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Preserving, improving and rediscovering: The role of the Research Centre of Viticulture and Enology in safeguarding grapevine genetic resources in Italy

Maria Antonietta Palombi [*,a], **Vittorio Alba** [b], **Marco Ammoniaci** [c], **Noemi Bevilacqua** [a], **A Raffaele Caputo** [b], **Roberto Carraro** [d], **Stefano Favale** [a], **Simone Garavelloni** [c], **Massimo Gardiman** [d], **Massimo Morassut** [a], **Marina Niero** [d], **Roberto Nuti** [a], **Giuseppina Pipitone** [d], **Sergio Puccioni** [c] **and Alessandra Zombardo** [c]

[a] *CREA-VE, Council for Agricultural Research and Economics, Research Centre for Viticulture and Enology, Velletri, 00049, Italy*
[b] *CREA-VE, Council for Agricultural Research and Economics, Research Centre for Viticulture and Enology, Turi, 70010, Italy*
[c] *CREA-VE, Council for Agricultural Research and Economics, Research Centre for Viticulture and Enology, Arezzo, 52100, Italy*
[d] *CREA-VE, Council for Agricultural Research and Economics, Research Centre for Viticulture and Enology, Conegliano, 31015, Italy*

Abstract: Grapevine is one the most cultivated species worldwide, with 8,000 estimated varieties. Protecting this biodiversity is of utmost importance, especially in countries historically devoted to viticulture, such as Italy. One of the richest Italian ampelographic collections, spread in different regions from the north to the south of the peninsula, is owned and managed by the Research Centre of Viticulture and Enology (CREA-VE).
Nowadays the collection, thanks to continuous enrichment, consists of more than 3,000 accessions, including not only wine and table grape varieties, rootstocks and other biotypes representing intra-varietal genetic variability of *Vitis vinifera* L., but also other species of the *Vitis* genus. Since 2004, the Italian Ministry of Agriculture, Food Sovereignty and Forestry has financed a specific programme named 'Risorse genetiche vegetali – Trattato FAO (RGV-FAO)' [Plant Genetic Resources – FAO Treaty] to collect, conserve, characterize and document plant genetic resources for food and agriculture.
This paper presents the processes undertaken to enhance the collection, characterize its accessions, preserve and foster the genetic diversity and adaptability in grapevines, with particular emphasis on how this material is managed, evaluated and valorized in terms of different perspectives and practical uses.

Keywords: plant genetic resources, *Vitis* spp, biodiversity, genebank, *ex situ* conservation

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Introduction

Grapevine is one of the most important crop species in the Mediterranean area and its value is not only economic but also historic. Archaeobotanical findings document the presence of wild grapes in the Old Continent since the Neolithic Age (Rottoli, 1993;

*Corresponding author: Maria Antonietta Palombi (mariaantonietta.palombi@crea.gov.it)

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Savo *et al*, 2016). Moreover, there is evidence of grape domestication during the VIII-VII century BCE, confirmed by the discovery of seeds of cultivated varieties in Central Italy (Motta, 2002). In this area, a well-established wine tradition probably began during the Etruscan period and Roman empire (Delle-Donne, 2017), which has continued until the present.

If historical aspects are important, the environmental value of plant biodiversity is especially relevant in marginal areas (Biasi and Brunori, 2015), where autochthonous grapevine varieties are traditionally grown and represent an ecosystem service with agroecological benefits (OIV, 2018; Giffard *et al*, 2022).

It is estimated that there are more than 70 *Vitis* species, with approximately 8,000 different cultivated varieties: 6,000 *Vitis vinifera* L. and 2,000 interspecific hybrids (Lacombe, 2023).

The most recent report from the UN Food and Agriculture Organization (FAO) on the status of global plant genetic resources for food and agriculture indicates that approximately 60,000 accessions of the *Vitis* genus are currently maintained in genebanks worldwide (FAO, 2010). This number will be soon updated since a new report is under revision (document n. CC5227/en).

The objective of germplasm conservation is to safeguard diversity through the implementation of effective techniques that reduce the risk of losses. The sustainable utilization and conservation of plant genetic resources relies on the efficient management of germplasm collections, which is essential to ensure the survival of the resources and their accessibility to relevant stakeholders, including researchers, breeders and farmers (FAO, 2014).

Currently, wine production is highly concentrated in a few grape varieties that dominate the market. It is estimated that in 2016, the top 17 varieties covered half of the world's grapevine planted area (Anderson and Nelgen, 2021), and within these, few clones are in use leading to a strong erosion of grapevine biodiversity.

