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Vitis genetic resources: current challenges, achievements and perspectives

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Summary

Management and knowledge of *Vitis* genetic resources aim to help address the multiple current and future challenges facing the professional sector: i) preservation of wild and cultivated grapevine heritage, ii) clonal selection of existing cultivars, iii) creation of new cultivars by hybridisation or other technologies, iv) progress in fundamental and applied sciences on all viticultural and oenological issues. It is therefore a vast field of study and actions that deals with all taxonomic levels within the *Vitis* genus (subgenus, species and their hybrids, cultivars and their variants, clones), in natural and cultivated environments, on all continents, both for fruit production (wine and table grapes) and for rootstocks. To meet the many expectations, different actors should ideally coordinate the following actions: 1) research and identification of sources of genetic diversity, 2) access, collection and sampling of this diversity, 3) *ex situ* and/or *in situ* conservation, 4) characterization, 5) dissemination of plant material and related information, 6) use of genetic resources by the different applicants. Following this outline, we will try to assess the challenges and achievements over the last 5-10 years, and discuss the perspectives of this disciplinary field taking into account elements of the wine-growing, scientific, institutional and regulatory context.

Keywords

grapevine, germplasm, collection, preservation, characterization, dissemination

Introduction

The *Vitis* genetic resources (VGR) or grapevine germplasm can be defined as “*all plant material of immediate or potential interest for the improvement of cultivated grapevine*” where:

- “*all plant material*” corresponds simultaneously to all taxonomic levels within the *Vitis* L. genus (subgenus, species, subspecies, varieties, clones), to wild and cultivated gene-pools, to traditional and new elite cultivars and to different biological forms (populations, plants, cell cultures, DNA, genes, etc.);

- “*immediate or potential interest*” refers to all the targets of adaptation: maintain grape diversity by limiting genetic erosion; fighting grape diseases; mitigating the effects of climate change; responding to consumer and citizen demands. The interest being for today and for the future;
- “*improvement*” refers to the different strategies available such as re-cultivation of forgotten varieties, acclimatization of foreign varieties, crossbreeding for creating new varieties, bud sport selection, clonal selection and biotechnologies;
- and “*grapevine*” refers to all the uses of this plant: wine grapes, table grapes, raisins, rootstocks and others.

This definition shows that the subject is vast and that VGR are at the heart of the vine and wine sector. To meet the many expectations, different actions must be carried out successively:

1. research and identification of sources of grape diversity;
2. access, collection and sampling of this diversity;
3. *ex situ* and/or *in situ* conservation;
4. characterization;
5. distribution of plant material and related information;
6. use of genetic resources by the different applicants.

Following this outline, we will try to assess the challenges and achievements over the last 5-10 years, and discuss the prospects of this disciplinary field taking into account elements of the wine-growing, scientific, institutional and regulatory context.

Sources of grapevine diversity

Deposits of grape diversity depend on the taxonomic level considered (Fig. 1). For instance, we cannot expect the same genetic and phenotypic polymorphism within one variety than within one species and *a fortiori* within interspecific hybrids. We also have to distinguish between existing and new diversity. The first one, accumulated over a long time span, can be found in natural or cultivated environments (ancient vineyards, trellises in gardens). The new diversity is recently created by breeders (introgression lines, elite cultivars, pre-breeding genitors) or during basic research works (mutants, transgenic lines, NBT). In this context, the challenges are both detecting the potentially useful missing genotypes and protecting the sources and the dynamic processes of di-



