



Genetic  
Resources

# Special Issue



## *Plant Genetic Resources* **Ex situ conservation in Europe**



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**Genetic Resources** is an open access journal disseminating global knowledge and tools used by the community of practitioners of plant and animal genetic resources involved in monitoring, collecting, maintaining, conserving, characterizing and using genetic resources for food, agriculture and forestry. **Genetic Resources** publishes original research, methods, strategies, guidelines, case studies and reviews as well as opinion and other papers on a variety of topics of interest on the present and future use of genetic resources. These may include the acquisition, documentation, conservation, management, assessment, characterization and evaluation of genetic resources and their link to broader biodiversity, socioeconomic practices, policy guidelines or similar, serving stakeholders within and across sectors. Occasionally, **Genetic Resources** publishes special issues with a focus on selected topics of interest for the genetic resources community. The journal has a focus on the European region and also welcomes contributions of wider interest from all world regions.

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# *Ex situ* conservation of plant genetic resources in Europe – A journey through history, mission, challenges and future opportunities

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**Abstract:** The Special Issue entitled: ‘*Ex situ* conservation of plant genetic resources in Europe – A journey through history, mission, challenges and future opportunities’ presents 16 original articles, including 11 genebank reports from 7 European countries (Belgium, France, Germany, Hungary, Italy, Norway and Russia) and 5 review and position papers presenting relevant concepts to improve plant genetic resources (PGR) conservation and access. They provide an overview of PGR *ex situ* conservation in Europe, and reflect on the history and future directions of the collections. Important topics in PGR conservation and use are explored, including quality management systems for genebanks, the role of community seedbanks, the importance of collaborative research projects and national and international research infrastructures for PGR, and the integration of *in situ* and *ex situ* PGR conservation. From this article collection, the key importance of genebanks clearly emerges – not only in the long-term conservation of plant biodiversity but also in supporting and enabling plant breeding, research in plant biology and *in situ* conservation initiatives, highlighting important topics that should be prioritized for the efficiency and continuous improvement of PGR conservation activities. This article collection sparks discussions on future directions of *ex situ* plant conservation to further increase the impact of genebanks and their contributions to sustainable development.

**Keywords:** agricultural biodiversity, crop diversity, genebanks, germplasm banks, plant conservation

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## Introduction

Human societies have created, organized and conserved living collections of plant species since ancient times, in all areas of the world and for multiple reasons (Granziera, 2001). Since the end of the 19th century, researchers have conceptualized and highlighted the importance of the conservation and availability of plant genetic resources (PGR) for crop breeding and research, especially of landraces and crop wild relatives (CWR) (Plucknett et al., 1987). The modern concept of a genebank – a facility for the long-term *ex situ* conservation of reproductive samples of PGR acces-

sible for breeding and research purposes – was first realized at the beginning of the 20th century at what is now the N. I. Vavilov All-Russian Institute of Plant Genetic Resources (VIR) in Saint Petersburg, which pioneered the collection, identification and description of the diversity of cultivated plants (Loskutov, 1999; Loskutov et al., 2025). Genebanks have since been established in many countries to preserve and keep available PGR and prevent the loss of wild plant populations and landraces due to substitution with modern high-yielding varieties (Lehmann, 1981). In Europe, one of the earliest genebanks was established in Gatersleben, then East Germany, after World War II, and subsequently became the German genebank after reunification in 1990. Other collections were established in several countries of Eastern Europe in the 1950s, including Bulgaria, Czechoslo-

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vakia, Hungary and Poland. Investments in national collections in Western Europe came later, after awareness was raised by the Food and Agriculture Organization of the UN (FAO) and technical conferences held in the 1960s, alerting about the risks of genetic erosion (i.e. “the loss of genetic diversity and variation in a crop”, van de Wouw et al. (2010)) due to displacements of old varieties by modern ones (Pistorius, 1997). The genebank of the Federal Republic of Germany in Braunschweig started operations in 1971, the Italian genebank in Bari in 1974, the Nordic genebank of the five Nordic countries in Lund, Sweden, in 1979 and the Dutch genebank in Wageningen in 1985. Many countries did not establish centralized genebanks, but their collections were created by universities as well as public and private research institutes. The splitting and sprouting of nations due to political changes after 1989, and the concept of sovereignty over genetic resources introduced by the Convention on Biological Diversity in 1992 (UNEP, 1992), opened the way to the expansion of conservation institutions. A large diversity of historical backgrounds and foundational motivations has given rise to a wide number (around 400) of institutes conserving PGR listed in the European Search Catalogue for Plant Genetic Resources (EURISCO, <http://eurisco.ecpgr.org>), summing up to more than 2 million accessions of PGR currently conserved *ex situ* in Europe. These accessions account for half of the total PGR accessions listed in the global Genesys database (Genesys PGR, [www.genesys-pgr.org](http://www.genesys-pgr.org)) and about one-third of all PGR accessions actively conserved in genebanks globally (FAO, 2025). These *ex situ* collections vary in their missions, expertise, financial sustainability, legal arrangements, size of collections, conservation and distribution methods, data information and quality management systems.

The *ex situ* conservation of PGR in genebanks is currently considered the most effective strategy to avoid losses in plant diversity and enhance the availability of these resources (Davies and Allender, 2017). This is particularly relevant considering that PGR represent a fundamental asset to widen and diversify the genetic basis of modern crop cultivars and provide useful traits for breeding in the current scenario of climate change and with the need to minimize negative impacts of agricultural production on natural ecosystems (McCouch et al., 2013; Pixley et al., 2023). Moreover, genebanks conserve and keep available landraces, old cultivars and neglected crops that are being rediscovered, after decades of genetic erosion in several European areas (see e.g. Hammer et al. (1996)), often linked with traditional foods and products and offering new opportunities for farmers and food industries (see e.g. Helicke (2024)).

Fostering collaboration among genebanks in different countries and involving different stakeholders can be an important strategy to strengthen the conservation and use of PGR (Engels et al., 2024). In the European scenario, the European Cooperative Programme for Plant Genetic Resources (ECPGR) is a collaborative

programme, active since 1980, among most European countries working together on multiple initiatives aimed at ensuring the long-term conservation and utilization of PGR in Europe. ECPGR recently published the *Plant Genetic Resources Strategy for Europe* (ECPGR, 2021). This document highlights gaps and necessary actions that should be addressed in the coming decade to ensure long-term PGR availability through their sustainable use and conservation. Consolidating and sustaining *ex situ* conservation is a priority action within the strategy, with several important targets identified that should be achieved by 2030.

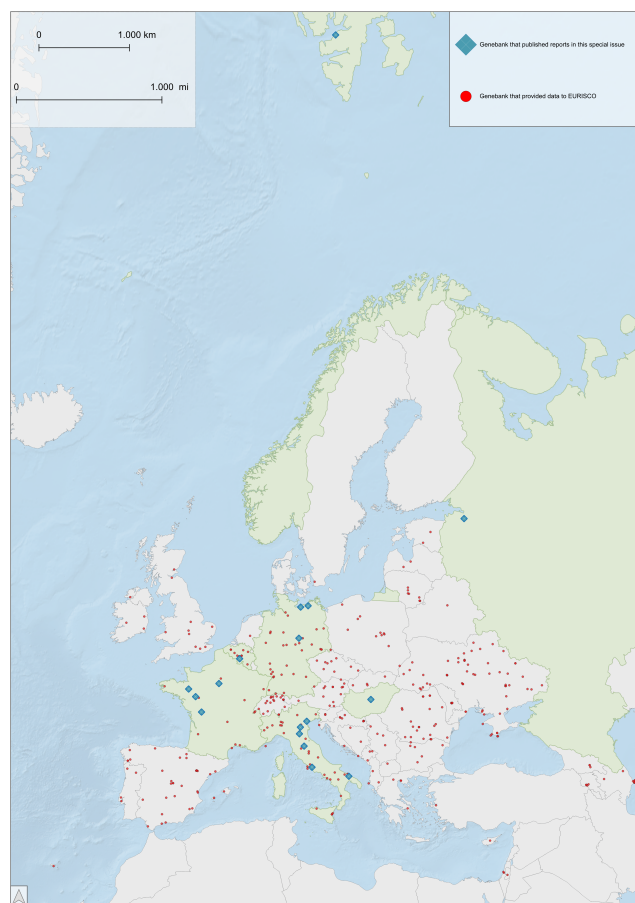
In this context, this *Genetic Resources* Special Issue aimed at providing the opportunity to disclose, at a so far unpublished level of detail, a comprehensive overview of the history, mode of operation, strengths and weaknesses of some exemplary European institutions conserving plant germplasm as well as related mechanisms influencing their operation. Most of these data and observations are of high relevance for the PGR community, yet they are often difficult to publish in regular research articles or remain scattered across various publications, often in national languages. This collection not only showcases examples of successful initiatives but also serves as a valuable resource for policymakers, helping them to understand the state of the art in view of identifying opportunities for better cooperation and sharing of responsibilities.

## Content of the Special Issue

This Special Issue is composed of 16 original articles in addition to this Editorial, grouped into two main categories: (1) reports from genebanks describing their history, composition of the collections, key activities and future perspectives, and (2) review and position papers on emerging topics aimed at enhancing the conservation and sustainable use of plant diversity in Europe.

Eleven genebank reports from seven European countries are presented in this issue, namely: Belgium (Dumont et al., 2025), France (Esnault et al., 2025; Feugey et al., 2025; Ricou et al., 2025; Sampoux et al., 2025), Germany (Weise et al., 2025), Hungary (Ay et al., 2025), Italy (Alberti et al., 2025; Palombi et al., 2025), Norway (Asdal, 2025) and Russia (Loskutov et al., 2025). These reports are just a snapshot of the more than 400 *ex situ* collections registered in EURISCO (Figure 1). However, they cover all the main *ex situ* conservation techniques for plant germplasm (long- and medium-term orthodox seed storage, *in vitro* conservation, cryopreservation and field conservation, see FAO (2014)) and vary significantly in terms of number of accessions and plant species conserved as well as management practices (covering multi-species as well as crop- and species-specific collections). While most of the genebank reports in this issue deal with the conservation of PGR for food and agriculture, reports on other collections are also included (i.e. the Versailles *Arabidopsis* Stock Centre, collections of ornamental species and non-food industrial crops, the Pannonian Seed Bank as a con-





**Figure 1.** Locations of European genebanks contributing reports to this special issue. In red dots, the location of all institutions providing data to EURISCO (extracted from FAO WIEWS). Blue diamonds indicate the locations of the eleven institutions that published genebank reports in the Special Issue (some collections are conserved in different sites). The seven countries where these contributing genebanks are located are highlighted in green.

servation seedbank for wild species), and the Svalbard Seed Vault (Norway), the largest global seed collection of safety duplicates of crop genebank accessions.

Review and position papers offer a view on important topics for PGR conservation and use: quality management systems for genebanks (van Hintum and Wijnker, 2024), an overview of community seedbanks in Europe (Bocci et al., 2025), the importance of collaborative research projects and national and international research infrastructures to promote PGR conservation and use (Bergheaud et al., 2025; Goritschnig et al., 2025), and the integration between *in situ* and *ex situ* conservation of PGR (Maxted et al., 2025).

### Key messages

This Special Issue provides an overview of the diversity and complexity of *ex situ* conservation activities of plant diversity across Europe and on emerging topics to enhance the long-term conservation and use of PGR. The presented genebanks started assembling their PGR collections during the 20th century, particularly after

the 1950s. Collecting activities and the acquisition of new accessions are still ongoing, focusing especially on CWR, landraces and crop species that have often been neglected and are now being re-evaluated by research and breeding, promising adaptation to changing environmental conditions. Most genebanks today focus on improving the documentation, characterization, evaluation, access and use of conserved accessions and their associated data.

The genebank reports highlight the diverse functions that these institutions have within their national PGR conservation programmes and seed systems. The distribution activities of conserved samples across the years highlighted the importance of these genebank collections for research and breeding, for both the private and the public sectors. Genebank collections are fundamental sources of useful agronomic and stress tolerance traits for plant breeding and also provide plant germplasm material for hobby growers, repatriation and rematriation activities (Ocampo-Giraldo et al., 2020). Genebanks are also important to support the registration of newly selected or conservation varieties and to conserve and keep available old cultivars formerly registered in national variety lists. They serve as central nodes in networks and collaborative programmes, including private and public institutions and on-farm networks, aiming at enhancing PGR conservation and use. Genebanks are often active in outreach activities, raising awareness about the importance of agricultural biodiversity and its long-term conservation, while also promoting the use of PGR.

Most genebanks are open to, and actively engage in, international collaborations for research and exchange of genetic resources. However, the opportunity to evolve towards a more integrated system for the conservation and management of genetic resources at the regional level is rarely acknowledged as a shared goal. Evidently, the benefits that could result from shared management and use of resources – such as economy of scale, reduction of redundancies and gaps, and integration of expertise – are not immediately recognized at the local level. This is in line with the challenges faced by initiatives like AEGIS (European Genebank Integration System) in gaining traction (van Hintum et al., 2021).

In the framework of this Special Issue, important topics emerged that should be considered priority actions for the continuous improvement of PGR conservation activities in Europe:

- Fully implement **quality management systems** for genebanks, including the creation of a certification agency specialized in genebank activities to continuously improve the efficiency, reliability and transparency of all genebank operations.
- Establish **safety duplicates**, not only for orthodox seed accessions but also *in vitro*, cryo and field collections. This is an important step to reduce the risk of losing these priceless resources.
- Coordinate and integrate *in situ* and *ex situ* **conservation strategies**, acknowledging the role

of community seedbanks and on-farm conservation programmes. This integration will foster a dynamic management of PGR to ensure that the highest degree of plant genetic diversity is conserved and accessible to users at all times.

- Support ongoing **multi-omics characterization and evaluation** of conserved accessions. This will help refocus conservation efforts, identify collection gaps and allow the mining of collections for useful traits.
- Improve **data management** and accessibility of accession data, integrating passport data with novel multi-omics characterization and evaluation data collected during routine regenerations and as part of collaborative research projects. Better documentation of PGR including CWR and landraces will increase their value and therefore their use for research and breeding.
- Test and employ new technologies to enhance **automation and digitization** of routine processes in the management of collections to reduce errors and increase data quality.
- Establish national and **pan-European research infrastructures** that can coordinate conservation activities and streamline scientific services and research on PGR conservation and use. Improved pan-European coordination will help align the diverse and often heterogeneous PGR conservation activities, improving financial and operational efficiency and access to services.

Achieving these ambitious targets will improve the long-term conservation and accessibility of our priceless natural resources, which are pivotal to face present and future challenges related to food security, environmental sustainability and the implementation of nature-based solutions.

Overall, the tangible and invaluable contribution of genebanks to the long-term conservation of, and access to, plant diversity clearly emerged from this article collection. Furthermore, as the importance of PGR in breeding continues to grow and with it the increasing volume of PGR-related data, the scope of genebanks is widening to becoming bio-digital genetic resources centres (Maxted et al., 2025; Mascher et al., 2019). The genebank reports underline the value of documenting and sharing the history of genebanks with the broadest community to inform collections' management and establish future priorities. We encourage more genetic resources centres to share the fascinating history of why and how their collections were assembled, how their conservation and research methodologies have evolved and reflect on challenges encountered over the years and their corrective actions. This Special Issue can also be a useful source for young professionals interested in PGR to obtain an overview of genebanking in Europe and its future goals. Finally, we hope that initiatives such as this article collection can spark discussions on the future directions of *ex situ* plant conservation to further

increase the impact of genebanks and their contribution to sustainable development.

### Authors' contributions

FG drafted the paper, all the authors contributed to the writing, revised and approved the final version.

### Conflict of interest statement

The authors declare that they have no competing interests.

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# Quality management in a genebank environment: Principles and experiences at the Centre for Genetic Resources, The Netherlands (CGN)

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**Abstract:** To enhance the management of plant genetic resources by genebanks, implementing a quality management system is essential. Such a system ensures the consistent quality of genebank operations through the establishment of a quality policy, the integration of quality planning and assurance, and the execution of continuous quality control and improvement measures. This structured approach also supports alignment with globally recognized standards, such as those established by the UN Food and Agriculture Organization (FAO). The Centre for Genetic Resources, The Netherlands (CGN), with its significant experience in quality management, is presented as a case study to illustrate the methodology and its impact on genebank operations. By detailing operating procedures, a quality management system provides transparency, fostering trust and facilitating collaboration between genebanks. Additionally, the potential for developing a certification system for genebanks – wherein an authorized body formally certifies that a genebank adheres to specific standards – is examined.