One of the primary challenges in the conservation of genetic resources is the necessity for long-term commitment and the integration of such activities into continuously funded, non-periodic programmes. The main objective is to guarantee the continued preservation of local viticultural genetic resources, which, regardless of potential commercial interests, represent a heritage of humanity and necessitate the involvement of specialized institutions capable of upholding internationally agreed standards.

In harmony with the Convention on Biological Diversity (CBD, 2005), the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) was adopted by FAO and came into force in June 2004. In Italy, the ITPGRFA was ratified in 2004 with a specific law (L. 06, April 2004, n. 101) and, consequently, all the collections maintained in the country under the supervision of the Italian Ministry of Agriculture, Food Sovereignty and Forestry (MASAF) were rationalized and included in a specific programme for collection, conservation, characterization and documentation of PGRFA, known as RGV-FAO (Vaccino *et al*, 2024). The main objectives are the conservation and sustainable use of agricultural plant genetic resources maintained in Italian repositories, essential for food security and safety.

In this specific framework, the main concern for public research in viticulture is the need to safeguard grapevine biodiversity.

Origin of CREA-VE repository

One of the main *Vitis* collections in Italy is maintained by CREA (Council for Agriculture Research and Economics) at the Research Centre for Viticulture and Enology. The primary, historic core of the ampelographic collection was established in the 1900s (Gardiman and Bavaresco, 2015) in Conegliano (Veneto). Other subcollections in Arezzo (Tuscany), Velletri (Latium) and Turi (Apulia) were included over time as the research centre evolved.

In Conegliano, the repository was founded in 1923 with the establishment of the Experimental Station of Viticulture and Enology, to provide the material necessary for the future work of the station. By the end of 1924, this collection included 350 European varieties, 246 direct-producer hybrids and 65 American rootstocks.

The other collections are more recent; in Velletri (Latium, Central Italy), a collaboration between ARSIAL (Agenzia Regionale per lo Sviluppo e l'Innovazione dell'Agricoltura del Lazio) and the CREA-VE Research Centre started in 1994, with the aim of recovering autochthonous grapevine material present in different area of Latium region. The exploration of the principal grape growing area in Latium contributed to collecting autochthonous varieties and, in 1998, after a minimum characterization and phytosanitary screening of all the plant material collected, an *ex situ* collection was established.

In Arezzo (Tuscany, Central Italy), the grapevine collection was set up starting in 1992. The vineyard covers an area of about 6ha and contains accessions mainly belonging to the autochthonous germplasm of Central Italy, collected and propagated from mother plants found mainly in Tuscany and Umbria. Over the decades, the management of the grapevine collection has been carried out thanks to funds from different regional and national projects.

In Apulia, the first collection was established in 1970 using regional funding and renewed in 2004; currently, it covers an area of about 10ha and includes both wine and table grape autochthonous varieties. During the last decade, the collection has therefore been implemented with additional accessions recovered within the FAO programme for the protection and valorization of genetic resources.

Over the years, the collection has been constantly updated and enriched with new accessions resulting from research in various cultivation areas and exchanges with other national and international institutes. As the

collection has been expanded and enriched over the years, it has also been characterized and rationalized through the identification of duplicates, synonyms, homonyms, and unique genotypic and phenotypic characteristics.

This paper focuses on the various activities carried out to maintain, characterize and utilize the grapevine genetic resources conserved in the CREA-VE ampelographic collection.

Plant genetic resources conservation at Council for Agricultural Research and Economics, Research Centre for Viticulture and Enology (CREA- VE)

Currently, the CREA-VE collection maintains over 3,000 accessions, including distinct species of the genus *Vitis* L., both cultivated and relatives, as reported in Table 1.

An exhaustive list of grape accessions maintained at CREA-VE is available in the European database EURISCO (http://eurisco.ecpgr.org/).

Due to the introduction of new accessions and/or a more in-depth identification of the material collected, the number of accessions reported should be considered very dynamic.

Of the 19 species of the genus *Vitis* conserved in the CREA-VE collection, the most represented is *Vitis vinifera* L.; the other species are exploited primarily to produce rootstocks or as genetic material for breeding. An example of morphological diversity of different species of *Vitis* spp. is shown in Figure 1.

Considering the main use of the accessions maintained at the CREA-VE repository, it is possible to distinguish wine grapes (61.6%), table grapes (17.7%), accessions used both as wine and table grapes (0.6%), rootstocks (5.7%) and grape material not defined (14.4%).

With respect to the biological status of the accessions, we have traditional varieties (70%), advanced varieties (1.5%), and breeding or research material (15%), with 13.5% of the accessions for which the biological status remains not defined.

All accessions are maintained *ex situ* in dedicated vineyards with a minimum of five vines. Part of the germplasm is maintained in containers in screenhouse facilities to comply with phytosanitary legislation.