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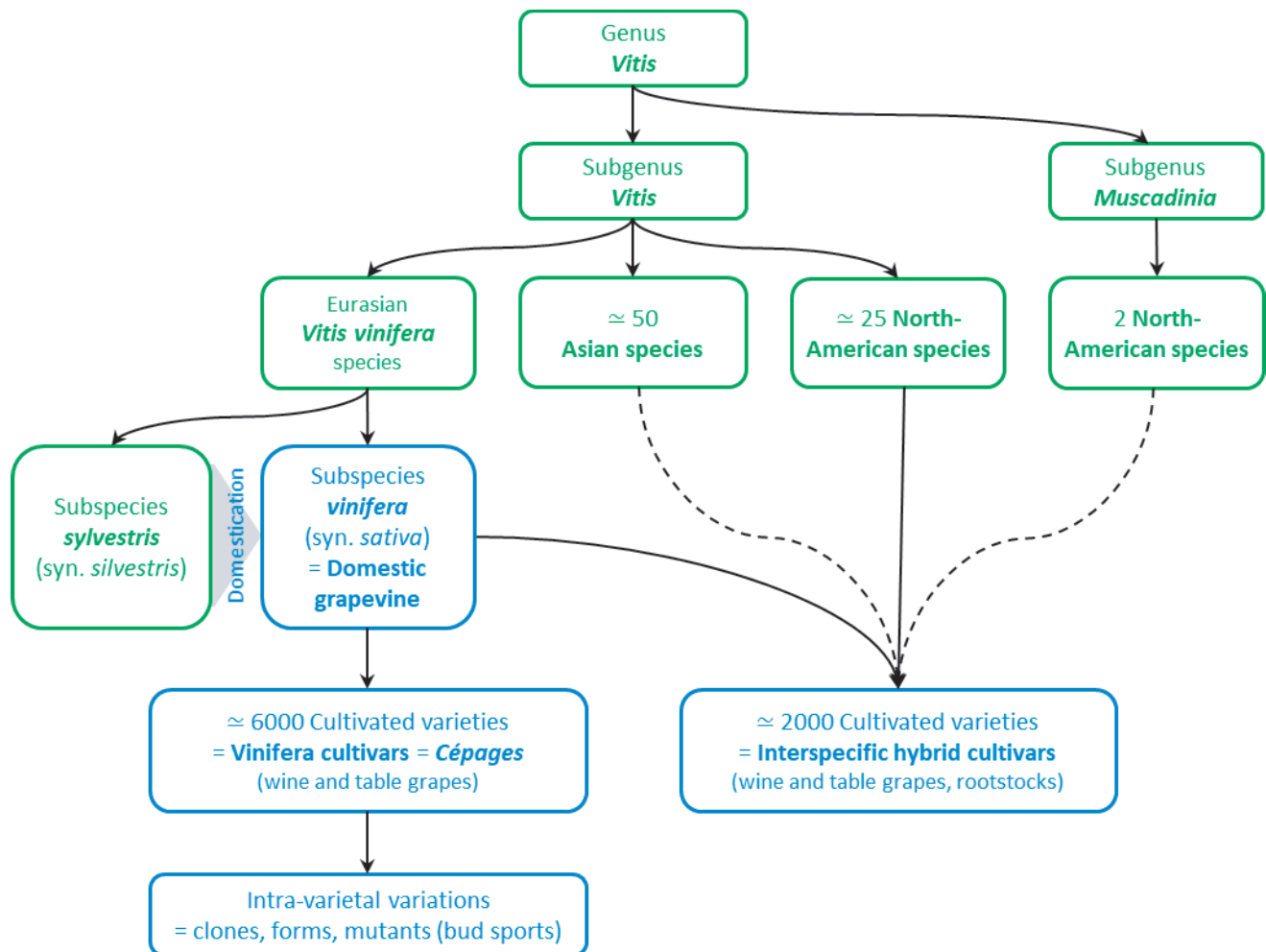


Fig. 1: Schematic taxonomy of the *Vitis* genus. Wild/cultivated compartments are indicated respectively in green and blue. Dashed lines indicate a lower contribution to the interspecific hybrids.

versity creation (*i.e.* natural selection, traditional or modern breeding and biotechnologies).

Many results have been achieved in the last 5-10 years for identifying sources of grape diversity through studies on *Vitis* sp. taxonomy (e.g. Péros *et al.*, 2011; Wen *et al.*, 2018; Klein *et al.*, 2018; Ma *et al.*, 2018; Fan *et al.*, 2018; Zecca *et al.*, 2020; Xu & Xu, 2021; Li *et al.*, 2021; Buck & Worthington, 2022), on diversity structure (e.g. Migicovsky *et al.*, 2017; Laucou *et al.*, 2018; Liang *et al.*, 2019; Ramos-Madrugal *et al.*, 2019; Cunha *et al.*, 2020; Magris *et al.*, 2021), on variety parentage (e.g. Maras *et al.*, 2020; Raimondi *et al.*, 2020; D’Onofrio *et al.*, 2021; Margaryan *et al.*, 2021; Röckel *et al.*, 2021), on clonal diversity (e.g. Gambino *et al.*, 2017; Roach *et al.*, 2020; Calderón *et al.*, 2021; Torres *et al.*, 2022), on mutant genotypes (e.g. Foria *et al.*, 2020; Röckel *et al.*, 2020; Tello *et al.*, 2021) or on phenotyping (e.g. Guo *et al.*, 2019; Gutierrez *et al.*, 2021; Flutre *et al.*, 2022). All these works have increased our knowledge and revealed unexpected VGR such as the cultivars Mgaloblishvili, Orsolina, Cocalona nera, Schwarze Zimmettraube, the Riesling 49 clone, the species *V. californica*, *V. piasezkii*, *V. pseudoreticulata*, etc. They also underlined the still ongoing genetic erosion everywhere in the world.