**Keywords:** ex situ genebank, genebank collaboration, quality management, certification system

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## Introduction

Genebanks play a vital role in safeguarding plant genetic resources (PGR) for future generations, ensuring that these resources remain accessible for developing crops essential to feeding the global population (FAO, 2010). In addition to their long-term conservation efforts, genebanks also provide crucial materials to crop scientists and plant breeders, supporting their research and breeding programmes. These responsibilities are significant and demand a high level of commitment. Consequently, it is essential that genebank operations maintain a high standard of quality, which must be consistently assured. Effective quality management is therefore critical to ensure that genebanks fulfil their responsibilities and operate at the appropriate level to meet global food security needs.

The Centre for Genetic Resources, The Netherlands (CGN) holds the statutory responsibility for managing PGR on behalf of the Dutch government. When this mandate was assigned by the Dutch Ministry of Agriculture in 2004, the ministry also required CGN to implement a quality management system to ensure that public funds were being used effectively and that the Dutch public could have confidence in CGN's ability to perform its duties at a high standard. As a result, CGN became the first genebank in the world to achieve ISO 9001 certification. As stated on the website of the International Organization for Standardization (ISO, 2015), "ISO 9001 is a globally recognized standard for quality management. It helps organizations of all sizes and sectors to improve their performance, meet customer expectations and demonstrate their commitment to quality. Its requirements define how to establish, implement, maintain, and continually improve a quality management system (QMS). Implementing ISO 9001 means your organization has put in place effective

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processes and trained staff to deliver flawless products or services time after time.”

Each genebank holds unique collections that often reflect regional crops and wild relatives adapted to specific climates and conditions. Preserving PGR is a shared responsibility that transcends borders. To fulfil this mission, genebanks must collaborate, sharing resources, data and expertise to prevent unnecessary duplication and ensure no vital genetic material is overlooked. Working together also enables coordinated efforts to store, regenerate and monitor seed viability over time. Successful collaboration relies on mutual trust, which in the context of genebanks means adhering to agreed-upon operating procedures and standards for managing PGR effectively. It is therefore not surprising that when the Global Crop Diversity Trust (GCDDT) took on the coordination of the CGIAR Genebank Platform and began contributing to the funding of CGIAR genebanks, it recognized the need for a mechanism to monitor the performance and quality of these institutions. Transparency in the operations of the genebanks was essential to assess their effectiveness and to identify areas for investment. Consequently, the GCDDT developed the ‘Genebank Quality Management System’ and provided support to the CGIAR genebanks in its implementation (Lusty *et al.*, 2021).

The various elements of quality management within a genebank context are described and illustrated through the experiences of CGN. Additionally, this discussion will introduce and explore certain aspects of the potential establishment of a Genebank Certification System.

## Quality management in a genebank environment

### Quality management

Quality can be managed, although it is difficult to define. According to the ISO 9001 standard for quality management systems (ISO, 2024), quality is defined as the “degree to which a set of inherent characteristics [or distinguishing features] of an object fulfils requirements”. An object is defined as “anything perceivable or conceivable, such as a product, service, process, person, organization, system or resource”. Based on this definition the quality of a genebank can be defined as ‘the degree to which the genebank fulfils its objectives’.

The objectives of a genebank are relatively well-defined and encompass two primary elements: the conservation of PGR for future generations and providing access to these PGR for the current generation of users (FAO, 2014). While these elements are broadly agreed upon, interpretations of what constitutes ‘conservation’ and ‘access’ can vary among genebanks. Discussions surrounding ‘conservation’ often focus on technical aspects, such as the required frequency of seed viability testing or the appropriate number of plants in a regeneration plot. In contrast, the concept of ‘access’ is more heavily influenced by policy considerations, addressing

questions like who should have access to the material and under what conditions.

Regardless of differing perspectives on these definitions, it is essential to ensure that the objectives established for a genebank are met in the most effective manner possible. A quality management system serves as a valuable tool to assist genebanks in achieving these goals optimally.

Quality management, and this will appear obvious, involves overseeing all activities and tasks necessary to sustain a desired level of excellence, specifically to achieve established objectives. This process typically encompasses several key components, including quality planning, quality control and quality improvement.

### Quality planning

In the context of a genebank, quality planning involves establishing methods to measure or assess the achievement of objectives using Key Performance Indicators (KPIs), defining and updating Standard Operating Procedures (SOPs), and ensuring that the desired quality level is maintained through an annual Quality Improvement Plan (QIP). These elements will be detailed below.

After clearly defining objectives, methods need to be established to quantify or otherwise assess the achievement of these goals. This is typically accomplished through the identification of KPIs, that can differ from genebank to genebank as they need to be tuned to the genebank’s organization and operations. The KPIs may include metrics such as the number of accessions, the quantity of samples distributed, the number of regenerations conducted, the percentage of successful regenerations, and the number of viability tests performed. It is important to view these KPIs as monitoring tools rather than strict performance assessments, as an overemphasis on these indicators could lead to unintended consequences. For example, a curator might feel compelled to distribute accessions solely to increase the number of samples sent out, potentially undermining the integrity of the genebank’s operations. The indiscriminate distribution of seed samples – such as fulfilling requests like “please send the entire lettuce collection” – can deplete inventory, necessitating earlier regeneration of the accession, which incurs additional costs and may impact the genetic integrity of the collection. Therefore, in cases of large requests, a genebank should work collaboratively with the requester to identify an optimal selection of accessions that meets their needs while preserving the collection’s resources.

The quality planning phase also encompasses the formulation of SOPs, which detail how essential operations are to be conducted. This is a critical component of effective genebank management and includes various elements such as protocols for acquisition, regeneration, seed processing (cleaning, drying, seed moisture content determination, viability testing, etc.), seed storage, distribution, information management and other

operational procedures. By clearly defining these SOPs, genebanks can ensure consistency and quality in their practices, thereby enhancing their overall effectiveness in achieving their objectives.

The FAO Genebank Standards for Seed Conservation (FAO, 2014) categorize genebank operations into ten key areas: 1) acquisition of germplasm, 2) drying and storage, 3) seed viability monitoring, 4) regeneration, 5) characterization, 6) evaluation, 7) documentation, 8) distribution and exchange, 9) safety duplication, and 10) security and personnel. Each of these essential elements can be associated with its own SOP. However, depending on the scope of the quality management system, this framework can be expanded to encompass the promotion of use, research activities and other genebank-related functions.

The level of detail in SOPs can vary significantly. In some instances, SOPs can serve as comprehensive guides, offering precise instructions on which actions to take and controls to operate in specific situations. In contrast, other SOPs may outline the principles and objectives of an operation without delving into the details of the actions required to achieve those goals. For example, a SOP for the acquisition of germplasm typically emphasizes the need to adhere to all legal and phytosanitary regulations and specifies certain criteria for selecting materials suitable for inclusion in the collection. However, it may not detail where and how the material should be obtained (although it could be beneficial to include guidelines on handling materials during collection missions).

SOPs not only clarify the processes involved but also serve as valuable resources for new staff members, helping them understand important considerations when performing genebank tasks.

It is essential that these SOPs are not created in isolation but rather reflect existing practices and the current operational reality. During the documentation of procedures, it may become evident that certain practices are not 'fit for purpose' and require improvement. The previously mentioned FAO Genebank Standards for Seed Conservation (FAO, 2014) can often serve as a valuable reference point, providing guidance on what constitutes a high standard and an appropriate level of operation for genebanks. This alignment ensures that the SOPs are not only functional as a reference and training material but also effective in enhancing the overall quality of operations.

Quality planning is an ongoing process rather than a one-time task. It is typically conducted annually, producing a QIP that is implemented throughout the year to achieve the desired quality standards. The QIP incorporates elements such as user feedback, non-conformities, assessment of evolving policies, application of new technologies, and potential risks.

## Quality control

Once the KPIs and SOPs have been established, the genebank can implement a quality control mechanism.

This process involves generating evidence that demonstrates compliance with the defined protocols, staff competency and user satisfaction. Documenting this evidence should be integrated into the SOPs and may, for example, include maintaining logbooks for regenerations, which could record instances where protocols could not be adhered to, accompanied by justifications and approvals from a supervisor.

Additionally, the quality control mechanism may involve maintaining an overview of all requests for material, documenting the dates of the requests, the actions taken, the shipment dates of seeds, and potentially including feedback from the requestors of seeds. This systematic approach to evidence production not only ensures accountability but also fosters continuous improvements in the operational efficiency of the genebank.

In addition to the user feedback, a significant and regular form of quality control comes from staff observations of potential deviations from established SOPs. These observations should be documented, processed and, together with other quality-related information, reviewed during internal audits to ensure comprehensive quality evaluation and drive continuous improvement through QIPs.

Ultimately, it should be feasible for an independent observer to assess and verify that the genebank is adhering to its established protocols. More critically, this observer should be able to ascertain that the staff possesses the requisite knowledge and skills to perform their duties as outlined in the SOPs. This principle is central to the certification process for the ISO 9001 standard for quality management systems.

As part of this ISO 9001 certification, an auditor, selected by the certifying agency, will conduct an annual evaluation of the genebank. During this assessment, the auditor will verify that the genebank is operating in accordance with its SOPs and that management effectively oversees organizational operations, including initiatives for quality improvement. This external validation not only reinforces accountability but also enhances confidence in the genebank's quality management practices.

## Quality improvement

The final component of quality management to be addressed here is quality improvement, which focuses on identifying operational flaws and implementing corrective measures. Staff observations and feedback from genebank users play a crucial role in this process. When activities deviate from established SOPs, these non-conformities necessitate a thorough analysis to identify their root causes and facilitate appropriate adjustments to improve the protocols. User reports that highlight issues such as not receiving requested materials, receiving incorrect materials, or experiencing difficulties in germination of the received material are critical indicators that something is amiss. These signals may suggest problems with the ordering system,



documentation errors, or seed viability concerns – all of which require immediate attention and action. This type of feedback from users can be asked when handling seed requests, but can also be collected in targeted questions and interviews.

It is vital that all forms of feedback are taken seriously and addressed promptly. Additionally, maintaining records of feedback and subsequent actions provides valuable information for auditors assessing the performance of a genebank. Also here, the presence of SOPs detailing procedures of how complaints and issues are identified, addressed and improvements implemented makes sure that the QMS itself fosters improvement.

Together, these elements constitute the quality management system of a genebank. Given that each genebank is unique and the implementation of quality management practices remains relatively uncommon in this sector, there is currently no standardized model for a genebank quality management system. A standard of potential interest was published in 2018 for the biobanking community (ISO 20387:2018) that through its focus on handling and storage of biological material, technical competence, risk management and data integrity may be of relevance to genebanks, albeit, to the best of our knowledge, no genebank currently uses this standard (ISO, 2018).

Genebanks that have adopted quality management are often hesitant to publish their SOPs and related documentation. To date, CGN is one of the few genebanks that has made its complete quality management system publicly available (ECPGR, 2024). In the introductory text accompanying the SOPs, CGN states:

“With these documents, CGN gives complete transparency regarding the reality of its genebank. As you will see, it is far from perfect. We hope this material will help others in setting up their quality management systems, and in providing transparency regarding their genebank operations. We also hope that it will start discussions and generate constructive feedback regarding our methods helping us to improve. In the end, we all want to conserve plant genetic resources as efficiently as possible, for the generations to come, and provide access to these resources for the current generation of users.”

### **Quality management at the Centre for Genetic Resources, The Netherlands (CGN)**

An important reason for implementing quality management, already referred to in the introduction section above, is to ensure the effective use of funding provided by supporting agencies. In 2004, CGN became the first genebank to achieve ISO 9001 certification when their funding body, the Dutch Ministry of Agriculture, mandated the establishment of a formal quality management system. PGR management was recognized by the Dutch government as a key responsibility arising from international commitments, such as the Convention on Biological Diversity (CBD) and the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA). The important responsibility of

managing genetic resources was delegated to CGN, a division of Wageningen University and Research, which had been responsible for operating the genebank for Wageningen's agricultural institutes already since 1986. To ensure that CGN was fulfilling this statutory role effectively, the Ministry required the implementation of a robust quality management system.

### **Setting up CGN's quality management system**

Already in 1993, CGN had produced an internal report titled CGN Genebank Protocol, which compiled the protocols followed by various curators, the seed manager and the documentation manager (van Hintum and Hazekamp, 1993). This report garnered considerable attention within the genebank community, as many institutions were keen to learn how a colleague genebank, CGN, conducted its operations. However, when the time came to formalize the SOPs for the quality management system, it became evident that the published CGN Genebank Protocol had outlined the procedures for an idealized scenario. For instance, the protocol might specify regenerating on the basis of a minimum of 50 plants, but in reality, if 55 were sown and 8 died, how should the curator proceed? The SOPs had to account not only for the ideal procedures but also specify the decision-making required in less-than-perfect circumstances. The process of drafting the SOPs sparked significant internal debate, curators learning from each other, asking the questions they never asked themselves, and ultimately leading to substantial improvements in quality.

The establishment of an ISO 9001-compliant quality management system at CGN was facilitated by an external consultancy firm. This firm provided expertise in the methodology, offering guidance on how to logically segment genebank activities, describe processes through flowcharts, and formulate the SOPs. As CGN was the first genebank to adopt a formal ISO 9001 quality management system, there was no pre-existing standard to follow. This allowed CGN to analyze its activities and make a system that was 'fit to purpose' to its circumstances and reality. However, when compared to the quality management systems now used by other genebanks, the terminology employed by CGN is somewhat unconventional, and the level of detail is occasionally either excessive or insufficient, as compared to other systems. Additionally, after two decades of operation and considerable evolution, the system's internal coherence has eroded, suggesting that a comprehensive revision may be necessary. Nevertheless, the system has significantly contributed to CGN's success as a genebank, and still does. It enabled CGN to maintain consistent quality, as reflected by user feedback.

The ISO 9001 standard mandates that the CGN conduct annual external audits, carried out by an ISO accredited conformity assessment agency. These audits generate reports that highlight areas requiring attention, including opportunities for improvement and, when applicable, instances of nonconformity with the ISO

standard. In cases of nonconformity, CGN must submit an improvement plan, complete with a timeline and supporting evidence.

In addition to the external audit, an internal audit is conducted annually. While CGN outsources this process to a specialized company, it retains the option to perform it in-house. The report of the internal audit serves as input for the subsequent external audit.

Every three years, recertification is conducted through a more comprehensive audit performed by the accredited auditing agency.

## Costs and benefits

Estimating the costs of ISO 9001 certification is nearly impossible. A general rule of thumb exists, but has very limited value. It suggests that establishing the system typically requires approximately €1,000 to €3,000 per employee, and to maintain it, 10–20% of the initial costs annually (personal observation). In the case of CGN, the estimate for the initial costs probably is conservative. The actual costs were never calculated and strongly depended on the significant staff time invested in drafting, revising and editing the SOPs. Conversely, now that the system has been in place for an extended period, the annual maintenance cost is likely on the low side of the rule of thumb estimate. In fact, when accounting for the cost savings achieved through more efficient operations, it could be argued that CGN is actually realizing financial savings as a result of its quality management system.

Another important aspect to consider is staff perception and acceptance. At CGN, initial resistance to the introduction of quality management was significant, as it was viewed as a constraint on creativity, reducing employees to mere components of a mechanized system. Additionally, the use of KPIs to monitor processes was perceived as akin to ‘Big Brother’ surveillance. However, over time, staff members came to recognize and appreciate the benefits of a structured organization, and the importance of well-documented procedures became particularly evident during instances of succession, such as when retiring employees were replaced by new hires.

## A Genebank Certification System

### Rationale for a Genebank Certification System

Effective collaboration and division of responsibilities are vital for the conservation of PGR required by future generations. However, successful collaboration necessitates mutual trust among genebanks. By adopting standardized practices and achieving a shared quality level, genebanks can establish reliance on one another’s efforts, thereby facilitating efficient collaboration. This partnership not only enhances the secure conservation and accessibility of PGR for users but also improves management efficiency by minimizing unnecessary redundancy; many genebanks currently conserve overlapping collections. The establishment of mutual trust enables

the principle that ‘if you undertake this task, I do not need to do so’, thereby reducing long-term conservation costs and reallocating resources to address gaps in collective PGR collections and investments in quality improvement. This, in turn, enhances PGR utilization through improved characterization, documentation and better user interfaces.