Different accessions of certain varieties are preserved to maintain some intra-varietal diversity.

Grape accessions at the CREA-VE repository have different origins, mainly from Italy (more than 60%). Many of these accessions represent rare or neglected grapevine varieties found throughout various wine-producing regions of Italy (Giust and Caputo, 2014; Bergamini *et al*, 2017; Gasparro *et al*, 2020; Zombardo *et al*, 2022, 2024; Palombi *et al*, 2023). Materials from other European countries, including Georgia and Armenia, represent a consistent percentage of the conserved germplasm (24%), and Americas (USA, Argentina and Brazil), Asia (China and Japan) and Africa (Algeria and South Africa) are represented in the collection (Figure 2).

Ordinary agronomic interventions are carried out during the cultivation cycle (winter pruning, soil management, pest control, fertilization, emergency irrigation, spring suckering, summer tying, and topping, crown management) to maintain the vines in a good vegetative-productive and phytosanitary state.

Management of *Vitis* genetic resources

The management of large germplasm collections is a complex task that requires a great deal of technical, agronomic and scientific expertise and it must be carried out in accordance with international standards (OIV, 2007; Maghradze *et al*, 2015). Primarily, the objective is to preserve the grapevine heritage, as well as to collect data on physiological and phenotypic characteristics of the germplasm (Boursiquot, 2000; Maul *et al*, 2012; Lacombe, 2023).

The first step in managing a collection is to correctly identify the collected plant material by carefully recording information about each accession, such as genotypic fingerprints and morphological characteristics. The next step is to collect information to record the 'passport data' according to the FAO Multi-Crop Passport Descriptor List for *Vitis* species (OIV, 2007; Alercia *et al*, 2015). These data include basic information such as a unique code, pedigree, origin, donor and others, in addition to specific descriptors that are relevant for the grapevine varieties and species.

According to ITPGRFA, every biological accession must also be linked to a Digital Object Identifier name (DOI), an international standard adapted to identify plant germplasm worldwide (Alercia *et al*, 2018), to facilitate the exchange of biological material and access to the information on crops and research around the world. The acquisition of DOIs for all conserved grapevine accessions at CREA is planned, and it is currently in progress.

The identification and characterization of the grapevine accessions represent the fundamental actions to be carried out in a rational germplasm conservation plan. These are achieved through a range of analytical techniques, including ampelographic description based on the analysis of traits that are highly heritable, DNA analyses, and agronomic and resistance trait evaluations.

The traditional approach for identifying and classifying grapevine varieties is ampelography (Galet, 1976; This *et al*, 2006), which relies primarily on the visual examination of morphological features. These observations are conducted by experts in the field, based on international standardized descriptors (IPGRI, 1997; OIV, 2007; UPOV, 2008). The International Organisation of Vine and Wine (OIV) experts have also introduced a 'primary priority descriptors list' encompassing only 14 descriptors (OIV, 2007; Maul and This, 2008), with a highly discriminating power, to reduce time in the characterization process.

Table 1. *Vitis* accessions maintained at CREA-VE

Species	Number of accessions
V. vinifera L.	2,906
Hybrids of *Vitis* spp.	393
V. aestivalis Michx.	1
V. andersonii Rehder	1
V. arizonica Engelm.	1
V. baileyana Munson	1
V. berlandieri Planch.	2
V. betulifolia Diels & Gilg	1
V. champinii Planch.	1
V. cinerea (Engelm.) Millardet	1
V. coignetiae Pulliat	1
V. doaniana Munson	1
V. longii Prince	3
V. monticola Engelm.	1
V. novae angliae Fernald	1
V. riparia Michx.	25
V. rubra Michx.	1
V. rupestris Scheele	12
V. slavinii Rehder	1
V. treleasei Munson	1

Figure 1. Some specific characteristics of different species of *Vitis* spp.: A, *Vitis doaniana* Muns.; B, *Vitis bayleiana* Munson; C, *Vitis berlandieri* Planch.; D, *Vitis vinifera* cv. 'Garnacha tinta'.

