOLD 2007).

Exploring the genetic relationship between the wild ancestor and cultivated grapevines is necessary to understand the domestication process. Thus, the identification of true wild grapevines and their characterization is becoming an increasingly common subject of scientific interest, including for exploring new sources of genetic variation that might be important for plant breeding (THIS *et al.* 2006).

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However very little is known about agro-biological and production characteristics of wild grapevine. The country reports given in the scope of the InWiGrape Activity of the European Cooperative Programme for Plant Genetic Resources (ECPGR - <http://www.ecpgr.cgiar.org/working-groups/vitis/inwigrape/presentations/>) in Split - July 5, 2016 and recent publications (BISCOTTI *et al.* 2015; SCHNEIDER *et al.* 2015; ZDUNIĆ *et al.* 2017) reveal that there is still a considerable potential for detection of further *Vitis sylvestris* populations in the wild which have not been reported and investigated at all. So far descriptors and guidelines for identification of true *Vitis sylvestris* plants, their characterization, evaluation and conservation were not harmonized, but rather conducted in different ways and with varying intensity in the European countries and thus became one of the objectives of the ECPGR InWiGrape Activity.

The importance of conserving grape genetic resources in Europe has been 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 conserving old and neglected varieties has been emphasized, while deploying SSR markers has been recommended as a complementary method for identification (MAUL and THIS 2008). Later, in the framework of 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 conserve wild grapevine continued in the COST FA1003 Activity (2010-2013), which resulted in a series of publications about 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 conservation requires adoption of legal measures to be integrated within formal state legislation (OCETE *et al.* 2015).

Following these previous studies on wild grapevine conservation and evaluation, the *Vitis* Working Group of the ECPGR (www.ecpgr.cgiar.org) initiated the InWiGrape Activity, to harmonize protocols referring to genetic resources of wild grapevine. In this paper, we propose a set of indicators that will assist in identification, conservation 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 *Vitis sylvestris* populations in Europe, partners within InWiGrape Activity compiled a bibliography on wild grapevine and produced the current distribution map of wild grapevine, accessible on-line from the European *Vitis* Database (<http://www.eu-vitis.de/index.php>).

Material and Methods

Sixteen institutional partners from 11 European countries participated in the InWiGrape Activity within the second call of the ECPGR Activity Grant Scheme. Liter-

ature on grapevine genetic resources was made available through the European *Vitis* Database generated during previous projects. For characterization and evaluation of wild grapevine 25 characteristics have been proposed (Table); 23 descriptors from the OIV descriptor list for grapevine varieties and *Vitis* species (OIV 2009), and two characteristics (colors of leaves in autumn and length of seed beak compared with whole seed length) which are not included in OIV descriptor list. 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 conserving and studying genetic resources of wild grapevine. The following aspects were considered: 1) compilation of bibliography/available information on habitats and wild grapevine research, 2) identification of subsp. *sylvestris* individuals in the wild, 3) *in situ* and *ex situ* conservation and characterization of agro-biological traits.


























Results

Bibliography on *Vitis vinifera* L. subsp. *sylvestris*: The bibliography covering wild grapevine available from usual bibliographic databases (VITIS-VEA, Web of Science, Scopus) was compiled. 155 publications were examined studying different aspects, methods and results with a focus on wild grapevine. The most common aspect was the identification and study of genetic diversity of wild grapevine populations using "Simple Sequence Repeats" (SSR) markers. In addition to this list of publications, 60 collected publications were produced outside of the traditional academic channels and included unpublished articles or materials published in local journals. Such material, although less available to the wider academic community, may provide necessary information or evidence of wild grapevine populations' existence and therefore could be very important for conservation. The distribution map of wild grapevine was generated on the basis of available GPS coordinates extracted from referred scientific publications. The full bibliography and distribution map are available from the European *Vitis* Database and will be open for continuous updating.

Identification and characterization of *V. 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, but applying the proposed morphological evaluation it should be possible to discriminate between wild grapevine, cultivated grapevine and other *Vitis* species. The Table shows recommended OIV descriptors for *in situ* identification of wild grapevine individuals. Morphological identification is recommended as a two-step process to examine in total 25 characteristics (23 OIV descriptors + 2 characteristics not included in

Table

Minimal check list of OIV descriptors for morphology evaluation of *Vitis sylvestris* individuals in the wild

1st screening: <i>Vitis vinifera</i> or other <i>Vitis</i> sp.		†Expression level for <i>V. vinifera</i> 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 (4 th 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 <i>Plasmopara</i>	Always none or very low
	OIV455 Leaf: degree of resistance to <i>Oidium</i>	Always none or very low
	OIV461 Degree of tolerance to <i>Phylloxera</i> (leaf)	Often high
2nd screening: subspecies <i>vinifera</i> (<i>sativa</i>) or <i>sylvestris</i>?		†Expression level for <i>sylvestris</i>
	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 flavor	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.

OIV list). In the first step 10 distinctive OIV descriptors are used to determine whether the observed individuals truly belong to *Vitis vinifera* species or not. In the second step 17 characteristics (15 distinctive characteristics + OIV076 and OIV078 already tested in first step) are recommended for determining whether the observed individuals belong to *vinifera* (synonym *sativa*) or *sylvestris* subspecies. Molecular analysis should follow-on from this morphological evaluation, using the 9 SSR markers (VVS2, VVMD5, VVMD7, VVMD27, VrZAG62, VrZAG79, VVMD25, VVMD28, VVMD32) agreed in GRAPEGEN06 project as a standard descriptor set for grapevine identification (THIS *et al.* 2004). Characterization and evaluation based on additional OIV descriptors from the OIV descriptor list (OIV, 2009) should be carried out on accessions deposited in *ex situ* collections (BENITO *et al.* 2017).

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

For conservation, 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.

Ex situ conservation: As with other plant genetic resources, particularly with those at risk of extinction, it is necessary to conserve 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* collection. In this case, we recommend checking individuals grown from seed using an appropriate number of SSR markers, because open pollination allows the possibility of a pollen donor

other than *V. 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* conservation 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). We recommend duplicating 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 small for effective grafting), cuttings should be rooted in pots, and grafting made later, when the plants are sufficiently developed. Rootstock remains at the discretion of the 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).

Conclusions

Wild grapevine (*Vitis vinifera* subsp. *sylvestris* Gmel.) is a valuable and endangered plant deserving full professional attention for conservation in its natural habitats (*in situ*) and in *ex situ* collections. Within InWiGrape ECPGR Activity, partners proposed a set of measures for identification and conservation of wild grapevine genetic resources. A distribution map of wild grapevine populations in Europe was produced on the basis of available bibliographic information. The map is accessible on-line through the European *Vitis* Database. Conservation of wild grapevine genetic resources requires the participation of different stakeholders, including research institutes, public/private forestry institutions/departments, botanical gardens and other state organizations working on biodiversity and ecosystems.

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