In Europe, the PGR community, organized under the European Cooperative Programme for Plant Genetic Resources (ECPGR), recognizes the necessity of implementing a genebank certification system. In its *Plant Genetic Resources Strategy for Europe*, launched on 30 November 2021, the European PGR community calls on the establishment of an economically sustainable certification system accessible to genebanks (ECPGR, 2021). Also, the FAO Intergovernmental Technical Working Group on Plant Genetic Resources for Food and Agriculture has underscored the importance of a quality assurance system, preferring the term “acknowledgment system” aligned with the FAO Genebank Standards (FAO, 2014). They have recommended that the FAO investigate capacity-building and evaluation mechanisms to support genebanks in adhering to these standards (FAO, 2023).

Beyond the benefits of quality management for individual genebanks, certification will provide a framework to ensure that these institutions meet community-agreed standards for conservation and access, and that continuity is guaranteed. The FAO Genebank Standards (FAO, 2014) are well accepted for the operating procedures concerning handling material and the Standard Material Transfer Agreement (SMTA) of the ITPGRFA could provide the basis for the distribution of PGR (FAO, 2024). In addition, procedures for guaranteeing continuity will need to be set up. Should a genebank lose its certification, another certified genebank should be able to assume responsibility for the material from the ‘lost’ institution, thereby ensuring that PGR once integrated into the system remains preserved and accessible. Consequently, a certification system is essential for enhancing efficiency, reliability, transparency and accountability, given that the conservation and accessibility of PGR represent a global responsibility that must be upheld by all credible stakeholders, including international, regional and national genebanks.

### Components of a certification system

Implementing a certification system for genebanks necessitates several key elements. Firstly, the genebanks seeking certification must establish a robust QMS that enables an external auditor to assess both the activities undertaken and the methodologies employed. Secondly, the SOPs utilized within the genebanks must align with community-agreed standards. Lastly, a certification mechanism must be developed and administered by an organization endowed with adequate authority.

An increasing number of genebanks are in the process of establishing QMS; however, international support and coordination remain limited. The absence of coordinated

international oversight for PGR activities has resulted in a lack of responsibility for guiding genebanks in these critical advancements. Consequently, there is a risk that genebanks will repeatedly reinvent processes and protocols. The establishment of a central hub to provide training materials, templates and examples of effective QMS tailored to various types of genebanks and operations could significantly expedite this process.

The FAO Genebank Standards (FAO, 2014) serve as an excellent foundation for defining minimum operational quality levels. While some adaptations will be necessary to incorporate current technology and evolving insights, the fundamental objectives – namely, to conserve plant genetic resources for future generations while ensuring their availability to present users – are clearly articulated. Moreover, details concerning access provision to PGR must be formulated, alongside procedures to ensure continuity. The groundwork has already been laid through the initial set of Genebank Standards (FAO, 2014).

A critical element still to be finalized in the development of a genebank certification system is the appointment of a Certifying Agency. This agency would have several key responsibilities. Firstly, it would need to establish the general competency requirements for genebanks, reflecting the consensus of both the scientific and genebank communities. Secondly, it would be tasked with creating a verification process to ensure these requirements are met. This process should outline how the requirements are to be fulfilled and how their fulfilment will be assessed. Typically, this includes certification audits every three to five years, along with intermediate audits to monitor ongoing quality management within the genebank. Several organizations are currently under consideration for the role of Certifying Agency, with the goal and expectation of arriving at a suitable solution.

## Discussion

The QMS of CGN was developed independently, without following a predefined standard, leaving scope for further improvement. Rather than positioning this QMS as an exemplary model, it has been made publicly accessible to provide transparency and encourage constructive feedback (ECPGR, 2024). An open dialogue regarding quality management practices and procedures in genebanks would benefit all involved by providing exposure to actual QMS approaches in genebanks and stimulating discussion about SOPs. Furthermore, it will inspire the harmonization of these systems and enhance the quality of all genebanks involved.

Recently, CGN initiated an evaluation of the alignment of its procedures with the FAO Genebank Standards (FAO, 2014), revealing certain divergences in practice. For instance, CGN's approach to seed viability testing, which employs fixed thresholds, contrasts with the FAO's recommendation to test for specific declines in viability (Wijnker *et al.*, 2024). While CGN's alternative approach is obviously based on a considered rationale,

feedback from the genebank community and possibly a certifying body could provide valuable input for further improvement.

In its commitment to ensuring the accessibility of PGR, CGN currently lacks a formal contingency plan should it cease operations or be unable to provide access to the genetic resources in its collections, thus jeopardizing access to PGR. A network of certified genebanks could play a critical role in these circumstances, taking over and potentially keeping the PGR currently in CGNs collection conserved and accessible. A genebank certification system would provide the credibility needed. Moreover, in combination with the legal assurances provided by the SMTA, it could provide a robust foundation for continued access to these resources in an open network of certified genebanks.

## Conclusions

Quality management serves as a crucial instrument for enhancing the effectiveness and efficiency of genebanks, establishing a foundation for collaboration. The experiences of various genebanks, particularly those of CGN, demonstrate the positive impacts of implementing quality management practices. By integrating quality management with community-agreed minimum standards for genebank operations, a foundation is established for genebank certification. This certification would represent a significant advancement toward ensuring the proper conservation of, and access to, PGR for both present and future generations of users, ultimately contributing to global food security.

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## Conflict of interest statement

The authors declare that they have no conflicts of interest.

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# History and current status of plant genetic resources conserved and maintained by the Hungarian central genebank

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**Abstract:** The predecessor of the National Centre for Biodiversity and Gene Conservation (NBGK) was established in Hungary in 1959. The 1950s were hectic times for Hungarian plant breeders, and many new genetic materials were registered in the National List of Varieties. In order to save obsolete genetic resources, in 1959 the government founded the Agrobotanical Institute at Tápiószele to prevent plant genetic erosion in the Pannonian region. The centre started its operation with 16,596 accessions. Their quantity continuously increased thanks to collecting missions and international connections. The NBGK collection is composed of cereals (37.3%), vegetables (18.7%), legumes (17.4%), industrial crops (5.29%), fruits and grapes (3.85%) and others. NBGK has operated under its current name since 2019 with the same mission as when it was first established. Today, it is the seventh largest genebank in Europe with 57,381 accessions of 1,745 plant species across 605 genera. Almost 95% of samples are maintained in the form of seeds in 15 cooled storage rooms (at temperatures of 5–8°C or -18°C), while the others are conserved *in vitro*, in the form of tubers or field collections. Sharing genetic materials has been a crucial part of the institute's activities since the beginning. Between 2019 and 2023, a total of 92,100 samples were distributed to a variety of partners, mainly gardeners and farmers (83.85%) and NGOs (14.63%). Researchers, breeders and universities account for only 1.52% of seed requests, which is the opposite of what is observed in other genebanks.

**Keywords:** plant genetic resources, gene conservation, *ex situ*, seed bank, Pannonian region

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## Foundation of the Hungarian genebank

The history of the National Centre for Biodiversity and Gene Conservation (Hungarian abbreviation NBGK) and its legal predecessor institutes started in the 1880s. Dr Lajos Szelényi, a Hungarian doctor born in Kismarton (today Eisenstadt, Austria), moved to Budapest after finishing his university studies in Vienna. Besides his healing activities, he was famous for his charity work. He donated large sums of money to Austrian and Hungarian medical and agricultural science. Dr Szelényi purchased 400 acres of land in Tápiószele, in the Central Hungarian Region, which he donated to the National Hungarian

Economic Association in his 1885 will, specifying that the land was to be used by future generations for agricultural experiments and vocational education in agriculture. The work of the first few decades was destroyed several times by the World Wars, revolutions and political transformations. The 1950s were hectic times for Hungarian plant breeders, and many new genetic materials were registered in the National List of Varieties. In order to save the old and obsolete varieties, collection departments were established at the breeding institutions. The Agrobotanical Institute was established on the land in Tápiószele left by Dr Szelényi under the leadership of Dr Andor Jánosy (1908–1975) based on a government initiative in 1959, with the aim of integrating the genetic resource collections of the country and preventing genetic erosion (Jánosy,

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1971). Despite several institutional reorganizations and changes of responsible organizations, the Hungarian genebank operated under its current name and form since 2019, and its function and scope of duties have changed little over the last 65 years. Currently, the NBGK operates as a central budgetary institution fulfilling public functions. It is maintained by the Hungarian Ministry of Agriculture, with 50% of its annual budget financed by the government and the other 50% covered through grants. The majority of these grants come from national sources, but some are financed by the European Union.

### Composition and expansion of the collection

The institute started its operation with 16,596 accessions of 871 plant species. According to our database, the starting number of accessions included both Hungarian and foreign materials. The number of accessions has continuously increased thanks to wide international scientific connections and collecting expeditions in Hungary and across the Carpathian Basin. Since its establishment, the institute has exchanged genetic materials with around 400 other institutes, but development through collecting missions also has a long tradition in the genebank (Guerrant *et al*, 2014). Our founder and first director, Dr Andor Jánosy, recognized already at the beginning of the 1950s that landraces were going to disappear from public production due to the spread of large-scale industrialized agriculture. He was one of the first in the world to collect cereals, fodder crops, maize and vegetable landraces and local varieties in cooperation with his colleagues before they became lost from production, replaced by high-yielding, intensively farmed improved varieties. This activity has always been regarded as an important duty of the institute, and it is still carried out today, for example in the case of fig genotypes. In 2023, we collected 22 fig (*Ficus carica* L.) genotypes from the northern shore of Lake Balaton, one from Budapest and another one from West Hungary. During the collecting trips, we measured and characterized fruits, leaves and branches. The collected twigs are propagated in our nursery. As part of the Pannon Seed Bank Project (a LIFE+ programme, LIFE08 NAT/H/000288), 2,064 accessions were collected between 2010 and 2014. These are vascular wild plants of the Pannonian biogeographical region and wild relatives of cultivated plant species (Hay and Probert, 2013; Walters *et al*, 2018). In the last 70 years, plant genetic resources have been collected from a total of 1,504 locations (Figure 1), adding 13,785 accessions of Hungarian origin to the collection of NBGK (Table 1). Today, collection work is also carried out abroad, thanks to collecting trips jointly organized with the genebanks of neighbouring countries (Slovakia and Romania). This has resulted in 2,477 landraces, local varieties, populations and ecotypes being collected from 340 locations. In addition, a further 237 accessions collected by foreign genebanks have been shipped to our institute through seed exchange.

With regard to the amount of conserved genetic material, NBGK is the seventh largest genebank in Europe today, preserving 57,381 accessions of 1,745 species from 605 genera. Our oldest accessions date back to the early 1950s. Both the number of accessions and taxa was continuously increased until the 2010s, with a levelling off in the 2020s (Figure 2).

Regarding the composition of the different plant groups (Table 2), our collection is dominated by cereals with 21,376 accessions, making up 37.3% of the collection. Besides the major spiked cereals like wheat (*Triticum* spp.) and barley (*Hordeum* spp.), maize (*Zea* spp.) and sorghum (*Sorghum* spp.), pseudocereals like amaranths (*Amaranthus* spp.), certain buckwheat species (*Fagopyrum* spp.), finger millet (*Eleusine coracana* (L.) Gaertn.) and teff (*Eragrostis tef* (Zucc.) Trotter) also belong to this group. Vegetables and grain legumes constitute 18.7% and 17.4% of the collection, respectively. The former group consists of the collections of tomato (*Lycopersicon* spp.), paprika (*Capsicum* spp.), Cucurbitaceae, onions (*Allium* spp.), root and leafy vegetables and other vegetables (e.g. *Physalis* spp.). Almost half of the grain legumes collection (4,322) is composed of *Phaseolus* species accessions originating from the American centre of origin and diversity, but pea (*Pisum* spp.), chickpea (*Cicer* spp.), lentil (*Lens* spp.) and soybean (*Glycine* spp.) accessions are also present. The three above-mentioned groups constitute 73.4% of the whole collection. The group of industrial crops containing 3,040 accessions includes genera such as sunflower (*Helianthus* spp.), flax (*Linum* spp.), poppy (*Papaver* spp.) and the neglected camelina (*Camelina* spp.) used for oil production. The group of forage legumes includes the accessions of 113 species from 20 genera, the most important of which are clovers (*Trifolium* spp.), alfalfas (*Medicago* spp.) and vetches (*Vicia* spp.) constituting 80.7% of this group. In terms of the number of taxa, Poaceae species are the second most diverse group (the first being herbs like medical plants from Lamiaceae and Asteraceae families) including 171 species of 51 genera. Almost half of the 2,298 accessions of grasses have been collected. Accessions of the Pannon Seed Bank are listed as a separate group.

The accessions of the above-mentioned utilization groups are stored generatively as seeds in the genebank, equating to 94.44% of the collection. Besides this method, the institute has other ways of preserving plant genetic resources. Although such samples are only a small part of the collection, they are very valuable. In recent years, the ratio of collections preserved in the form of field collections has increased. Woody fruit crops, grapes and ornamentals are in this group, composing 3.85% of the collection. The collection of potato (*Solanum tuberosum* L.) and its wild relatives are preserved *in vitro* (Engelmann, 2011), constituting 1.24% of the genebank collections. From the group of tuber crops, Jerusalem artichoke (*Helianthus tuberosus* L.), sweet potato (*Ipomoea batatas* L.) and onions

(*Allium* spp.) are preserved vegetatively, making up 0.47% of the collections.

### Multiplication and maintenance of genebank accessions

Genotypes stored in the form of seeds, tubers, bulbs or *in vitro* are reproduced in the field if the minimal number of seeds required for genebank storage has to be reached, viability has declined, taxonomic analyses are necessary, the accession needs to be multiplied for distribution purposes, or experiments are required to be performed. Accessions conserved in the form of field collection are kept always outside in the fields. Three professional teams carry out the work related to such duties: the Department of Arable Crops, the Department of Horticultural Crops and the Department of Fruit Crops. The annual sowing plan is defined by the number of accessions requiring regeneration for the above-mentioned reasons and the number of spatially isolated field plots available for sowing. The number of accessions regenerated by growing new individuals in the field in the last 10 years is shown in Table 3.