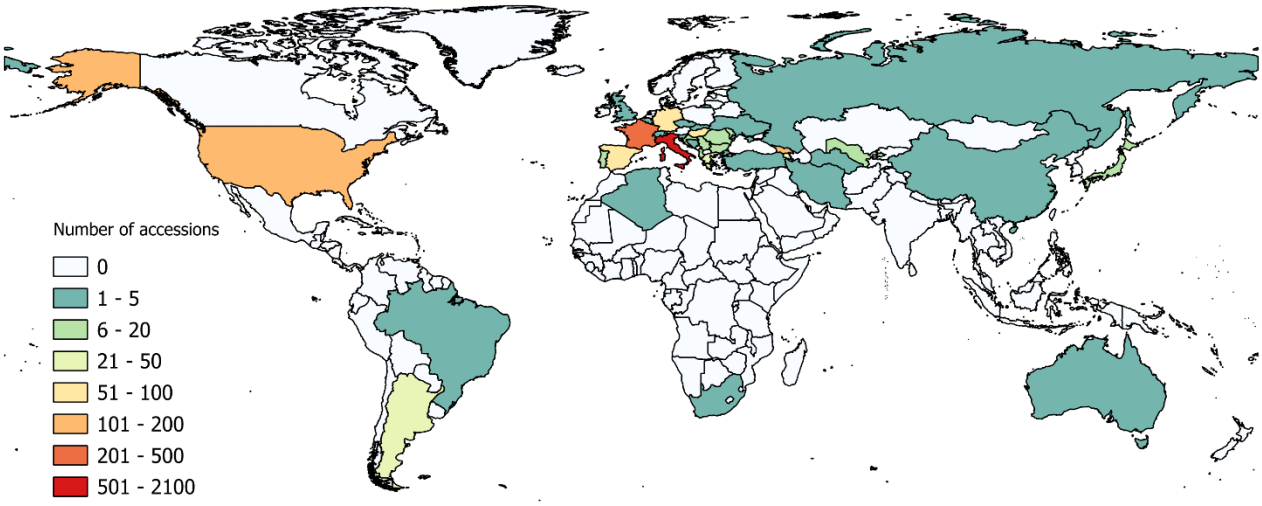

Figure 2. Country of origin of the *Vitis* accessions maintained at the CREA-VE repository.

Using the descriptors and methods defined by OIV and UPOV, the ampelographic characteristics of many accessions, maintained at CREA, have been recorded over the years (Alba *et al*, 2014, 2015; Labagnara *et al*, 2018; Zombardo *et al*, 2021; Palombi *et al*, 2023) and their information was useful for conducting distinctness, uniformity and stability (DUS) tests, studies on somatic variants (Crespan *et al*, 2016) and characterization of Italian variety families distributed in the peninsula (Costacurta *et al*, 2003, 2004).

The phyllometric method (also known as leaf ampelometry) is based on the measurement of specific leaf characteristics, such as the length of the veins and the angles formed between them (Bodor-Pesti *et al*, 2023). This technique, firstly proposed by Goethe (1876) and then set up by Ravaz (1902), is performed using specific ampelometric software (Soldavini *et al*, 2006), although, in recent years, it has been improved with the adoption of leaf morphometric methods (Chitwood, 2021) and image analyses by means of artificial intelligence (Liu *et al*, 2021; De Nart *et al*, 2024).

Ampelography was long the only method for identifying varieties, but DNA fingerprinting, especially if performed by microsatellites has proved suitable for both the rapid and reliable identification of varieties and the comparison of data between different laboratories, using reference data codification (Sefc *et al*, 2001; This *et al*, 2004).

The CREA grapevine collection was genetically characterized during the last decade using at least 11 Simple Sequence Repeats (SSR or microsatellites) markers (Migliaro *et al*, 2013). This work unveils duplicates, cases of mislabelling, homonyms and synonyms (Cipriani *et al*, 2010; Storchi *et al*, 2016; De Lorenzis *et al*, 2019; Pipitone *et al*, 2024).

A more detailed genetic characterization using 18K Single Nucleotide Polimorphic (SNP) markers was recently carried out on a subset of the Conegliano collection, comprising more than 600 accessions (D'Onofrio *et al*, 2021).

Genotyping is also useful for defining the pedigree of varieties and the area of origin, and accessions from CREA's ampelographic collection have been successfully used in phylogenetic studies of many varieties (Crespan *et al*, 2009; Bergamini *et al*, 2012, 2016; D'Onofrio *et al*, 2021).

The recording of characteristics, the expression of which is often influenced by environmental conditions (agronomic traits and quality), is another action that will be undertaken. These data are crucial for the potential use of the material in breeding programmes.

Moreover, during the vegetative season, visual inspections are conducted to evaluate the health status of the vines and to find out fungal disease (mildew and esca, in particular), virus infections and grapevine yellows symptoms.

In Figure 3, a scheme highlighting the primary processes of grape collection and conservation activities is shown.