In the next years, it seems a priority to deepen the studies on taxonomy of *Vitis* species from Asia, Mexico and Central America, on *V. v. sylvestris* at global level, on the parentages of interspecific hybrids and Eastern *vinifera* varieties, on clonal identification and diversity and on nucleotide diversity for identified genes. We also need common monitoring indicators for better estimates of the genetic erosion of each gene pool (Bonneuil *et al.*, 2012). In parallel to this scientific work, we have to promote any action to preserve reservoirs of diversity. Actions on the wild compartment have to include areas still not legally protected, yet sometimes rich. As to the cultivated compartment, the “old vines” initiatives (e.g. South Africa, Australia, Italy) have to be supported and coordinated with other public programmes. Last, breeders and researchers must be encouraged for safeguarding the most strategic material they generated.

Collecting grape germplasm

Based on these sources of diversity, we have to collect the strategic VGR, that is to say finding, sampling and grouping the missing genotypes of interest. Whenever possible, it is obviously preferable to perform original on-site surveys rather than exchanging plant material between repositories. This

prospecting work can be done following a general method that is classical but must still meet the following basic requirements:

- Prioritize actions according to taxa rarity and risk of their loss (Maletic *et al.*, 2015; Maul *et al.*, 2019);
- Preliminary survey (documents, interviews) to determine the best collection sites;
- Obtaining administrative authorizations for sampling and formalities relating to intellectual property rights;
- Effective access to source plots and mapping. Gathering local knowledge from the neighbourhood (owners, growers, elderly people, etc.);
- Inventory and on-site identification (ampelography) of individual vines;
- On-site visual estimation of sanitary status;
- Sampling and marking of individual vines;
- Harvest, tagging and transport of plant material in accordance with national and international procedures in force.

This process is not technically complex, but requires time, qualified staff and adequate financial means.

Recently, different facilitating tools have been released such as grape recognition field guides (Zdunic *et al.*, 2017; André *et al.*, 2020), international models for passport data recording (“MCPD” from FAO also adopted by OIV) or integrated smartphone apps for field recording (GPS, photos, notes, audio). All around the world, several collecting campaigns have been conducted in the last years at different grape taxonomic levels: wild *Vitis* species (e.g. Heinitz *et al.*, 2019; Huerta-Acosta *et al.*, 2022; Mata-Alejandro *et al.*, 2022), *V. v. sylvestris* subspecies (e.g. Naqinezhad *et al.*, 2018; Kupe *et al.*, 2021; Lukšić *et al.*, 2022), traditional *vinifera* cultivars (e.g. Maras *et al.*, 2020; Miazzi *et al.*, 2020; Akram *et al.*, 2021; Margaryan *et al.*, 2021; Zombardo *et al.*, 2021; Gago *et al.*, 2022; Mendoza *et al.*, 2022; Pszczolkowski *et al.*, 2022; Torres *et al.*, 2022; Yilmaz *et al.*, 2022) and clones (e.g. Grigoriou *et al.*, 2020; Gonçalves & Martins, 2022). For the latter level, the low number of academic publications should be seen in the light of the many local private initiatives for mass selection, which also aim to aggregate intra-varietal diversity. These works made it possible to uncover original VGR as in the case of new species or subspecies (e.g. *Vitis shizishanensis*, Ma *et al.*, 2021), presumed extinct varieties (e.g. Citronelle, Plant de Chaudefonds) and unknown varieties, that is to say traditional but yet undescribed varieties. Even if we can take pleas-

ure in the finding of such patrimonial varieties, many are no longer associated with a name or a local empirical knowledge (Table 1) because elderly growers sadly departed without passing on the related information to the next generations. This situation was less common 20 to 30 years ago.