The number of accessions sown from the different plant groups fluctuates yearly. This is not only because the different collections contain a different number of accessions, but also due to the diversity of factors that need to be considered during multiplication. Between 2014 and 2023 plant groups with large collections – such as grain legumes, cereals, herbs and onions – dominated the multiplication process every year. Consequently, members of the *Phaseolus*, *Triticum*, *Allium*, *Lycopersicon*, *Origanum* and *Capsicum* genera reached the highest levels of multiplication in the last ten years. In this period, common bean (*Phaseolus vulgaris* L.) and winter wheat (*Triticum aestivum* L.) were the most frequently multiplied species. The genebank established its fruit crop collection in 2013. Due to its continuous expansion, today it contains 1,212 accessions. Besides the members of the Maloideae subfamily (apple (*Malus domestica* Borkh.), pear (*Pyrus communis* L.), medlar (*Mespilus germanica* J.B. Phipps), quince (*Cydonia oblonga* Mill.), stone fruits (plum (*Prunus domestica* L.), sour cherry (*Prunus cerasus* L.), cherry (*Prunus avium* L.), peach (*Prunus persica* L.), apricot (*Prunus armeniaca* L.)) and other fruit species – such as Cornelian cherry (*Cornus mas* L.) and service tree (*Sorbus* spp.) – are also found in the almost 9ha plantation. Furthermore, the collection is complemented by a 1ha walnut (*Juglans regia* L.) plantation and a 0.3ha grape (*Vitis vinifera* L.) plantation. Usually, three individual plants are conserved for each genotype. The first plants started to produce fruit in 2020. Our institute has conserved a vegetative collection of ornamental plant varieties (*Iris*, *Hemerocallis*, *Hibiscus*, *Hosta* spp.) of 976 accessions in the nurseries of the genebank since 2017. There is also a rare and old woody plant stand in the central site of the genebank, which functions as a locally protected arboretum. Some of the oak trees of the garden have been planted around

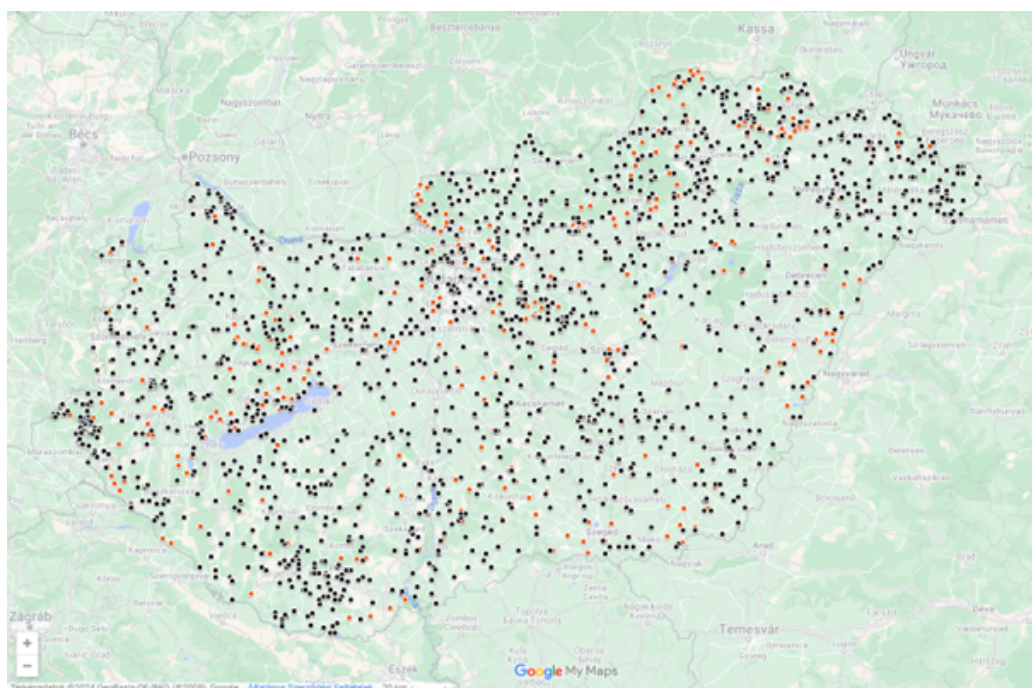
the mansion (currently the main building) already in the time of Dr Lajos Szelényi. Unfortunately, the majority of the original English park died. The current garden was planted by the employees of the institute in the 1960s and 1970s. About 350 tree and bush species live here, including several rare ones like the Californian white oak (*Quercus lobata* Née), the Oregon cypress (*Hesperocyparis bakeri* Bartel), the Algerian fir (*Abies numidica* de Lannoy), the Trojan fir (*Abies nordmanniana* subsp. *equi-trojani* Spach), the Cilician fir (*Abies cilicica* Carrière), the Spanish fir (*Abies pinsapo* Boiss.), the Turkish fir (*Abies bornmuelleriana* Coode & Cullen), the Lebanon cedar (*Cedrus libani* subsp. *libani* A. Rich.) and the mountain pine (*Pinus uncinata* Turra).

The agrobotanical analysis and the assessment of the morphological biodiversity of plants sown in the fields are carried out by taxonomists according to Hungarian and international guidelines (Table 4). The descriptors used for characterization are based on the descriptors lists of the International Union for the Protection of New Varieties of Plants (UPOV, 2005), the International Board of Plant Genetic Resources (IBPGR) (Thormann et al, 2018), the European Cooperative Programme for Plant Genetic Resources (ECPGR) and in the case of grapes the International Organisation of Vine and Wine (OIV) (Hannin et al, 2006). For some plant species, we have refined or complemented the criteria system for agrobotanical analysis. For example, in the case of carrot, the UPOV guidelines have been integrated with the IBPGR descriptors and as a result, the following features are also recorded: colour of core, colour of cortex, root diameter of core relative to total diameter, and homogeneity of flesh colouring throughout root length. Today these characterization data are almost always confirmed by photos as well. Data are documented electronically and then assessed and stored in the database of the institute. The digitalization of the former paper-based agrobotanical analyses is a great challenge for us.

### Human resources, infrastructural developments

The Hungarian genebank started its operation with 81 permanent workers in 1959. The number of employees exceeded 200 within ten years. Our first director, Dr Andor Jánosy, put great emphasis on scientific research, so he hired many researchers. Being a member of the genebank's staff was prestigious in the 1970s. Political transformations that occurred in Hungary in 1990 led to the decline of the institute. Due to financial reasons, the number of employees was reduced to only 40 people within a few years, and the survival of the institute was at risk. Fortunately, since 2010 the Hungarian government has considered conservation of plant genetic diversity as an important issue once again, with a public function of strategic importance. Today the institute has 130 employees (Figure 3), six of which are scientists (dealing with research and development). More than 35% of workers have a university degree.

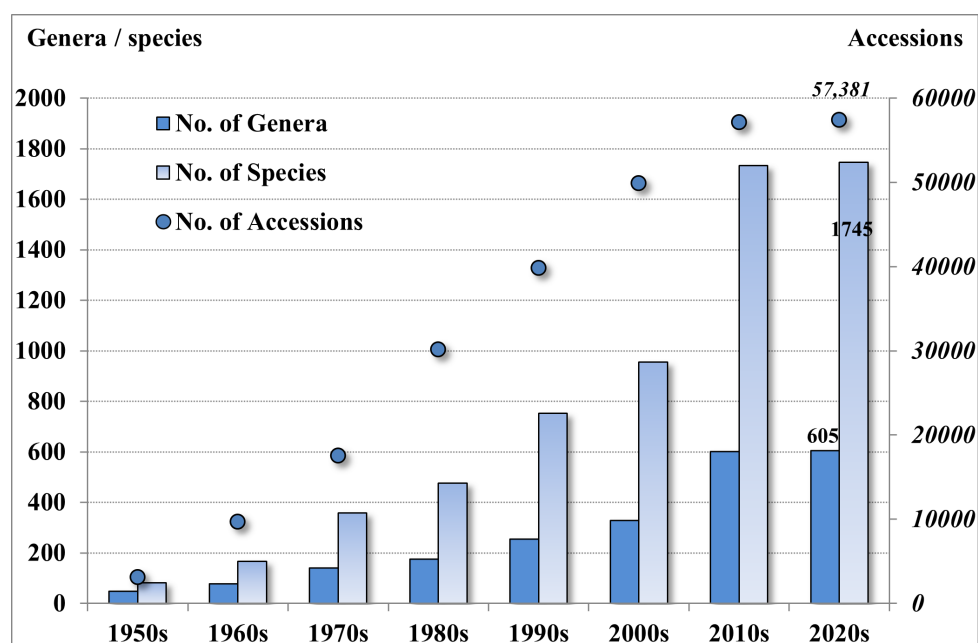




**Figure 1.** Collection activity of the National Centre for Biodiversity and Gene Conservation (NBGK) in Hungary (1950–2023). Black, cultivated plants; red, wild plants (Pannon Seed Bank). End of data collection: 31 December 2023 (Google Maps).

**Table 1.** Division of collected accessions by place of origin

Places of collection	NBGK		Pannon Seed Bank		Total	
	Accessions	Locations	Accessions	Locations	Accessions	Locations
Hungary	11,802	1,322	1,983	416	13,785	1,504
Neighbouring country	2,396	319	81	21	2,477	340
Other country	237	166	0	0	237	166
Total	14,435	1,807	2,064	437	16,499	2,010



**Figure 2.** The increasing number of genera, species and accessions maintained by the National Centre for Biodiversity and Gene Conservation (NBGK) from the date of its founding until now. End of data collection: 31 December 2023.



**Table 2.** Division of the National Centre for Biodiversity and Gene Conservation (NBGK) collection by utilization groups. \*, roots and tubers, ornamentals and fruit crops.

Method of conservation	Plant utilization group	Accessions	Ratio of the whole collection (%)
Generative (94.44%)	Cereals	21,376	37.3%
	Vegetables	10,739	18.7%
	Grain legumes	10,002	17.4%
	Industrial crops	3,040	5.3%
	Fodder legumes	2,823	4.9%
	Grasses	2,298	4.0%
	Herbs	1,163	2.0%
	Others *	687	1.2%
	Wild species (Pannon Seed Bank)	2,064	3.6%
Vegetative (0.47%)	Tuber crops	63	0.1%
	Onions	209	0.4%
<i>In vitro</i> (1.24%)	Potato and its wild relatives	709	1.2%
Plantation (3.85%)	Woody fruit crops	1,051	1.8%
	Ornamentals	996	1.7%
	Grapes	161	0.3%
<b>Total</b>		<b>57,381</b>	<b>100,0%</b>

**Table 3.** Yearly number of accessions regenerated in the field between 2014 and 2023 divided by plant groups. DAC, Department of Arable Crops; DHC, Department of Horticultural Crops.

	Plant group	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023
DAC	Grain legumes	2,984	2,286	2,647	2,166	1,717	1,952	2,040	2,550	2,320	1,879
	Cereals	1,657	1,071	1,712	1,445	2,542	2,163	2,049	2,089	2,725	2,582
	Fodder legumes	720	566	484	382	646	583	491	360	367	330
	Grasses	395	364	367	478	827	621	499	239	235	296
	Industrial crops	296	207	214	231	314	384	418	274	229	289
	Cucurbitaceae	370	155	218	126	120	261	238	140	98	130
	Root and tuber crops	96	98	110	128	158	120	102	79	98	100
	Pseudocereals	34	69	78	51	16	18	52	27	20	48
	<b>Total</b>	<b>6,552</b>	<b>4,816</b>	<b>5,830</b>	<b>5,007</b>	<b>6,340</b>	<b>6,102</b>	<b>5,889</b>	<b>5,758</b>	<b>6,092</b>	<b>5,654</b>
DHC	Onions	235	294	286	321	288	441	403	346	327	345
	Tomato	67	56	82	51	49	102	138	70	50	71
	Paprika	84	54	53	47	44	80	112	53	26	53
	Other Solanaceae	66	26	56	7	11	17	30	4	8	6
	Root vegetables	123	81	85	99	90	181	184	132	114	128
	Leafy vegetables	161	100	51	60	61	160	160	98	87	124
	Brassicaceae	50	27	56	45	29	52	67	30	41	30
	Cucurbitaceae	149	42	19	5	14	51	47	72	39	39
	Herbs	53	69	234	221	207	270	708	561	602	467
	Ornamentals	33	12	23	25	43	65	52	192	140	151
	Other vegetables	9	3	0	0	0	0	8	0	0	0
	<b>Total</b>	<b>1,030</b>	<b>764</b>	<b>945</b>	<b>881</b>	<b>836</b>	<b>1,419</b>	<b>1,909</b>	<b>1,558</b>	<b>1,434</b>	<b>1,414</b>

**Table 4.** Descriptors for morphological diversity – an example using the agrobotanical description of a pear landrace according to the UPOV guidelines (2023).

Name of variety	‘Hidegkúti nyári’ summer pear
Shoot shape	Straight, the internodes are long
Shoot colour	Brown-red on the sunny side with few lenticels
Vegetative bud	Rounded, markedly held out from shoot
Bud support size	Medium
Average shoot length	62cm
Average internode length/thickness	27.4mm/4.24mm
Average leaf length/width	61.2mm/32.9mm
Average petiole length/thickness	36mm/0.56mm
Flower bud	Short, mainly on spurs
Petals	Long, overlap, small in size, ovate in shape
Position of stigma	Mostly above the level of the anthers
Time of maturity	End of July – beginning of August
Fruit size	Short in height, small in diameter, the height-to-diameter ratio is approximately 1:1
Average fruit height/diameter	51.9mm/55mm
Average fruit weight	68.1g
Fruit shape	The maximum diameter is in the centre of the fruit, the fruit is longitudinally symmetrical and the lateral shape is convex
Fruit ground colour	Yellow
Fruit cover colour	Absent
Area of russet	Small around the eye basin, none on the cheeks and around the stalk attachment
Stalk shape	Moderately long and thick, slightly curved, straight in relation to the axis of the fruit
Average stalk length/thickness	28.5mm/9.5mm
Average stalk cavity depth/width	1.1mm/12.8mm
Average eye basin dept /width	3.2mm/16.4mm
Flesh	Soft, medium juicy, fine structure
Sugar content	17.4Bx°
Seed	Elliptic

**Figure 3.** Staff of the National Centre for Biodiversity and Gene Conservation (NBGK) in summer 2022. Photo by Gergely Gócsa, w [www.gocsafoto.hu](http://www.gocsafoto.hu)

The infrastructure of the genebank – including the buildings and equipment – has continuously developed over time. During the first decade, seed conservation was carried out by storing the seeds in paper bags at room temperature. The first cold storage room – operating at 4°C – was built in 1971. At the time, this was one of the first seed storage rooms of its kind in Europe. In order to make seed storage more effective, paper bags were replaced by aluminium bags and glass jars. As a result of large investment in recent years, one of our old buildings has been completely renovated (Figure 4). From 2024, all the cold storage rooms, seed drying rooms, and germination testing laboratory are located in the same place. Currently, 15 cold storage rooms are operating for direct seed conservation purposes. There are nine active storage rooms running at a temperature of 5–8°C ensuring medium-term conservation, and we have six base storage rooms cooling seeds to -18°C for long-term storage of genebank accessions of orthodox species (Dickie *et al.*, 1990; Nagel and Börner, 2010). From the latter base storage rooms, three rooms have a special status: the National Base Storage Room, the Pannon Seed Bank and the Safety Duplicate Storage Room.

The National Base Storage Room has been used since 1996, with the aim of maintaining safety duplicates from the collections of the other Hungarian gene conservation institutes at no cost. So far, 26 Hungarian gene conservation institutes have sent genetic materials totalling 16,966 accessions of 219 plant species from 124 genera. The Pannon Seed Bank project was a LIFE+ programme running between 2011 and 2015, aiming at the long-term conservation of native plant species of the Pannonian Biogeographical Region whose seeds can be stored with this technology. After a transition period, we rethought the project, and continued the monitoring and collection of wild plant species from the region in 2017. This cold storage room contains 2,064 accessions of 921 species from 430 genera today. In 2014 our genebank built the Safety Duplicate Storage Room with the support of the Hungarian Ministry of Agriculture and the Aggtelek National Park in the strictly guarded passage of a dripstone cave. The role of this storage room is to duplicate the seeds of the most important plant genetic resources for food and agriculture (25% of the whole collection), including landraces, local populations and ecotypes collected from the Carpathian Basin and also old varieties that have disappeared from public production. So far, 6,733 accessions of 289 species from 159 genera have been put in the Safety Duplicate Storage Room.

Our equipment has been continuously modernized over the last 10 years, with the decreasing availability of physical labour force, driving us to purchase modern machines. Our former plot seeder and harvester have been replaced by new machinery in the last 1–2 years. For decades, we used Russian tractors and implements, but today we work with those produced in Western Europe and Hungary. Our germination testing laboratory

has been upgraded by purchasing new incubators. The analytical, genetic and tissue culture laboratory has been equipped with state-of-the-art devices (Figure 5). In the future, we plan to renovate the greenhouses built in 1961 and also to obtain phenotyping systems.

## Breeding and maintenance of varieties

Since its establishment, NBGK has regarded plant breeding as an important secondary activity. Our institute used to be one of the sites of the national variety testing network, where the performance testing of candidate varieties (plant materials under a 3-year registration process) took place. By applying the methods of positive individual selection and crossbreeding, the researchers of the genebank have developed 28 new varieties (Table 5). The qualifying certificates of our listed varieties are kept in our library (Figure 6). Many of them have been on the National List of Varieties for decades. The maintenance and propagule production of these varieties also take place in Tápiószéle. Today, three Jerusalem artichoke (*Helianthus tuberosus*) varieties ('Tápiói korai', 'Tápiói sima', 'Tápiói piros') and one sweet potato (*Ipomoea batatas*) variety ('Tápiói 96') are included in this process.

In 2023, our genebank applied for the registration of 17 candidate varieties on the National List of Varieties in the following categories: variety, landrace and variety developed for growing under particular conditions.

Our candidate varieties are: one peanut (*Arachis hypogaea* L.), two chickpea (*Cicer arietinum* L.), two cowpea (*Vigna unguiculata* (L.) Walp.), one teff (*Eragrostis tef*), one coracan (*Eleusine coracana*).