The data collected are partly included in various databases and can be accessed via the websites of the European Vitis Database (http://www.eu-vitis.de), the Vitis International Variety Catalogue (https://www.vivc.de), the EURISCO web catalogue (https://eurisco.ecpgr.org). The maintained accessions show very high phenotypic variability for many characters, including leaf (Figure 4) and cluster shape and size (Figure 5), berry colour, shape and size, seed presence, berry skin thickness, sugar accumulation, phenological periods (Alba *et al*, 2023) and susceptibility to various

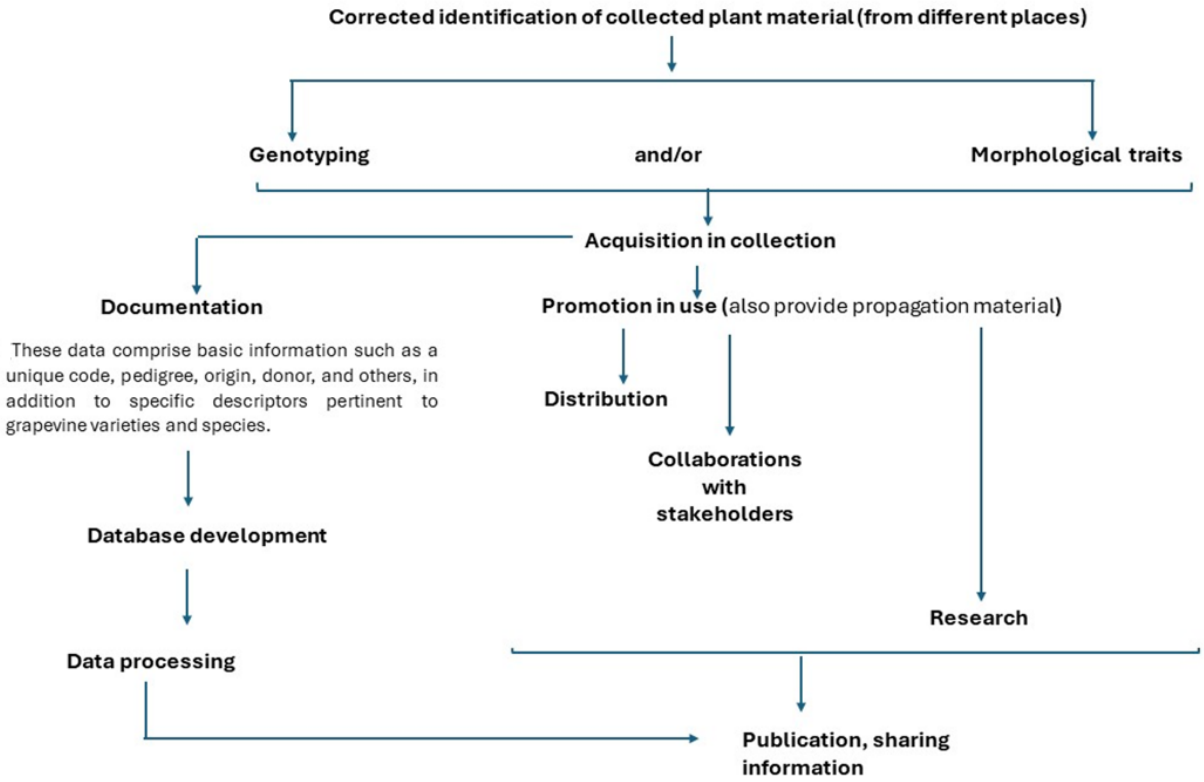

Figure 3. Scheme of genebank activities.

pathogens. Another indicative example is the average weight of the bunch: in some accessions, it is less than 50g, while in others it can exceed 600g. Examples include fruit colour (Table 2) and range of phenological stages (Figure 6).

The final stages of the development of a common and shared information system among the repositories concerned by the RGV-FAO programme are currently underway. At present, the various facilities of CREA-VE collate their data in shared spreadsheet files, where passport data, morpho-phenological data and genetic data, when available, are reported.

Over the past two decades, grape accessions have been exchanged for various purposes at the national and international levels. At the national level, autochthonous cultivars have been requested with the objective of reintroducing them into cultivation (growers) or incorporating them into national breeding programmes (researchers); at the international level, the majority of germplasm of foreign origin was received from several research centres, using specific agreements. The number of exchanges is estimated at 100 accessions.

Valorization of the collections

Within the ITPGRFA framework, recovery, characterization and conservation are to be considered priority components in the management of the collection (FAO, 2010). Moreover, another cornerstone is the sustainable use of agrobiodiversity. Italian biodiversity of autochthonous vines represents the heritage of a territory, due to its long history from the first domestication to modern cultivation. The discovery and rescue of autochthonous grape varieties promote the valorisation of the wine-growing territory and thus preserve its traditional and cultural legacy. Public and private research centres have undertaken this challenge with the primary objective of safeguarding the Italian ampelographic assortment.

Measures for the sustainable use of genetic resources include expanding the genetic base of cultivated varieties and increasing the diversity available to farmers.