For now and in the near future, we really have to consider the germplasm collecting activity as a race against time and as our major priority: tomorrow it will be too late, tomorrow only exchanges between *ex situ* repositories will be possible. This is the same for the empirical knowledge related to ancient varieties that is disappearing at the same speed and urgently needs to be recorded through ethnobotanical works. In the next years, we also have to recruit and train a new generation of ampelographers, given the shortage of such skills in many grape countries and the retirement of former specialists everywhere in the world. Based on this assessment, OIV launched a new round of international courses of ampelography (2021 in Madrid, 2022 in Montpellier and 2023 planned in Chile). Another perspective would be to benefit from new field tools for immediately identifying grape varieties by image capture, NIRS or DNA analysis. Recent works suggest that this dream is not unreachable. Portable tools allowing viral status testing directly *in situ* during collecting campaigns would also be of high interest. Last, we have to reduce all the difficulties in acquiring new protected varieties, pre-breeding lines and research material both from breeders and researchers; this is achievable through the generalize use of efficient “material transfer agreements” (MTA).

Conservation of grape accessions

The goals of VGR preservation are i) to maintain alive the grape accessions we decided to keep long term via an efficient process of cultivation and regeneration, ii) with a good sanitary and physiological status, a good identity, a good traceability, iii) in compliance with national and international agreements and iv) with the best ratio cost/effectiveness/risk. Different options of conservation are possible according to the specific objectives and the means available (Table 2). These strategies can be conducted at local, regional or national level. Stakeholders can be public, private collective or private individual, and have different professional status (inter-branch organisations, growers, amateurs). They can possibly be grouped into collaborative networks at different levels. In any case, one general question still arises: what to preserve? Indeed,

Table 1: Examples of unknown traditional grape cultivars, i.e. without any name or previous description, found during recent collecting campaigns

Nb cv. total	Nb cv. unknown	Country	Reference
33	10	Italy	(Miazzi <i>et al.</i> , 2020)
39	15	Italy	(Zombardo <i>et al.</i> , 2021)
45	26	Argentina	(Torres <i>et al.</i> , 2022)
18	4	Chile	(Pszczolkowski <i>et al.</i> , 2022)
15	4	Peru	(Mendoza <i>et al.</i> , 2022)
101	51	Montenegro	(Maras <i>et al.</i> , 2020)
221	67	Armenia	(Margaryan <i>et al.</i> , 2021)

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Table 2: Types of *Vitis* genetic resources conservation

Gene pool	<i>In situ</i> conservation	<i>Ex situ</i> conservation
Wild grape relatives	Protection of natural areas	Collections:
Cultivated grapevines	« On farm » preservation	– Vineyard-repository – Insect-proof greenhouse – In vitro culture – Cryopreservation – DNA bank

if the theoretical question “should everything be preserved?” remains open, the practical issue “can everything be preserved?” leads to a negative answer due to the lack of means (funding, staff, etc.). Thus, any conservation action must define its priorities.

It is difficult to determine whether the number of initiatives has been stable or declining in recent years compared to previous decades. Finally, few –yet significant *in situ* initiatives have been achieved on *Vitis sp.* in the USA (e.g. Pavék *et al.*, 2003; Callen *et al.*, 2016; Heinitz *et al.*, 2019), on *V. v. sylvestris* in Europe (Germany, Switzerland, France, etc.), on rare local varieties (ECPGR project “Grape on-farm”, Maul *et al.*, 2019) and on “old vineyards” private projects (Australia, California, Chile, France, Italy, South Africa, etc.). For *ex situ* preservation, at the same time, we had both good and bad news. On one hand, grape genebank standards have been set up (Maghradze *et al.*, 2015), many accessions were sanitized (*i.e.* rendered virus-free) in USA, France, Spain, etc. (e.g. Gisbert *et al.*, 2017) and major online databases have been released for sharing the information related to preserved VGR (e.g. Armenia: <http://www.vitis.am/eng>; Balears: <https://varietatslocalsib.com/ca/webcataleg>; Italy: <https://vitisdb.it>). On the other hand, some field collections have been closed down or are endangered (Butler, 2014); this can be interpreted as a disengagement of the public authorities, as well as an increase in private curators. However, more risk is taken with private initiatives compared to public ones because they usually lack long-term stability. In addition, we can note that conservation networks built in the late 20th century are not active enough, either at national, European or international levels. The same is true for intra-varietal diversity (*i.e.* clones) for which conservation efforts are insufficient, even in major traditional wine-producing countries where this diversity still exists.