Our landrace candidates are: one sunflower (*Helianthus annuus* L.), one sorghum (*Sorghum bicolor* (L.) Moench.), one millet (*Panicum miliaceum* L.), two flax (*Linum usitatissimum* L.), one barley (*Hordeum vulgare* L.), one maize (*Zea mays* L.), one safflower (*Carthamus tinctorius* L.), one fodder watermelon (*Citrullus amarus* Schrad.), two tomato (*Lycopersicon esculentum* Mill.), one paprika (*Capsicum annum* L.).

Our candidate variety developed for growing under particular conditions is: one lentil (*Lens culinaris* Medik.).

Our tomato and paprika landrace candidates were selected from genebank collections of 2,097 accessions and 3,615 accessions, respectively. We also perform preparatory and monitoring activities for variety development for other species, such as winter wheat, rye, common bean, kidney vetch (*Anthyllis vulneraria* L.), crested wheatgrass (*Agropyron cristatum* (L.) Gaertn.), timothy grass (*Phleum pratense* L.), smooth brome (*Bromopsis inermis* (Leyss.) Holub), poppy, onion, beetroot (*Beta vulgaris* L. subsp. *vulgaris*), parsley (*Petroselinum crispum* (Mill.) Fuss), carrot (*Daucus carota* subsp. *sativus* (Hoffm.) Arcang.), calendula (*Calendula officinalis* L.), summer savory (*Satureja hortensis* L.), dill (*Anethum graveolens* L.) and oregano (*Origanum vulgare* L.). In the case of these species, we currently do not have enough seeds for variety certifying analyses and for





**Figure 4.** Ceremonial handover of the new genebank building of the National Centre for Biodiversity and Gene Conservation (NBGK) on 10 July 2024. We plan to use this new building for at least 50 years for professional conservation of plant genetic resources in Hungary. Photo by Anikó Gál Soltész, NBGK



**Figure 5.** (a) Oat (*Avena sativa* L.) accessions in the active storage room at a temperature of 5–8°C in 2015; (b) Sowing of spiked cereals with a plot seeder in autumn 2019; (c) Measuring crude protein content in the biochemical laboratory in 2022; (d) DNA isolation from tetraploid wheat accessions in 2022. Photos by Attila Simon, Lajos Horváth and Dóra Bárdos.





**Figure 6.** Qualification certificate of the soybean variety called ‘Pannonia 10’ from 1967. The breeder was Viktor Ferenczi. Digitalized by NBGK Library.

super-elite multiplication, and the different varieties still need to be fully described. We plan to apply for the registration of these candidate varieties on the National List of Varieties within the next five years.

The registration of landraces on the National List of Varieties is difficult in Hungary due to bureaucratic reasons, since these varieties cannot fulfil the criteria of uniformity during the DUS tests (Distinctness, Uniformity and Stability), or they often do not exceed the yield level of modern improved varieties used as control varieties during performance tests.

### Hungarian and international scientific and social relations

The institute has always worked to meet international professional requirements (Cromarty *et al*, 1982; FAO, 2014). Director Dr Andor Jánossy organized the EUCARPIA congress in Budapest in 1974 (Jánossy and Lupton, 1974), during which the participants also visited the genebank in Tápiószéle, which by that time already had an international reputation (Figure 7). In addition, our institute published the scientific journal *Agrobotanika* between 1959 and 1975, presenting the results of research colleagues and describing collecting trips.

Hungary signed the Convention on Biological Diversity (CBD), ratified the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA), and joined the quality assurance programme of A European Genebank Integrated System (AEGIS). Our institute is member of the Promoting a Plant Genetic

Resources for Europe (PRO-GRACE) consortium, created within the Horizon Europe programme in 2023. We participate in ECPGR Working Group activities, and our collection is available in the *ex situ* database of the European Search Catalogue for Plant Genetic Resources (EURISCO). NBGK provides the presidency of the Hungarian Plant Genebank Council, a consultative platform established in 2011. It works as an independent professional advisory board besides the Minister of Agriculture. It is responsible for the professional representation of plant gene conservation, as well as related research and development issues in Hungary. Additionally, it provides expert opinions on legislation, applications and national programmes related to plant gene conservation, including to the Minister of Agriculture. We have contacts with several universities and around 30 university students spend their professional practices in the genebank every year, who may become future employees of the institute. We participate in joint research and development projects together with universities and innovative enterprises from the commercial sector. We perform field experiments with drought-resistant alternative species, edible grain legumes, fodder cereals, oil crops and Jerusalem artichoke, winter and spring lentil, chickpea and poppy. We study the chemical composition of apple, pear, apricot, pumpkin, beetroot, celeriac, carrot, tomato and herb accessions stored in the genebank by analytical methods (Table 6). In our genetic laboratory, we analyze tetraploid wheats (Röder *et al*, 1998) and the genetic relationship between *in vitro* conserved potatoes by using SSR (simple sequence repeats or microsatellite) markers.

**Table 5.** Registered plant varieties in Hungary bred/maintained by the National Centre for Biodiversity and Gene Conservation (NBGK) (1954–2024). Year, year of registration in Hungary.

Denomination of species		Hungarian name of variety	Breeders	Year
Scientific name	Common name			
<i>Avena sativa</i> L.	Oat	Tápláni csupasz	Mr Miklós DEUTSCH, Mrs Zsuzsa WESEL	1973
<i>Capsicum annuum</i> L.	Sweet pepper	Kocsolai zöldhúsú	NBGK	1970
<i>Glycine max</i> L.	Soybean	Pannonia 10	Mr Viktor FERENCZI	1967
<i>Glycine max</i> L.	Soybean	Tápláni takarmány	Mr Árpád SZÜCS, Mrs Zsuzsanna KANYÓ	1968
<i>Helianthus tuberosus</i> L.	Jerusalem artichoke	Tápiói korai	Mr Lajos HORVÁTH, Mrs Ágnes BÁRDY, Mr László HOLLY, Mr József BARTA	2003
<i>Helianthus tuberosus</i> L.	Jerusalem artichoke	Tápiói sima	NBGK	2003
<i>Helianthus tuberosus</i> L.	Jerusalem artichoke	Tápiói piros	NBGK	2022
<i>Hordeum vulgare</i> L.	Spring barley	Tápláni tavaszi	Mr Miklós DEUTSCH, Mr Andor JÁNOSSY Dr, Mrs Józsefné NÉMETH	1968
<i>Ipomoea batatas</i> L.	Sweet potato	Tápiói 96	NBGK	2003
<i>Lycopersicon esculentum</i> Mill.	Tomato	Tápláni konzerv	Mr János ÁVÁR, Mrs Jánosné ÁVÁR	1970
<i>Medicago sativa</i> L.	Alfalfa	Bánkúti	Mr Andor JÁNOSSY Dr, Mr Zoltán CSÁK, Mr Zoltán BÖJTÖS	1961
<i>Medicago sativa</i> L.	Alfalfa	Békésszentandrás	Mr Andor JÁNOSSY Dr, Mr Zoltán CSÁK, Mr Zoltán BÖJTÖS	1961
<i>Medicago sativa</i> L.	Alfalfa	Nagyszénási	Mr Andor JÁNOSSY Dr, Mr Zoltán CSÁK, Mr Zoltán BÖJTÖS	1961
<i>Medicago sativa</i> L.	Alfalfa	Szarvasi	Mr Andor JÁNOSSY Dr, Mr Zoltán CSÁK, Mr Zoltán BÖJTÖS	1961
<i>Medicago sativa</i> L.	Alfalfa	Tápiószelei 1	Mr Andor JÁNOSSY Dr, Mr Árpád SZÜCS	1970
<i>Melilotus albus</i> Medik.	Sweet clover	Kecskeméti kétéves	NBGK	1969
<i>Oryza sativa</i> L.	Rice	Nucleoryza	Mr Zoltán SAJÓ, Mr József SIMON	1979
<i>Panicum miliaceum</i> L.	Millet	Topáz	NBGK	1986
<i>Phaseolus vulgaris</i> L.	Bean	Nagykállói étkezési	Mr Ambrus SZABÓ, Mrs Ambrusné SZABÓ	1979
<i>Phaseolus vulgaris</i> L.	Bean	Tápiói cirmos étkezési	Mr Árpád SZÜCS, Mrs Árpádné SZÜCS, Mrs Józsefné NÉMETH	1980
<i>Phaseolus vulgaris</i> L. var. nanus	Bean	Tápiószelei barnabab	Mr Árpád SZÜCS, Mrs Zsuzsanna KANYÓ	1967

Continued on next page

Table 5 continued

Denomination of species		Hungarian name of variety	Breeders	Year
Scientific name	Common name			
<i>Phaseolus vulgaris</i> L. var. <i>nanus</i>	Bean	Tápiószelei fürjbab	Mr Árpád SZÜCS, Mrs Zsuzsanna KANYÓ	1967
<i>Phaseolus vulgaris</i> L. var. <i>nanus</i>	Bean	Tápiói gyöngybab	Mr Árpád SZÜCS, Mrs Zsuzsanna KANYÓ	1970
<i>Trifolium incarnatum</i> L.	Crimson clover	Kemenesaljai	Mr Andor JÁNOSSY Dr, Mr Miklós DEUTSCH	1968
<i>Trifolium pratense</i> L.	Red clover	Táplánszentkereszti diploid	NBGK	1954
<i>Trifolium pratense</i> L.	Red clover	Táplánszentkereszti	Mr Miklós DEUTSCH	1955
<i>Trifolium pratense</i> L.	Red clover	Hungaropoly tetraploid	Mr Andor JÁNOSSY Dr, Mr Miklós DEUTSCH, Mrs Lajosné HORVÁTH Dr, Mr Árpád SZÜCS, Mr László BÁNYAI	1966
<i>Trifolium pratense</i> L.	Red clover	Tápiói tetraploid	Mr Andor JÁNOSSY Dr, Mr Miklós DEUTSCH, Mr Árpád SZÜCS, Mrs Lajosné HORVÁTH Dr, Mr István SÜLYÖK	1970

Additionally, we coordinate our own on-farm network. Within a given landscape and agricultural district, plant populations adapted to local biotic and abiotic factors are often the most stable varieties (Holly *et al*, 2009). The on-farm programme was launched with four farmers in 2018. The network is expanding; in 2024, we are working with 20 farmers. Collecting seeds in their fields or gardens and recording information concerning the motivation of farmers in growing these varieties has contributed to our knowledge of agricultural biodiversity. Landraces of crops such as maize, vetches, cucurbits, beans, paprika, rye (*Secale cereale* M. Bieb.) and some underutilized species (i.e. safflower) are used in on-farm conservation in various regions within Hungary. Farmers and gardeners taking part in the programme can try those landraces which had been collected from their area decades ago. Our partners worked with 430 landraces between 2018 and 2023. Since they report on their experience, the genebank gets useful first-hand information on the actual producibility and marketability of these plant genotypes.

Our results are published in an open-access format (Gyurkó *et al*, 2023; Kis *et al*, 2023). Besides international scientific articles, our Hungarian popular publications are also well-known among the local people. We also organize and take part in seed swaps run by NGOs in different parts of the country. Our institute is open for groups of visitors by prior arrangement. Every year we present the genebank to hundreds of professionals and lay visitors in Tápíószele.

## Distribution

The National Gene Conservation Strategy sets measures for the accessibility, mobilization and distribution of genebank samples. Increasing and keeping this activity at a high level has become the most important tool for the utilization of the collections.

Distribution has been one of the goals of our institute since the beginning (Figure 8). In the first ten years of operation, the genebank disseminated about 5,000 seed samples for plant breeding purposes. Between 2019 and 2023, we registered 10,136 seed requests, from which 9,915 have been fulfilled by shipping 92,100 samples.

We operate a separate website for this activity (www.mintakeres.hu), which works as a webshop. There are two seed dissemination campaigns each year (one in spring and the other in autumn). The majority of seed distributions (83.85%) are directed to hobby gardeners and farmers, with the remaining part to NGOs, breeding and research institutes (Table 7). According to the research coordinated by the Centre for Genetic Resources, The Netherlands (CGN) within the PRO-GRACE consortium in December 2023, the situation is the opposite for the majority of European genebanks for which the majority of distributions are directed to breeding and research institutes (van Hintum, unpublished). For scientific, research-related, educational or cultural seed requests, our whole collection is available in addition to those varieties

listed in the webshop. However, in this case, a standard material transfer agreement (SMTA) (Correa, 2006) needs to be signed, and the number of stored propagules should not fall below our critical threshold limit as a result of the request (2,000 seeds on average). The most distributed species in the last five years were paprika (10,289 samples), tomato (9,234 samples), maize (4,225 samples), basil (*Ocimum basilicum* L.; 1,916 samples) and common bean (1,865 samples). We shipped 425 samples abroad. About 60% of them were requested by research institutes, while the remaining others by hobby gardeners. The number of seed requests reached its peak in 2023, while the number of distributed samples peaked in 2021. We aim to maintain these same high levels of distributions in the future. This is not a profitable activity for our institute since those requesting seeds only have to pay a 5 EUR handling fee. Seed dissemination for research and educational purposes is free of charge.

The fruit gardener agreement – ‘Agreement on cooperation in the conservation of fruit varieties long cultivated in the Carpathian Basin and adapted to local circumstances’ – coordinated by NBGK is connected to our fruit varieties collection. This initiative aims at the reintroduction of old fruit varieties, landraces and local varieties to municipal sites, church gardens and schoolyards. Fruit saplings are provided free to the applicants by NBGK. So far a total of 329 gardens have been established throughout the country using 13,728 fruit tree scions. Children are also involved in the process of planting trees, and in this way, the next generations will be more engaged in the conservation of genetic resources (Figure 9).

## Future plans

Our new seed cold storage facilities, opened in 2024, provides the basis for further expansion of our collection. We aim to make full use of this infrastructure and store at least 70,000 accessions within the next few years. We will increase the current number of employees from 130 to 150. We plan to invest in a new building dedicated to *in vitro* conservation activities and in a new greenhouse suitable for performing research activities and plant physiology analyses. We intend to decrease the average age of our machines, especially tractors, and to obtain new equipment for our laboratory, including a capillary electrophoresis instrument and a huller for glumaceous cereals. These would actively contribute to increasing the number and quality of our scientific publications. We will apply for the registration of many new candidate varieties on the National List of Varieties in the next few years. We would like to further develop our database and elaborate a quality assurance system that is consistent with other European genebanks.

## Acknowledgements

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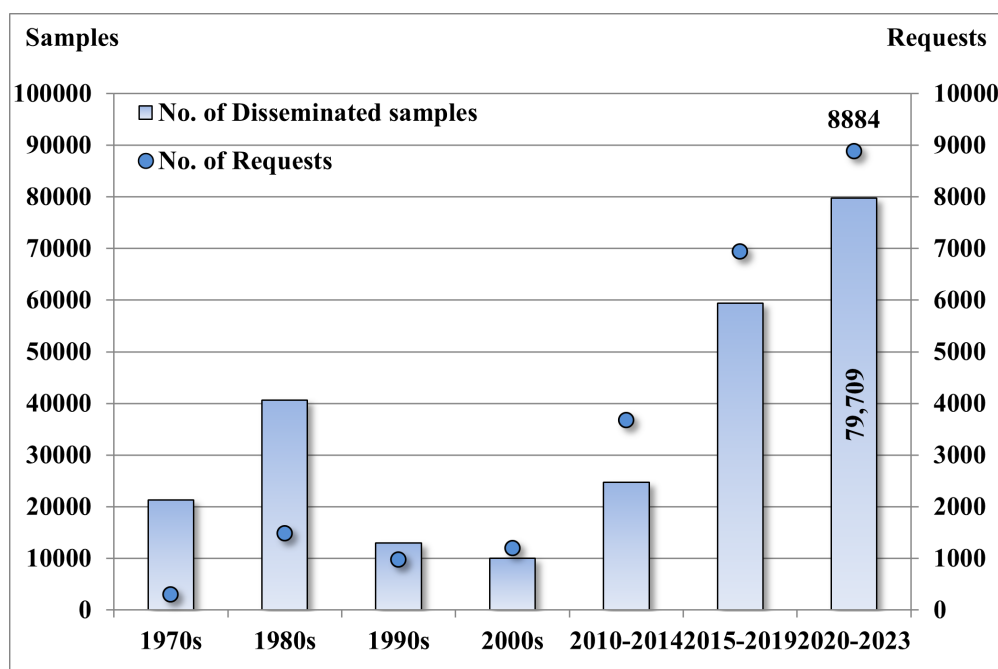




**Figure 7.** Participants of the international EUCARPIA conference study hemp (*Cannabis sativa* L.) plots in the nursery of the genebank in Tápiószéle in June 1974. Photo: NBGK Archives.