To be commercially employed, a grape variety must first be listed in the National Register. For wine grapes, this is additionally contingent upon classification at the regional administrative level, while varieties intended for fresh consumption (e.g. table grapes) require only registration to obtain the certification of vegetative propagation material.

The process of registration and classification is accomplished through the morphological, physiological and agronomic characterization of the variety, which must be conducted in accordance with precise legislative national guidelines.

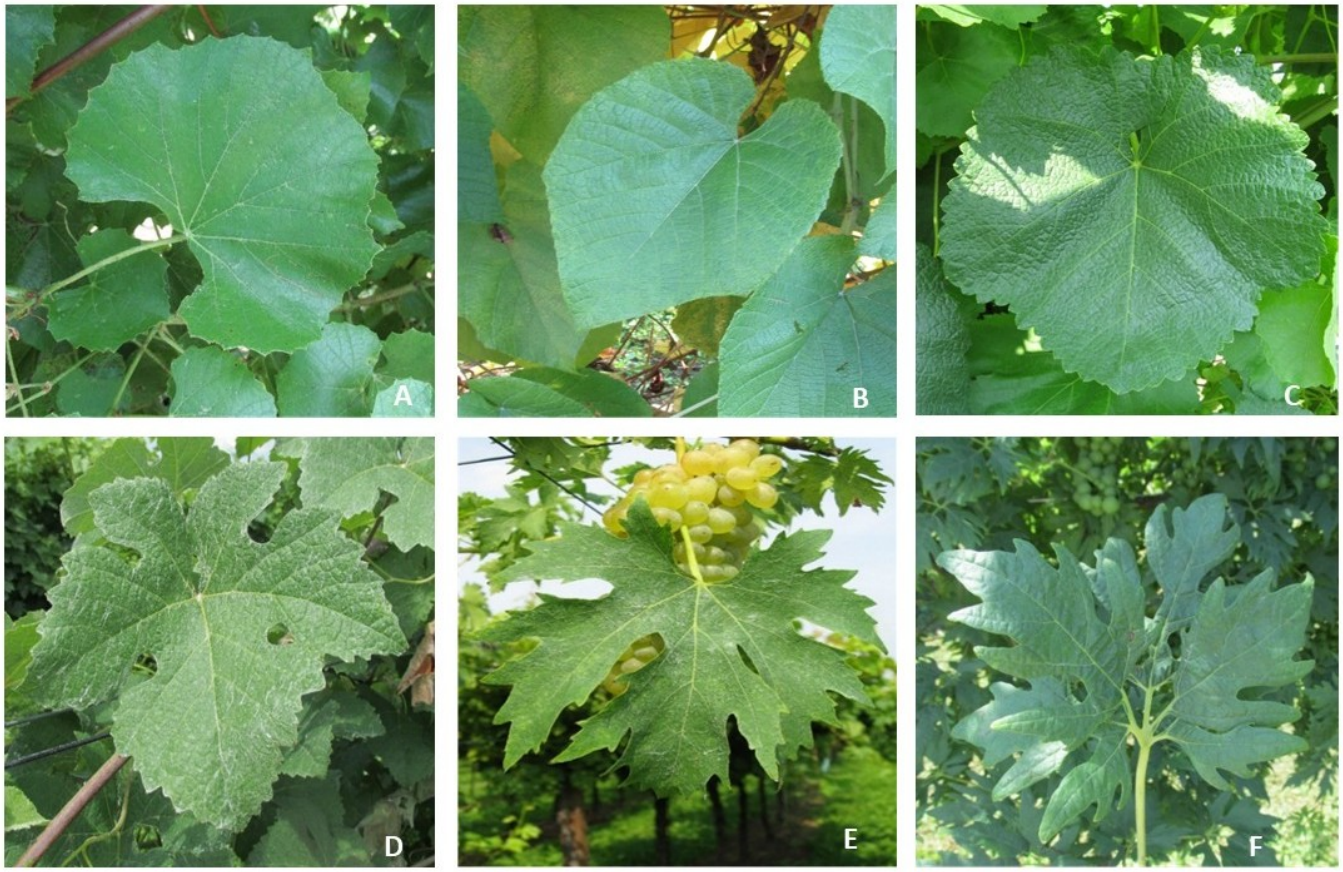

Figure 4. Variability in leaves can be observed in blade size and shape, number and depth of lobes, shape and size of teeth, petiolar sinus, hairs, etc. A, 'Ramsey'; B, *Vitis cinerea* Engelmann; C, 'Malbo gentile'; D, 'Pinot meunier'; E, 'Badacsonyi somszoeloe'; F, 'Chasselas cioutat' .