One current tendency is to build insect-proof greenhouses to prevent virus infections (e.g. GPGV, GRBV) of healthy grape material by insects or mites. Such projects already exist in France (IFV) and in the USA (UC Davis). Nonetheless, despite the technical and sanitary relevance of this strategy, the issue of investment costs arises when considering its generalisation, as does that of operating costs in a context of global energy crisis. Another technical trend for long-term VGR preservation would be cryopreservation (e.g. in liquid nitrogen at -196°C). Different countries are currently considering this option: Brasil, Croatia, Egypt, France, New Zealand, and the USA (e.g. Markovic *et al.*, 2015; Bi *et al.*, 2017; Haggag *et al.*, 2018; Bettoni *et al.*, 2021). Although the plant material is not immediately available when stored with this method-

ology, it is attractive because the technicality involved and related investments are limited while conservation operating costs are reduced. But for a large-scale implementation, the main obstacle is the staff time needed to incorporate hundreds or thousands of accessions into a cryobank (Ph. Chatelet, personal communication). In any case, this methodology is promising for the sanitation of virus-infected grape accessions instead of alternative sanitation methods such as apex micro-grafting (Pathirana *et al.*, 2013; Markovic *et al.*, 2015). Biotechnologies and related facilities are now sufficiently widespread to consider the creation of a common international grape DNA bank, knowing that today this kind of storage only partially exists in dispersed laboratories without coordination. And indeed it is difficult to create or manage a collaborative network over a long period. Whether it is centralised or distributed (Bonneuil *et al.*, 2007), the network strategy is nevertheless of great interest for genetic resources safeguarding, because it reduces the risks of loss and the costs for each party. Our grape community must therefore make significant and sustainable efforts to strengthen and manage the existing networks. All the actions deployed to achieve the VGR conservation objectives (*ex situ*, *in situ*, networks) obviously require financial resources. These must certainly be appropriate but above all constant (Gepts, 2006), which is hardly compatible with the current principle of project-based research funding.

Characterization of *Vitis* genetic resources

After preservation, the next step is VGR characterisation, which is required for their rational management and exploitation by direct use or crossing. Ideally, this means gathering all the information potentially useful on each grape accession available: identity, taxonomy, pedigree, morphology, anatomy, physiology, sanitary status, agronomic and technologic traits, genetic data and bibliography. In effect, full characterisation of plant material does not only correspond to phenotyping. For an efficient utilization, these scientific and technical data should be “FAIR” that is to say Findable, Accessible, Interoperable and Reusable (Pommier *et al.*, 2019).

During the last decade, this field of research has been very active resulting in many publications (articles, books, websites) and new tools (sensors, dataflow, software) in many countries, dealing with various traits of interest: phenology; fertility; drought, heat or frost resistances; biotic tolerances; berry characteristics; sugar, acidity and polyphenol contents; rooting ability; etc. Summarizing these numerous advances would require another article (for review, see Martinson &

Cadle-Davidson, 2019; Cadle-Davidson *et al.*, 2019; Delrot *et al.*, 2020; Vezzulli *et al.*, 2022). Nevertheless, we can note that a current tendency is to perform VGR characterisation at medium or high throughput, even if low-speed science stays sometimes compulsory for complex traits.

In order to make further progress on this topic, we have first to finalize and stabilize the international harmonization of ontologies and formats for data recording and sharing. This still represents a complex task and we should take advantage of the work already launched by different programmes (e.g. OIV, Integrape, DataCrop, VitisGen2). We also have to pay attention to the many unpublished data that are sleeping in labs. They represent indeed a significant amount of work already produced that can now be more easily exploited thanks to the new online facilities dedicated to data deposits and data papers. These new strategies of data release before publication of the related scientific papers is promising for our grape community that is used to accumulate multi-year field observations. Last, the multiplication of grape databases at different levels (labs, institutes, countries and international) obliges us to build, reinforce or facilitate their interoperability. All the cross-links between normalized ID for plant material, phenotypes and genotypes must be promoted, failing which the different information systems will work separately.