**Table 6.** An example of biochemical diversity – sugar, crude protein and crude fibre content – of some celeriac varieties maintained in Tápiószéle from the harvest of 2022 (all data is the average of three replications, and they refer to dry matter).

Name of variety	Fructose (g/l)	Glucose (g/l)	Sucrose (g/l)	Fructose + glucose + sucrose (g/l)	Crude protein (%)	Crude fibre (%)
Albin	0.172	28.175	17.369	45.716	11.78	7.76
Apia	0.138	33.404	27.683	61.225	11.42	8.18
Balder	0.131	34.826	22.008	56.964	14.23	7.40
Brilliant	0.332	30.419	20.702	51.453	10.10	8.80
Bükkzsérci	0.289	30.334	19.081	49.704	13.29	8.98
Erdőhorváti	0.634	30.494	18.268	49.396	11.58	8.56
Frigga	0.052	33.547	28.188	61.788	9.56	8.87
Hegykői	0.130	32.694	25.698	58.523	12.63	7.39
Imperator	0.252	34.446	23.667	58.365	13.26	8.46
Kecskeméti	0.133	31.141	22.745	54.018	11.02	7.99
Kéki	0.219	32.215	17.727	50.161	10.76	7.82
Kisteleki	0.129	36.822	20.417	57.367	11.23	8.04
Maxim	0.027	30.111	27.605	57.744	11.93	8.23
Neon	0.042	29.174	22.858	52.074	10.79	8.14
Nyíregyházi	0.925	37.688	20.200	58.813	11.72	8.15
Prágai óriás	0.157	29.142	16.604	45.903	11.04	8.49
Sótonyi	0.236	33.636	25.449	59.320	9.97	8.90
Taktaharkányi	0.313	43.278	23.438	67.029	13.19	9.27
Tarpai	0.644	32.368	18.054	51.067	12.09	8.94
Trizsi	0.123	31.512	20.378	52.013	12.60	7.72



**Figure 8.** Propagules disseminated from the National Centre for Biodiversity and Gene Conservation (NBGK) between 1973 and 2023. End of data collection: 31 December 2023.

**Table 7.** Number of propagule requests and distributed samples by type of requesters between 2019 and 2023.

		Hobby gardeners	Farmers	Research institutes Public	Research institutes Private	Educational institutes	NGOs	Museums	Municipalities	Total
2019	Requests	1,157	17	15	4	5	5	2	0	1,205
	Samples	9,474	58	148	41	31	2,638	1	0	12,391
2020	Requests	1,376	21	3	2	6	3	0	0	1,411
	Samples	10,919	99	39	16	191	2,945	0	0	14,209
2021	Requests	2,394	24	2	4	4	1	4	1	2,434
	Samples	19,150	259	3	53	58	2,500	42	3	22,068
2022	Requests	2,287	30	3	5	9	4	2	0	2,340
	Samples	16,615	134	34	276	321	3,964	25	0	21,369
2023	Requests	2,481	29	8	1	2	1	1	2	2,525
	Samples	20,027	487	133	1	58	1,250	2	105	22,063
Total	Requests	9,695	121	31	16	26	14	9	3	9,915
	Samples	76,185	1,037	357	387	659	13,297	70	108	92,100

Resource Community for Europe – ProGRACE’ project (identification number: 101094738) carried out within the Horizon Europe programme.

collected the data on arable crops. Borbála Baktay collected data on the history of the institute.

### Declaration

The authors declare that the photos do not infringe on any personal or property rights, and that all people shown in the pictures have given their consent for publication.

### Author contributions

Zoltán Áy edited and wrote the paper. Attila Simon conceived, designed and performed the analysis. Adrienn Gyurkó collected the data on fruit crops. Evelin Fekete collected the data on horticultural crops. Balázs Horváth

### Conflict of interest statement

The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speaker’s bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript. This statement indicates that the above information is true and correct.



**Figure 9.** Even the youngest ones can be engaged in gene conservation. Planting of landrace fruit saplings provided by the National Centre for Biodiversity and Gene Conservation (NBGK) in Sokorópátka (Northwest Hungary, December 2021). Photo by Balázs Csapó, [www.kisalfold.hu](http://www.kisalfold.hu)

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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

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**Abstract:** Grapevine is one of 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;

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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 greenhouse 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.

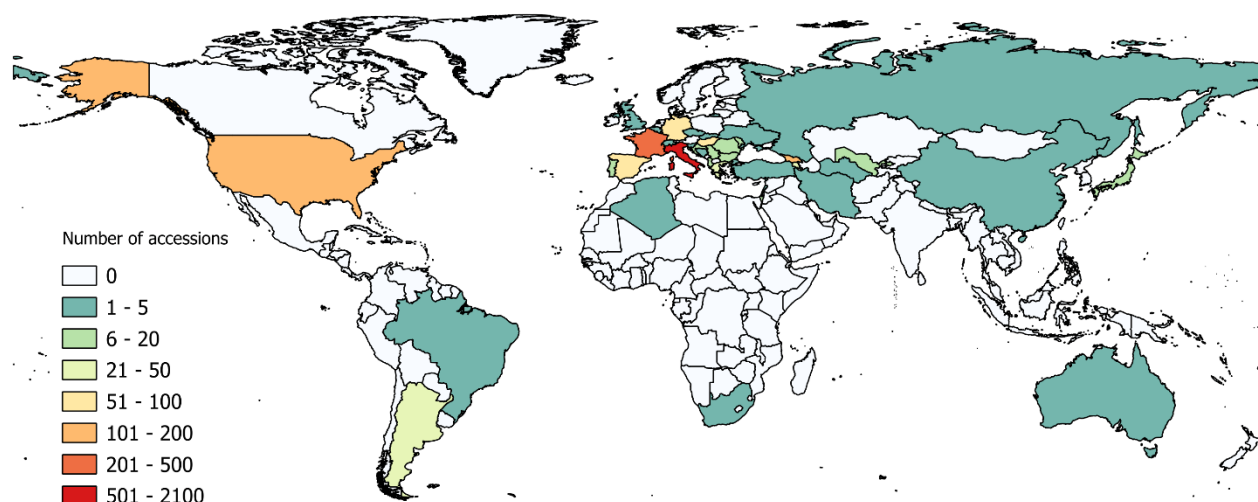


**Table 1.** *Vitis* accessions maintained at CREA-VE

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

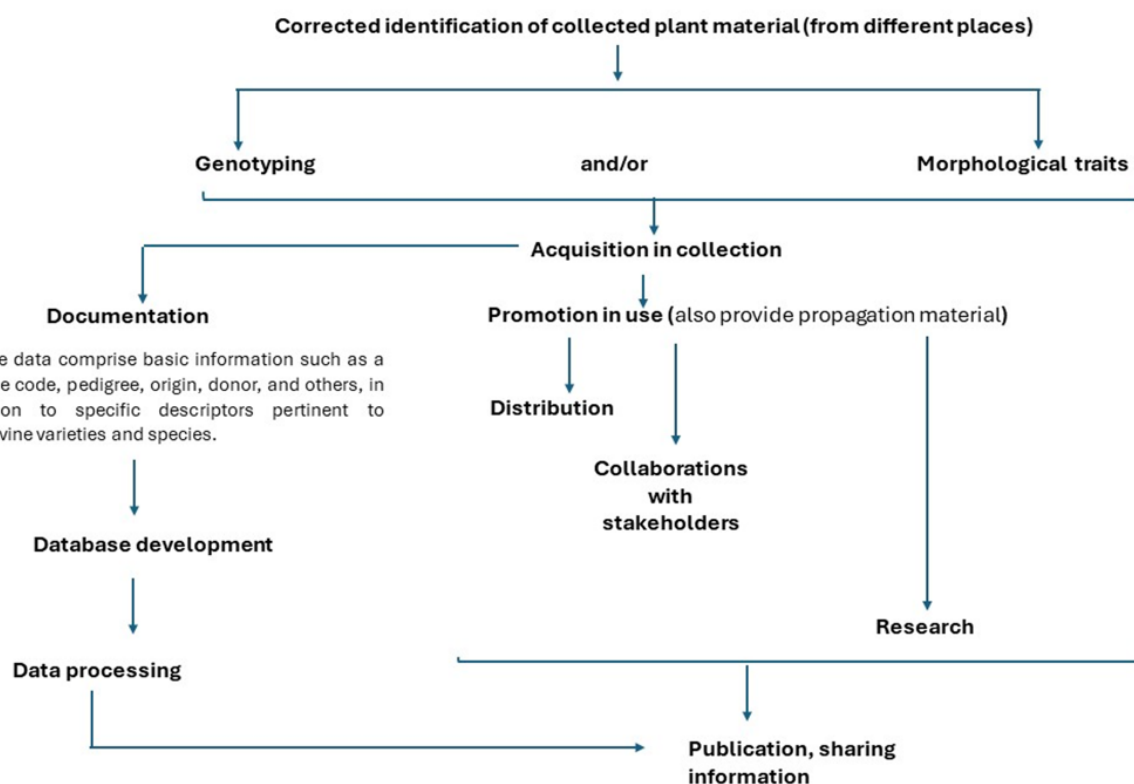


Figure 3. Scheme of genebank activities.

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 collec-

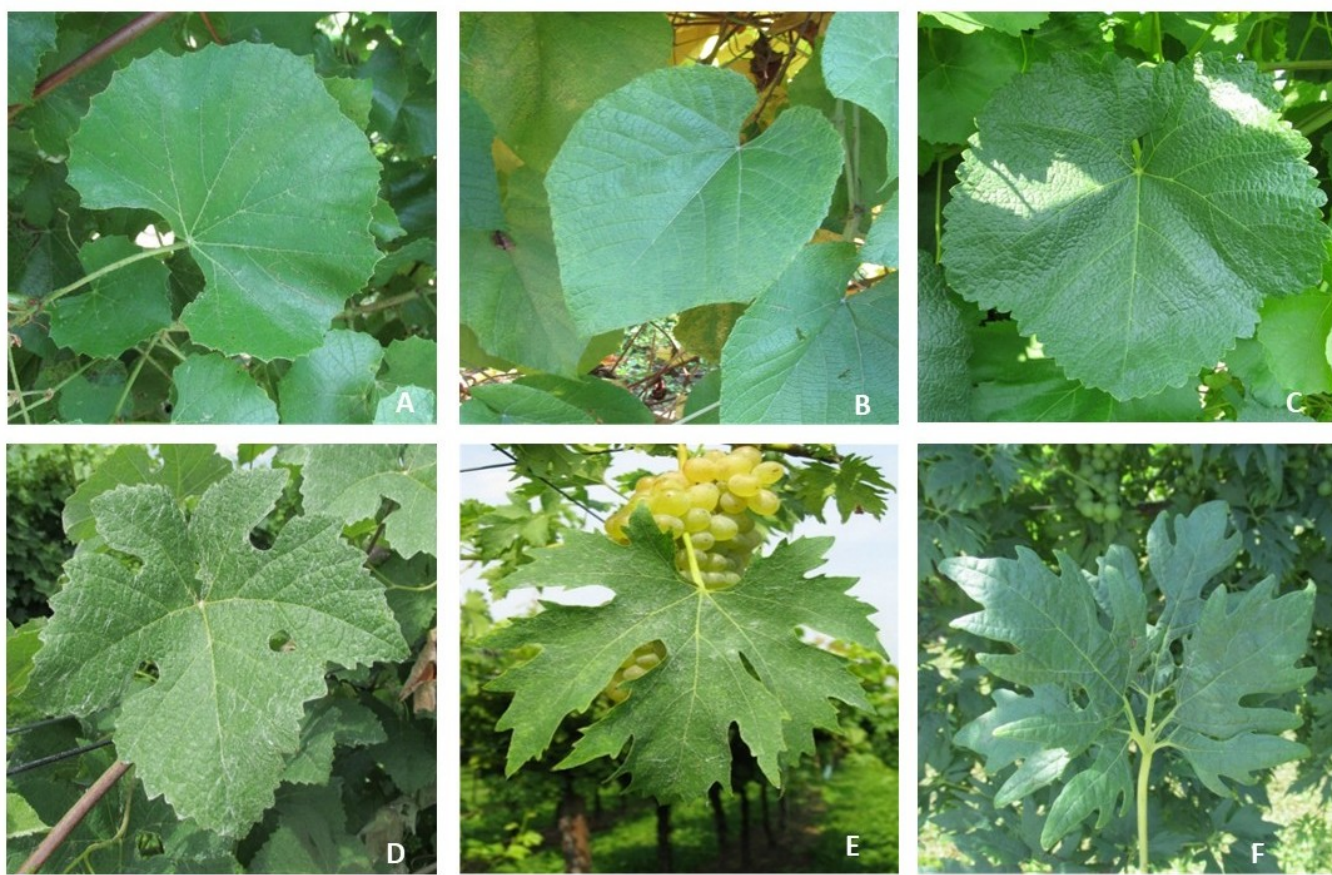
tion (FAO, 2010). Moreover, another cornerstone is the sustainable use of agrobiodiversity. Italian biodiversity of autochthonous vines represents the heritage of a territory, due to its long history from the first domestication to modern cultivation. The discovery and rescue of autochthonous grape varieties 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* Engelm; C, 'Malbo gentile'; D, 'Pinot meunier'; E, 'Badacsony somszeloe'; F, 'Chasselas cioutat'.



Nehelescol B



Piccola nera Rs

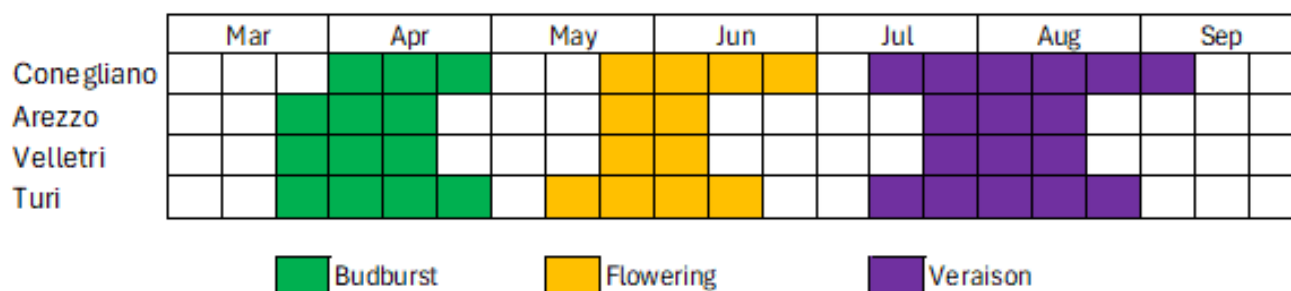


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**Figure 5.** Morphological variety differences in grapes for colour, shape, size and other traits.

**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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# The INRAE Biological Resource Center ‘BrACySol’: a French centre of valuable *Brassica*, *Allium* and *Solanum* genetic resources for breeding

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**Abstract:** The INRAE Biological Resource Center ‘BrACySol’ belongs to BRC4Plants, the plant network of the French Research Infrastructure of Agronomic Biological Resource Centers (AgroBRC-RARé). It preserves more than 15,000 accessions belonging to different cultivated genera: *Brassica* (cabbage, turnip, rape and mustard), *Allium* (shallot and garlic) and *Solanum* (potato and crop wild relatives). The *Brassica* genetic resources are conserved as seeds in freezers or liquid nitrogen. The *Allium* resources are maintained by vegetative propagation in fields or greenhouses and the *Solanum* resources are maintained by vegetative propagation in fields, greenhouses, *in vitro* or in liquid nitrogen. These collections include old landraces, widespread cultivars, crop wild relatives and original scientific material. The accessions are described with passport, morphological or agronomic descriptors or traits. They have been included in various research programmes, at the national or international level, aiming at characterizing the diversity of these collections, studying the genetics of agronomic traits, developing molecular tools and creating pre-breeding lines helpful for breeding programmes.