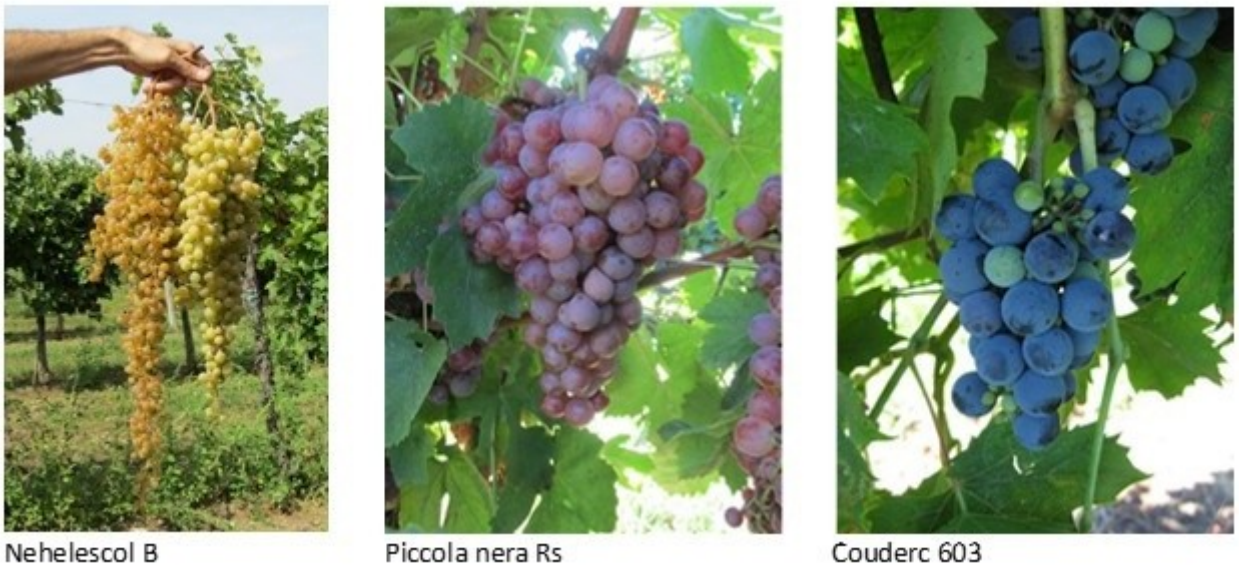

Figure 5. Morphological variety differences in grapes for colour, shape, size and other traits.

Table 2. Distribution in colour classes of the accessions maintained in the different subcollections (%).

	Black	White	Rose	Red	Grey
Conegliano	48	47	3	1	1
Arezzo	59	37	4	-	-
Velletri	28	65	2	5	-
Turi	37	59	4	-	-

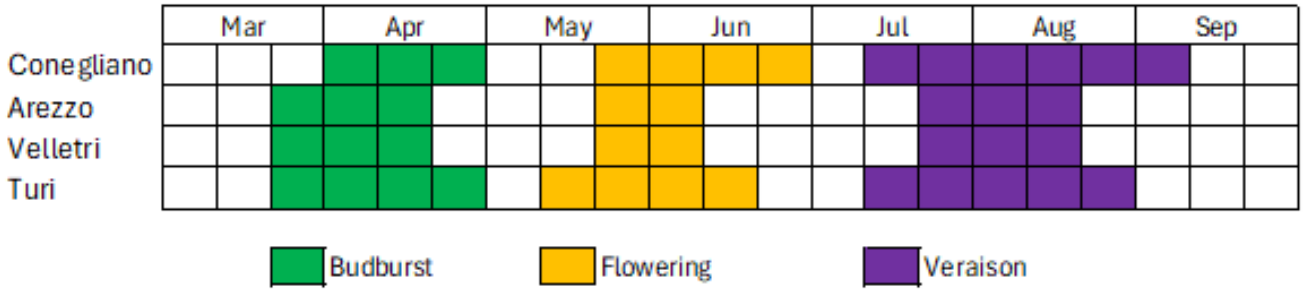

Figure 6. Variability in the date of occurrence of the main phenological periods.

In Italy, the interest of winegrowers in local varieties linked to the history of their territory is growing steadily and significantly: varietal wines are appreciated niche products and marketed profitably. As a result of the various national and regional projects for the conservation and valorization of vine genetic resources carried out by CREA, many local and historical Italian varieties have been included in the National Register, and it is now possible for winegrowers to use them commercially.

In Central Italy (Tuscany) significant examples are 'Orpicchio', 'Morellone', 'Nocchianello bianco', 'Nocchianello nero', and 'Gralima', while other varieties are currently being evaluated for their interesting characteristics, for example, 'Tané', a variety from Massa Carrara (Tuscany) with a bright rose berry colour, that at first evaluation seems to be suitable for producing easy-to-drink rosé wines. Also, in Central Italy (Latium) example are nine different clones of 'Cesanese di Affile'.