Distribution of grape plant material

VGR distribution requires first to have a large enough quantity of plant material (buds, seeds, pollen, explant, etc.). This means a prior process of multiplication or production that can take time and/or staff. Second, the plant material has to achieve a satisfactory sanitary status to fulfil the national or international official requirements. This calls for testing capabilities; many countries further require a quarantine period before the material can be released. Third, the legal status of the plant material must be considered to properly apply intellectual property principles (commercial vs. research; private vs. public; autochthonous vs. exogenous; etc.). International agreements must be implemented, such as the Rio Convention on Biological Diversity (CBD 1992) and the Nagoya Access and Benefit Sharing (ABS 2010). These three conditions must be met to allow for grape germplasm distribution with the relevant documentation (phytosanitary passport, material transfer agreement, etc.). In conclusion, it is not so easy to send VGR properly.

These rules are obviously justified by the associated sanitary risks and by a moral duty to share the benefits of plant material (Gepts, 2006). But they cause a significant increase in paperwork for each VGR exchange and thus an evolution of the curator skills and workload. Many researchers and breeders are not fully aware of these regulations: there is a clear need for additional information and training. Nowadays there is finally a risk of reducing or blocking VGR exchanges due to this regulatory context, whereas an effective circulation of VGR is necessary for them to provide the expected uses

Uses of grape genetic resources

Expectations regarding grapevine diversity are numerous as they relate to all the objectives of grape breeding (cf. introduction) that do not have the same relative importance in different areas or types of production. To meet these goals, several strategies for using VGR are applied:

- Re-cultivation of local traditional varieties;
- Acclimatization of foreign varieties;
- Bud sport selection;
- Clonal and mass selection;
- Use of biotechnologies for modifying existing varieties;
- Cross-breeding of chosen genitors for creating new varieties.

Not only the goals and the strategies are diverse but also the profiles of the users. In fact, there are sometimes strong debates (scientific, economic, politic or even philosophic) between pros and cons of this or that technique, this or that orientation. And colleagues working on other crops can be surprised by the virulence and precision of controversies in the viticulture sector, and thus on grape plant material. Faced with this multiplicity of objectives, methods and actors, there is only one legal framework (often complex) in each country for allowing the deployment of grapevine varieties and clones. Regulatory procedures exist at international, national and sometimes regional levels for experimentation, naming, protection, registration, classification, multiplication and cultivation of grape varieties. Even if justified for protecting the growers and consumers, this legal framework can place a serious limitation on VGR use.

In this constrained context, we can underline that much has been done for grapevine diversification in the last ten years, for local, foreign and new grape varieties altogether as well as for wine grapes, table grapes and rootstocks. But despite the many varieties recently made available to growers, we can note some paradoxes. The first is the gap between the recent interest in minor traditional varieties (at least in the old World) and their actual use. On one hand, there are many initiatives and communications around these varieties (variously termed ancestral, ancient, native, extinct, historical, local, minor, modest, rare, forgotten, heritage, traditional, old, neglected, endangered, etc.) with a real demand from certain consumers in search of local, original and heritage products (Maul, 2017; Doncieux *et al.*, 2022). For example, this has resulted in the registration between 2012 and 2021 of 39 traditional varieties in the French official catalogue (source: <https://plantgrape.plantnet-project.org>) and 80 in the Italian one (source: <http://catalogoviti.politicheagricole.it/catalogo.php>). But on the other hand, these varieties are very rarely planted (e.g. only 23 ha in total in France) while the share of international varieties is still increasing: 60% of the global vineyard were cultivated with 35 varieties in 2000 and with only 28 in 2016 (Anderson & Nelgen, 2020). A second paradox concerns the narrow genetic basis of newly bred varieties: despite the great number of potential genitors available in grape collections, only a few are actually used in several breeding programmes. For instance, French, German, Italian and Swiss breeding programmes using only four genitors (*i.e.* Bronner, Kozma 20-3, Regent, Solaris) have generated more

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than 30 new cultivars: Artaban, Baron, Cabernet blanc, Cabernet Cantor, Cabernet Carbon, Cabernet Carol, Cabernet Cortis, Cabernet Volos, Cabertin, Calandro, Coliris, Divico, Divona, Fleurtaï, Galante, Garantás, Julius, Merlot Kanthus, Merlot Khorus, Monarch, Muscaris, Opalor, Osella, Pinore, Pinotin, Regent, Sauvignon Kretos, Selenor, Sirano, Soreli, Vi-doc, etc. (source: <https://www.vivc.de>).

In prospect, a continuous and collective work still has to be done for reducing discrepancies at each step between the available grape material and the material actually used by all stakeholders (Fig. 2).