**Keywords:** Vegetative propagation, seeds, conservation, diversity, characterization, breeding, genebank

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## Introduction

The French National Research Institute for Agriculture, Food and Environment (INRAE) manages the Biological Resource Center (BRC) ‘BrACySol’ which was set up in 2012 with the aim of establishing a collective management system of the different genetic resources collections maintained by INRAE in Ploudaniel (Brittany, Western part of France). The BRC BrACySol is managed

by two INRAE units: the Joint Research Unit Institute of Genetics, Environment and Plant Protection (IGEPP, FRA010) and the Experimental Unit Genetic Resources in Oceanic Conditions (RGCO, FRA179). Currently, 18 permanent staff members are involved in the activities of the BRC, representing about nine full-time equivalents. Its operations are financed mainly by national or European research projects or by partnerships with private companies. It belongs to BRC4Plants (Bergheaud *et al*, 2025), the plant network of the National Research Infrastructure of Agronomic Biological Resource Centres

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(BRCs) named RARe, for Agronomic Resources for Research (AgroBRC-RARe).

### Description of the collections

The BRC BrACySol maintains collections of genetic resources of different genera: *Brassica* (cabbage, turnip, oilseed rape and mustard), *Allium* (shallot and garlic) and *Solanum* (potato and related species). These collections were set up by researchers over the course of their research programmes.

#### Brassica collection

The *Brassica* collection is composed of:

- 1,094 accessions of vegetable crucifers, including mainly landraces that were cultivated before the development of hybrid varieties. They were collected in France on farms in the 1980s (Table 1). This material is not present in any other genebank.
- 1,749 accessions of oilseed crucifers including lineage varieties representing the world variability (Table 1).

It also includes original scientific material like pre-breeding lines presenting traits of agronomic importance such as resistance to different pests (*Leptosphaeria maculans* (Desmazières) Cesati & de Notaris or *Plasmodiophora brassicae* (Voronin)) or seed quality, but also mapping populations, core collections, doubled haploid plants or *Rlm* (Resistance to *Leptosphaeria maculans*) genes differential set (Balesdent et al, 2005).

The accessions of this collection are long-term stored as seed samples in freezers (-18°C). For each accession, three seed lots are formed according to an internal protocol: the first one is used for distribution or germination tests, the second one is a reserve lot which is used to make new distribution batches when the first seed lot is empty, and the third one is a safety lot which is conserved in another place than the first two seed lots. For the accessions that are landraces collected on farms, a fourth seed lot was formed and is conserved in a cryotank (-196°C). The seed lots conserved in the freezers are regenerated every 10 to 15 years, depending on their germination performance which is tested the year of their obtention, after 4 and 8 years of conservation, and then every 2 years, using a germination method in Petri dishes developed in house (100 seeds are placed in a Petri dish on a paper soaked with 2ml of sterile water; the number of germinated seeds is counted after incubation at 20°C for 7 days). Until now, the seed lots that are in liquid nitrogen have never been regenerated. The regeneration protocol depends on the biological status of the accessions. Below is a summary of the regeneration protocols for landraces and lines.

**Landraces:** The regeneration protocol for the landraces was determined to avoid genetic drift during successive multiplications (Divaret, 1999). For each accession, 120 individuals are planted in insect-proof cages

(Figure 1). Pollination is carried out by bumblebees and the seed lot is accepted if at least 75 plants have flowered and produced seeds. Nevertheless, if morphological observations reveal genetic drift after several generations, a new cycle of multiplications can be performed, starting from the cryopreserved seeds which constitute a safety long-term conservation stock.

**Lines:** The seeds are produced by bagging a few inflorescences of each line and pollination is performed using flies.

A final validation of the new seed lot is performed by observing the plants in the field obtained from this new seed lot and plants obtained from the previous seed lot.

The collection is maintained in facilities including 3,000m<sup>2</sup> of greenhouses, 1,500m<sup>2</sup> of field space, a seed drying chamber, 16 freezers for a total capacity of 3,700L and a 170L cryotank.

#### Solanum collection

The *Solanum* collection includes about 11,000 accessions of potato and its wild relatives. The collection has been formed from research programmes since 1949. It is composed of:

- 737 genotypes belonging to 29 wild potato species collected in South and Central America (Table 1). These accessions were introduced into the BRC in the form of seeds provided mainly by the US Potato Genebank (Sturgeon Bay, USA). They are maintained by vegetative propagation as clones (Figure 2). This way of maintaining these potato wild species constitutes a specificity of our BRC. Each clone was evaluated for resistance to different pathogens (mainly *Phytophthora infestans* (Montagne) de Bary and cyst nematodes). These characterization data are therefore available for INRAE researchers and project partners.
- About 1,400 varieties representing world variability, including old varieties not maintained in any other European genebank (Table 1).
- Original scientific material like interspecific hybrids, mapping populations, a core collection or dihaploid plants.

This collection is maintained by vegetative propagation in the form of tubers (produced each year in the fields or in greenhouses for the wild species), *in vitro* plantlets (subcultured every 12 to 15 months) or cryopreserved shoot tips. The cryopreservation of the shoot tips is performed using a droplet vitrification method (Kim et al, 2006). The *in vitro* collection is a safety duplicate of part of the field and greenhouse collections. Some accessions are present only *in vitro*. So far, a small number of accessions is cryopreserved (123 clones). This long-term conservation method is used for the most valuable genetic resources (core collection, wild relatives clones, national collection).

The facilities used to maintain this collection consist of 1,500m<sup>2</sup> of greenhouses, 2ha of field space, *in vitro*

culture facilities, cold storage rooms (950m<sup>3</sup>) and a 170L cryotank.

## Allium collection

The *Allium* collection includes 108 garlic accessions and 246 shallot accessions (Table 1). The collection has grown through research programmes since the 1970s. It is composed of landraces collected in France before the creation of the National catalogue in 1991, old and new French varieties and original scientific material for the selection of agronomic characteristics such as disease resistance (resistance to *Botrytis squamosa* (Walker) or *Peronospora destructor* (Berkeley) Caspary) or dry matter content of bulbs.

The accessions are maintained by vegetative multiplication in the form of bulbs produced every year in the field (for shallot) or in a greenhouse (for garlic).

The facilities used to maintain this collection consist of 250m<sup>2</sup> of greenhouses, 500m<sup>2</sup> of field space and cold storage rooms (20m<sup>2</sup>).

## Associated data

The accessions of these collections are described with passport data using the Multi-Crop Passport Descriptors (MCPD) (Alercia *et al.*, 2015), with morphological descriptors defined by international experts according to the Union for the Protection of New Varieties of Plants (UPOV) guidelines and/or International Plant Genetic Resources Institute (IPGRI) format (IBPGR, 1990; IPGRI, ECPGR, AVRDC, 2001; Bioversity International, International Potato Center (CIP), 2009) and with agronomic traits evaluated according to specific protocols of the research projects.

The characterization data are recorded in MS Excel files and stored on a local server. We are currently working on the development of a local database in which all these data will be gathered, facilitating the management of the available information and its subsequent transfer to the French portal Florilège (<http://florilege.arcad-project.org>).

The BRC BrACySol contributes to the French national collection of genetic resources (Duval *et al.*, 2023) that is made available as part of France's international commitments in contributing to the implementation of the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) (FAO, 2001). So far, 132 potato varieties and 60 rapeseed lines are included in this national collection.

Part of these collections can be viewed on the French portal Florilège (the garlic collection, the traditional cultivars and landraces of the shallot collection, the cabbage landraces of the *Brassica* collection and the potato national collection). We are currently working on formatting the data for other parts of the collections in order to increase the number of accessions that can be viewed on this portal (starting with the potato variety collection and the rapeseed national collection). Similarly, data on part of these collections can be viewed in the EURISCO database (<http://eurisco.ecp>

[gr.org](http://gr.org)). We are currently working with the French national coordination for conservation of plant genetic resources (Duval *et al.*, 2023) to update these data and upload further data in EURISCO.

The Management System of the BRC BrACySol has been certified under the ISO 9001-2015 quality standard (ISO, 2015) since 2021.

## Distribution service

The accessions maintained within the BRC BrACySol can be ordered via the French portal Florilège. The distribution is performed according to the access conditions mentioned on the portal. The *Brassica* genetic resources are distributed as seeds, the potato genetic resources as tubers or *in vitro* plantlets and the *Allium* genetic resources as bulbs. The signature of a Material Transfer Agreement is required (SMTA for the accessions included in the ITPGRFA or INRAE MTA for the others). From 2019 to 2023, the BRC BrACySol distributed more than 6,000 accessions to users: 36% to INRAE teams, 6% to French public institutes other than INRAE, 13% to international public institutes, 28% to French private companies, 6% to international private companies and 11% to farmers, associations or private individuals.

These distributions are performed by the BRC BrACySol in compliance with international regulations concerning sanitary issues (Regulation (EU) 2016/2031, EU (2016)). In order to ensure the healthy status of the collections, various measures are taken and health diagnostics carried out. Regarding the potato and *Allium* collections, detection tests are carried out each year by an external service provider for the following pathogens: *Ralstonia solanacearum* (Smith), *Clavibacter michiganensis* spp. *Sepedonicus* (Spieckermann & Kotthoff), *Globodera pallida* (Stone) and *G. rostochiensis* (Wollenweber), *Meloidogyne fallax* (Karssen) and *M. chitwoodi* (Golden, O'Bannon, Santo & Finley) (for potato); *Ditylenchus dipsaci* (Kuehn) (for *Allium* accessions). Furthermore, Enzyme-linked Immunosorbent Assays (ELISA) (Gan and Patel, 2013) are also regularly performed to detect the main viruses that can infect these crops (Potato Virus Y, Potato Virus X, Potato Virus A, Potato Virus S, Potato Virus M and Potato Leafroll Virus for potato accessions, Onion Yellow Dwarf Virus and Leek Yellow Stripe Virus for *Allium* accessions). If one of these viruses is detected in one plant, this plant is eliminated or isolated. Recently, we showed that virus elimination in potato can be obtained with the routine cryopreservation method (Souchet *et al.*, 2024). However, for now, we have not implemented a cleaning programme for virus-infected accessions. Finally, all the accessions imported from non-EU countries are subjected to quarantine.

The distributions are also performed in compliance with international regulations concerning access and benefit sharing. We are supported for these legal issues by lawyers from INRAE and use a dedicated decision support system developed in a project managed by AgroBRC-RARE ([http://golo.cirad.fr/ABS4BRC\\_WEB](http://golo.cirad.fr/ABS4BRC_WEB)).

**Table 1.** Composition of the collections.<sup>a</sup>, *Solanum* taxonomy according to Hawkes (1990)

Genera	Species	No. of accessions	Biological status (%)			
			Wild	Traditional cultivar/ Landrace	Breeding/ research material	Advanced/ Improved cultivar
<i>Brassica</i>	<i>Brassica carinata</i> A. Braun	2				100
	<i>Brassica juncea</i> (L.) Czern.	111		100		
	<i>Brassica napus</i> L.	1,706		5	76	19
	<i>Brassica oleracea</i> L.	972	4	92	1	3
	<i>Brassica rapa</i> L.	50		95		5
	<b>Total</b>	<b>2,841</b>				
<i>Solanum</i> <sup>a</sup>	<i>Solanum tuberosum</i> L.	10,000		1	84	15
	<i>Solanum tuberosum</i> subsp <i>andigena</i>	123	100			
	<i>Solanum andreanum</i> Baker	2	100			
	<i>Solanum albicans</i> Ochoa	1	100			
	<i>Solanum alandiae</i> Card.	4	100			
	<i>Solanum berthaultii</i> Hawkes	13	100			
	<i>Solanum bulbocastanum</i> Dun.	6	100			
	<i>Solanum brevidens</i> Phil.	46	100			
	<i>Solanum brachistotrichum</i> (Bitt.) Rydb.	6	100			
	<i>Solanum chacoense</i> Bitt.	89	100			
	<i>Solanum commersonii</i> Dun.	2	100			
	<i>Solanum cardiophyllum</i> Lindl.	3	100			
	<i>Solanum demissum</i> Lindl.	115	100			
	<i>Solanum etuberosum</i> Lindl.	10	100			
	<i>Solanum fendleri</i> Asa Gray	3	100			
	<i>Solanum gourlayi</i> Hawkes	21	100			
	<i>Solanum hougasii</i> Corr.	5	100			
	<i>Solanum kurtzianum</i> Bitt. et Wittm.	2	100			
	<i>Solanum oplocense</i> Hawkes	4	100			
	<i>Solanum phureja</i> Juz. Et Buk.	42	100			
	<i>Solanum polyadenium</i> Greenm.	6	100			
	<i>Solanum polytrichon</i> Rydb.	14	100			
	<i>Solanum schenckii</i> Bitt.	33	100			
	<i>Solanum spegazzinii</i> Bitt.	40	100			
	<i>Solanum sparsipilum</i> (Bitt.) Juz. et Buk.	14	100			
	<i>Solanum stenotomum</i> Juz. et Buk.	25	100			
	<i>Solanum stoloniferum</i> Schlechtd. et Bché.	37	100			
	<i>Solanum tarijense</i> Hawkes	17	100			
	<i>Solanum trifidum</i> Corr.	8	100			
	<i>Solanum vernei</i> Bitt. et Wittm.	46	100			
	<b>Total</b>	<b>10,737</b>				
<i>Allium</i>	<i>Allium cepa</i> var. <i>aggregatum</i> G. Don	246		9	81	10
	<i>Allium sativum</i> L.	108		28	64	8
	<i>Allium oschaninii</i> B. Fedtsch	4	100			
	<i>Allium roylei</i> Stearn	1	100			
	<b>Total</b>	<b>420</b>				





**Figure 1.** Regeneration of *Brassica* landraces under insect-proof cages



**Figure 2.** Culture of potato-related wild species in the greenhouse

### Partnership activities

The BRC BrACySol is involved in various EU-funded projects related to the conservation and sustainable use of plant genetic resources: H2020 G2P-Sol (<http://www.g2p-sol.eu>); Prima BrasExplor (<https://brasexplor.hub.inrae.fr>); Horizon Europe NemEmerge (<https://nem-emerge.eu>) and ProWild (<https://www.pro-wild.eu/>). It takes part also in various projects financed by national funds or private partners (including Promosol, GIE Colza, Association des Créateurs de Variétés Nouvelles de Pomme de Terre (ACVNPT), Fédération Nationale des Producteurs de Plants de Pomme de Terre (FN3PT/Inov3PT)). The objectives of these projects are 1) to explore, describe and analyze the genetic diversity of the collections (Esnault *et al*, 2014; Missinou *et al*, 2022; Spanoghe *et al*, 2022), 2)

to develop core collections (Esnault *et al*, 2016), 3) to carry out genetic association analyses to identify the regions of the genome involved in resistance traits to different pests or abiotic stresses (Kumar *et al*, 2018), 4) to introduce this diversity into pre-breeding material by exploiting recombination (Boideau *et al*, 2021), 5) to develop markers that can be used in marker-assisted selection.

Recent results showed that the genetic resources maintained in the BRC BrACySol proved to be of great value to identifying sources of stable late blight resistance in potatoes and to introduce efficiently new variability in oilseed rape using its diploid progenitors (Esnault *et al*, 2023). The most noteworthy current research activities are:

- Exploitation of a genomic dataset (Leuenberger *et al*, 2024b) developed on a panel of potato pre-breeding

clones maintained in the BRC BrACySol in genome-wide association studies to identify genes involved in resistance to cyst nematodes (Leuenberger et al, 2024a) or to late blight disease (PhD work of Leuenberger J.).

- In the framework of the European project BrasExplor, collect, genotyping and phenotyping of *Brassica oleracea* and *B. rapa* wild populations and cultivated landraces extending from the North Atlantic coast to the southern Algerian desert. The taxonomy of these collected accessions was checked by combining cytogenetic and molecular methods (Falentin et al, 2024). This plant material is used to investigate the genomic regions involved in adaptation to climate change (Wagner et al, 2023).

As mentioned, BRC BrACySol has established long-standing collaborations with private partners, contributing in particular to breeding programmes.