In Southern Italy, we can mention 'Santa Sofia', a white grape variety that was registered in 2019 and can now be grown in Basilicata and Campania, 'Sabato', a black grape variety, and 'Agostina', with white grapes, to cite a few examples. As far as Apulia is concerned, 'Negro Dolce' is an interesting variety in the process of being registered.

In Northern Italy, the most recent cases concern varieties that are characteristic of the Veneto region, including 'Rabosa bianca', 'Recaldina', 'Pecolo scuro', 'Pattaresca', 'Mattarella' and 'Benedina'.

The vines present in the vineyard collection also served as plant material used for the propagation and subsequent planting of the varieties in other germplasm collections, hopefully also with custodian winegrowers, or to bring back some varieties to the territories of origin with a past viticultural vocation (i.e. 'Biancone', transferred back to the place of origin, Elba Island in Tuscan archipelago; Zombardo, personal communication). In addition to its primary function as a genebank, the grapevine germplasm collection is indispensable for the implementation of numerous national and international research projects and the activities of the *Vitis* Working Group of the European Cooperative Programme for Plant Genetic Resources (ECPGR), as it ensures the availability of essential samples and data.

The multi-year phenological data set is of paramount importance for the creation and validation of phenological models, as well as for the investigation of vine adaptation to climate change (Parker *et al*, 2011; Tomasi *et al*, 2011; Fila *et al*, 2012; Valori *et al*, 2023).

Finally, the grapevine germplasm collection allowed us to raise awareness of the existence of almost forgotten vines that deserve attention, at a scientific but also general public level (Pagano *et al*, 2014; Zombardo *et al*, 2017; Storchi *et al*, 2022).

Conclusions and perspectives

Over the years, a great deal of effort has gone into characterizing the preserved varieties, using the most advanced phenology, morphology, biochemistry and molecular tools for various groups of varieties. However, many phenotypic and ampelographic aspects need to be explored further, to understand also better how some of these could influence interesting traits (i.e. berry quality, resistance/tolerance to biotic and abiotic stresses) to help researchers to better understand the genetic basis of traits and, consequently, for useful traits introgression for varietal constitution. However, several aspects remain to be fulfilled. These include creating a core collection, duplicating unique accessions to enhance security, filling the gaps in the genetic and

geographical distribution of conserved biodiversity, and improving the sharing and dissemination of information.

As reported, the CREA-VE repository maintained the collection as a field genebank. However, this conservation strategy is financially demanding due to the intensive management requirements, and there is an inherent risk of material loss due to pests and diseases. To address these challenges, novel approaches combining *in vitro* storage (slow growth) and cryopreservation may offer a solution for the long-term maintenance of grape genetic resources. These techniques represent the optimal strategy for the long-term storage of plant genetic resources, offering the greatest safety and cost-effectiveness. However, in the case of vegetatively propagated species, such as grapevine, they present the disadvantage of being genotype dependent. Should these issues be resolved in the future, cryopreservation could be effectively applied at the CREA-VE repository.

Finally, researchers involved worldwide in different topics of grape could work for a possible 'Global Grape Diversity Platform' to secure the long-term conservation and use of these genetic resources. In this perspective, CREA-VE (and other research centres of CREA) is involved in the European project PRO-GRACE (https://www.grace-ri.eu/). The project addresses different topics, such as developing and testing unified strategies, procedures and standards for evaluating phenotypic traits of plant genetic resources both *in situ* and *ex situ*, and providing the information to end-users, including breeders and farmers. The aim is to create a new concept and governance model for sharing information on plant genetic resources at the European level, enabling the construction of an integrated European plant genetic resources information system. This approach seeks to ensure the safeguarding, use, valorization and cost reduction of grape genetic resources.

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Author contributions

M. Antonietta Palombi: Conceptualization and ideation; Vittorio Alba, A. Raffaele Caputo, Roberto Carraro, Massimo Gardiman, M. Antonietta Palombi and Alessandra Zombardo: writing, original draft preparation and data analysis; Massimo Gardiman, tables and figure preparation; Roberto Carraro: pictures; Vittorio Alba, Roberto Carraro, Massimo Gardiman, M. Antonietta Palombi and Alessandra Zombardo: writing, review and editing; Marco Ammoniaci,Noemi Bevilacqua, Roberto Carraro, Stefano Favale,Simone Garavelloni, Massimo Morassut, Marina Niero, Roberto Nuti, Giuseppina Pipitone, Sergio Puccioni: collect data; M. Antonietta Palombi: Supervision. All authors have read and agreed to the published version of the manuscript.

Conflict of interest statement

The authors declare that they have no competing interests.

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