Conclusion

The current challenges regarding VGR seem relatively stable compared to the past decades, since the sanitary crisis of the late 19th century (Hewitt & Rives, 1979), and they remain important. During the last 5-10 years, our scientific and professional community has reached significant achievements, especially in diversity analysis and phenotypic characterisation of grape germplasm. In the near future, we have to focus all together on the *in situ* protection of endangered gene pools at all taxonomic levels and on the actions for collecting the threatened grape diversity. These priorities should be addressed collectively, inasmuch no grape genetics, breeding or selection are possible without genetic resources. The period ahead of us will clearly be favourable to all the characterisation works, thanks to new methods and tools. Further good news is that the context of the sector, from growers to consumers, is finally open again to varietal diversification and changes.

Managing VGR amounts to dealing with a multiplicity of taxonomic levels, varieties, goals, priorities, heritage, traits, stakeholders, strategies, regulations, empirical knowledge, agreements, geographies, threats, publications, expecta-

tions, pools, applicants, genes, data, etc. This requires permanent integration. But to do so today, we don't have to re-invent the existing tools, infrastructures, bodies or methods; we'd better promote their implementation, modernisation and coordination. A key point is the recurring issue of funding, which must be appropriate and above all stable, even if obviously in competition with other grapevine research expenses (e.g. genomics, phenomics, metabolomics). We also have to recruit and train a new generation of young colleagues involved in this strategic and exiting area of research and action.

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Conflicts of interest

The author declare that he does not have any conflicts of interest.

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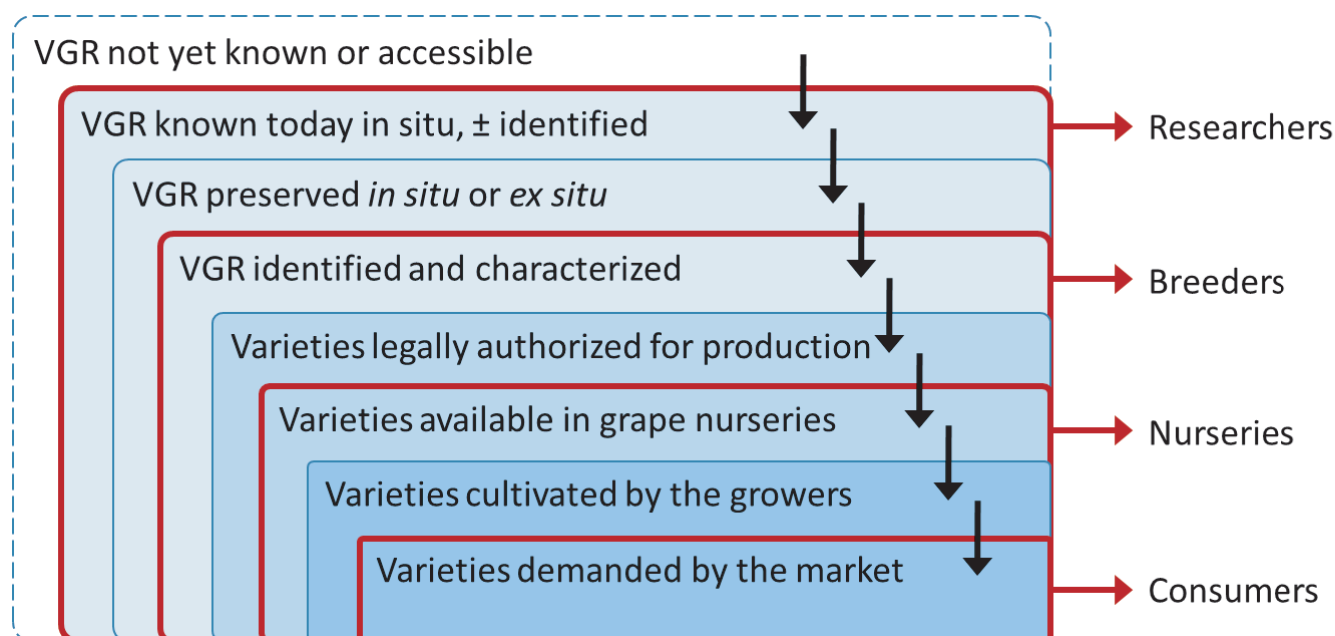


Fig. 2: Gaps between potentially available *Vitis* genetic resources (VGR) and those actually used by grapevine breeding stakeholders, from initial genetic diversity to grape product consumers.

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