One of these collaborations consists of a convention signed in 1995 between INRAE and the four French potato breeders gathered within ACVNPT. ACVNPT provides financial support to INRAE for the conservation and characterization of the potato genetic resources maintained within the BRC BrACySol and in return gets free access, with a 5-year exclusivity period, to the pre-breeding material generated by INRAE within the framework of its research activities using these genetic resources (Kerlan et al, 2017). Since 1995, INRAE has selected 994 pre-breeding clones, improved mainly for resistance to different pathogens (including *Phytophthora infestans*, *Globodera pallida*, *Pectobacterium* sp., *Meloidogyne incognita* (Kofold & White) or Potato Virus Y). So far, 41 potato varieties have been registered by the French breeders who used these pre-breeding clones, maintained by the BRC, in their crossing programmes.

Promosol is another important partner, who funded several projects including ProBiodiv. In this project, it was demonstrated that it is possible to introduce efficiently new variability in oilseed rape using its diploid progenitors conserved in the BRC BrACySol. Pre-breeding oilseed rape populations were created and seeds of 1,600 introgressed lines were provided to the breeders belonging to Promosol (Esnault et al, 2023).

### Network and Working Group participation

The BRC BrACySol coordinates two national networks for the conservation of plant genetic resources: the ‘oilseed crucifers’ network and the ‘potato’ network. These networks involve private and public partners and have defined the lists of accessions to be included in the national collection.

In addition, the BRC BrACySol is part of the European Cooperative Programme for Plant Genetic Resources (ECPGR) and is a member of the *Brassica*, *Allium* and Potato Working Groups. It takes part in the following ECPGR activities that are currently being funded: ‘GarlicCS’ (Genotyping-by-sequencing of the European garlic collection to develop a sustainable *ex situ* conservation

strategy) and ‘Euro-Potatoes’ (Collaboration action for updating the virtual European potato collection).

### Conclusion

The *Brassica*, *Allium* and *Solanum* genetic resources maintained in the BRC BrACySol proved to be of great value in tackling agronomic issues currently faced by these crops. The BRC aims at conserving the diversity and the good quality of these genetic resources and distributing them to researchers and breeders to further contribute to the development of more agroecological agriculture in a context of climate change.

To achieve these goals, the BRC BrACySol works currently to improve the management of the characterization data associated with the accessions and enhance the visibility of these genetic resources.

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### Author contributions

FE managed and contributed to the overall writing of the manuscript. MPC, MAD, LALV, RP, JP, JQ and CS contributed to the writing of the *Solanum* collection description, its associated data and distribution service. SD, PG, VR, and ST contributed to the writing of the *Brassica* collection description, its associated data and distribution service. DK and JQ contributed to the writing of the *Allium* collection description, its associated data and distribution service. FE, JEC, AMC, MCK, AL, MMD, MT and NN contributed to the writing of partnership activities and network participation.

### Conflict of interest statement

The authors have no conflicts of interest to report.

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# The Svalbard Global Seed Vault – conserving plant genetic resources for European and global food security

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**Abstract:** The Svalbard Global Seed Vault is the largest safety backup of the world's crop diversity. It offers safe storage for duplicates of seed samples conserved in genebanks worldwide. Since its opening in 2008, 123 institutes located in 87 different countries have deposited 1,331,458 seed samples of 6,297 crop and crop wild relative species in the Seed Vault. European institutes have very actively taken advantage of the opportunity to back up their genetic seed material. As of today, 38 institutes located in 30 different European countries have deposited 178,999 seed samples in the Seed Vault. Details about seed samples, depositors and species are publicly available through the Seed Portal website. The seed samples that are deposited in the Seed Vault remain the property of the depositing institute. The germplasm is at their disposal if they should need it, e.g. if the material in their home collections is damaged or inaccessible. The Seed Vault has already proved its value and importance for securing plant genetic resources when the International Center for Agricultural Research in the Dry Areas (ICARDA), formerly having its headquarters and genebank in Aleppo, Syria had to relocate their genebank activities, due to the Syrian Civil War. Deposited seeds withdrawn from the Seed Vault formed the basis for establishing new genebank functions at ICARDA units in Morocco and Lebanon.

**Keywords:** Svalbard Global Seed Vault, plant genetic resources, genebank, *ex situ* conservation

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## Rationale and history

Plant genetic resources (PGR) are the raw material needed for the development of improved crop plant varieties, and therefore a precondition for securing future food supplies. Plant genetic material is conserved and made available for research and plant breeding by national, regional or international genebanks and other institutions and organizations holding plant and seed collections (Sanchez *et al*, 2023).

The first genebanks for seed conservation were established as early as in the 1920s, but most countries established their genebanks or genetic resource centres from the 1960s onwards. The Nordic Genebank (NGB) for plants was established in 1979 as a regional genebank for the five Nordic Countries (Denmark,

Finland, Iceland, Norway and Sweden). NGB was merged with Nordic programmes for domestic animal genetic resources (AnGR) and forest genetic resources (FGR) in 2008, into the Nordic Genetic Resource Centre (NordGen) located at the Swedish Agricultural University in Alnarp Campus outside Malmö in Sweden.

Many genebanks are vulnerable to different types of natural and man-made disasters, war and conflicts or simply to lack of resources, and over the years valuable genetic material has been lost. Insurance policies are needed to ensure their protection (Asdal, 2021). The simplest and best way of securing this kind of material is to ensure the conservation of duplicates in storage facilities located in other geographical locations. The further away, the lower the risk for the same accident, natural disaster, political unrest etc. to occur both at the genebank and at the deposit location.

Considerations along these lines were the basis for the Nordic Genebank decision in 1984 to begin backing

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up the Nordic seed collection in an abandoned part of a coal mine in the Svalbard Islands (Figure 1), an Arctic archipelago administered by Norway, halfway between the mainland and the North Pole (Asdal (2021), Figure 2). The coal mine offered stable permafrost (i.e. permanently frozen ground) between -3 and -4°C that secured the freezing of seeds without any cooling equipment, energy supplies or even regular surveillance (Asdal, 2021). Over the years, around 13,000 seed samples of important Nordic agricultural and horticultural crops were placed in the coal mine and stayed there until the Seed Vault opened in 2008.

This low-tech and rather cheap solution gained extensive attention among genebanks and institutions committed to the conservation and use of PGR worldwide, FAO among these. The idea of storing backup copies of seed collections in a similar type of setting also from other national and international genebanks was launched quite soon and discussed and elaborated further through the 1990s (Qvenild, 2005).

When the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) came into force in 2004, the international framework for this kind of international cooperation became established and discussions about a global seed storage facility in Svalbard were revived. A report was commissioned by the Norwegian Ministry of Foreign Affairs to investigate the feasibility of establishing an international seed facility in Svalbard based on political, legal, technical and practical considerations (Fowler *et al*, 2004).

Based on the recommendation of this report, Norway presented the offer to build and manage a global seed vault in Svalbard at the FAO Commission on Genetic Resources for Food and Agriculture (CGRFA) meeting in Rome, Italy in 2004. The offer comprised secure black-box storage of backup seed samples of genebank collections conserved in optimal storage conditions at -18°C. Black-box conservation in this case implies that the deposited seed samples remain the property of the depositing genebank, the seed material is conserved in sealed boxes and the material can only be returned to the owner if needed.

After comprehensive international support, orally expressed already in the CGRFA meeting, the Seed Vault was funded and built by the Norwegian Government in 2007 (Asdal, 2021) (Figure 3). The broad support also materialized in the fact that many genebanks quite quickly began to prepare seeds for the opening event. Already at the Seed Vault opening on the 26 February 2008, 19 genebanks deposited 237,106 seed samples, which was a significant manifestation of the international commitment to increase the security of PGR and an acknowledgement of the role of the Svalbard Global Seed Vault.

The main objective of the Svalbard Global Seed Vault is to conserve security duplicates of unique seed samples that are conserved in regular genebanks. Over the years, the Seed Vault has also become a strong and iconic symbol of the importance of conserving PGR, and it

is now actively used in public and media outreach to increase public awareness about the importance of PGR conservation and use. This is illustrated by the fact that the Seed Vault today is, even when the interior is closed to visitors, one of the top tourist destinations in Svalbard.

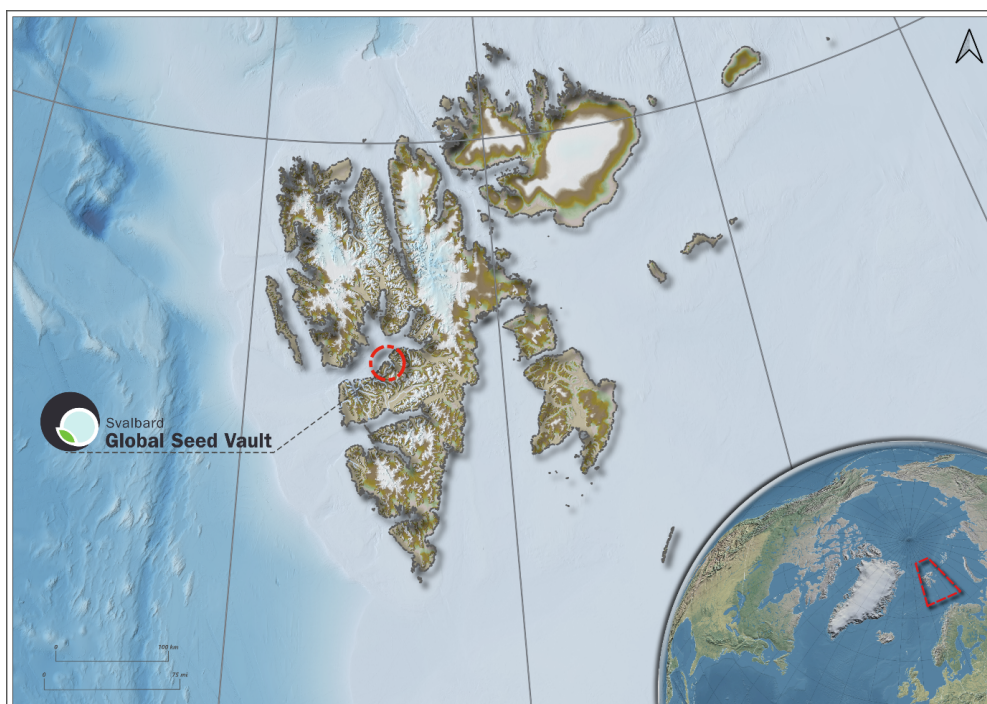
Building the Svalbard Global Seed Vault fits in a long-term Norwegian commitment to support international efforts and projects on conserving biological and genetic diversity. Svalbard is and will, despite severe climate change in the Arctic, also in the future be a cold place suitable for frozen storage for seeds. Together with good infrastructure and public services in Svalbard and international confidence in Norwegian management, Svalbard appears to be an ideal location for a facility like this.

## Ownership and cooperation

The Svalbard Global Seed Vault is owned by Norway and the Norwegian Government guarantees its continuous long-term management and conservation of the seeds. Seed operations, i.e. anything related to the dispatching and depositing of the seeds, are managed in partnership between the Norwegian Ministry of Agriculture and Food, the Global Crop Diversity Trust and the Nordic Genetic Resource Centre (NordGen). Communication with current and potential depositor genebanks, seed deposit routines and logistics are taken care of by NordGen while the Crop Trust supports seed multiplication and shipment from genebanks in developing countries. The three partners cover the management costs with larger parts of the budget coming from the Ministry and Crop Trust.

The three partners work together on increasing awareness of the Svalbard Global Seed Vault and encouraging more genebanks to back up their genetic resources in Svalbard. Information is disseminated on different platforms, through websites and social media, by conferences and online lectures, through genebank visits and media interviews. Despite access inside the Seed Vault has been closed for all visitors due to security reasons since 2018, a broad diversity of visitors goes to Svalbard on the occasions of Seed Vault openings when seeds are brought into the Seed Vault, including genebank representatives, scientists, politicians, international organization officials, journalists and TV teams.

The Seed Vault operates in close connection with FAO and its CGRFA and ITPGRFA. Information about the Seed Vault and its offer to secure genetic resources from genebanks worldwide is regularly conveyed in meetings and side events in FAO bodies and ITPGRFA governing body meetings. The Seed Vault mission is reported and presented in documents and reports from these organizations. The ITPGRFA Benefit-Sharing Fund has supported several projects aiming at securing seed collections in the Vault.



**Figure 1.** The location of the Global Seed Vault and the location of the Svalbard archipelago (map developed by Claudio Ballerini, using QGIS).



**Figure 2.** The history of conserving seeds in permafrost started in 1984, when backup samples of the Nordic Gene Bank seed collection were deposited in a steel container placed in an abandoned shaft of Cole Mine #3. The container is still in the mine, hosting some experimental seed samples while the backup collection has been moved to the Seed Vault. (Photo: NordGen)





**Figure 3.** The Svalbard Global Seed Vault was constructed in 2007 and opened in February 2008. It is located on the hillside of the Plateau Mountain above Longyearbyen airport, around 4km outside Longyearbyen town centre. (Photo: NordGen)

### The facility

The Seed Vault consists of an outer so-called Portal Building, situated 130m above sea level, leading into an 80m-long tunnel ending in a large transverse hall with doors leading into three seed chambers. The total depth of the construction is about 130m, which includes the Portal Building, the tunnel, the transverse hall and the seed chambers, each 12m wide and 27m long. The seed chambers have between 40 and 60m of solid rock between the roof and the hillside surface (Figure 4). Natural permafrost in the ground provides between  $-3^{\circ}\text{C}$  and  $-5^{\circ}\text{C}$ . The seed chambers are artificially cooled down to  $-18^{\circ}\text{C}$ , which is the recommended temperature for long-term conservation of seeds in genebanks (FAO, 2014).

During the first years of operation, melting water in the hillside in the summer seasons and rainfalls caused water intrusion in the entrance tunnel. Pumps were installed in the lower part of the tunnel to pump out the water. Heavy rainfall in October 2016 caused more water to enter the tunnel than ever before. Although the chambers containing the seeds were not affected by the water, the incident convinced the partners and the Norwegian Government of the need for a new watertight entrance tunnel. Knowledge about expected increased temperatures in Svalbard also contributed to the decision to construct an improved entrance tunnel. A new watertight tunnel was funded by the Norwegian Government and completed in 2019.

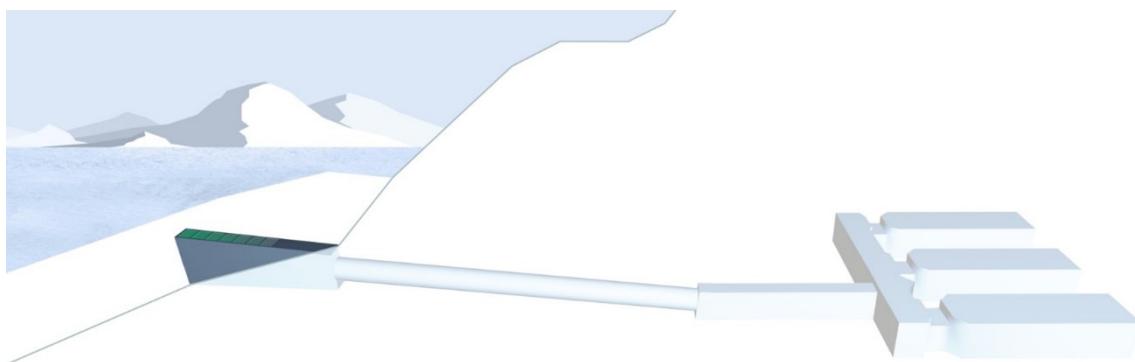
In the same project, a new building for housing technical equipment such as ventilation and new cooling

machines was constructed, and a range of security measures and surveillance systems were installed. The renewed Seed Vault is now well-prepared for all kinds of threats including climate change and possible hostile actions. Warmer temperatures in the Arctic will not threaten the security of the seeds in the Seed Vault but will require some more electric power to cool down the interior of the Seed Vault.

Each of the three seed chambers in the Seed Vault has the capacity to store 1,5 million seed samples, which is approximately twice the number of unique samples that are currently maintained globally in one or more genebanks (FAO, 2010) (Figure 5). However, genebanks are expected to expand their seed collections through new collecting missions aimed at securing crop wild relatives from natural habitats and farmer material in regions that so far have not yet been fully covered. In addition, research and plant breeding programmes will create new genetic material, and older varieties that are replaced will need to be conserved. Nevertheless, the Svalbard Global Seed Vault will have the capacity to conserve duplicate samples of unique genebank accessions for several decades to come.

Over the years, the Seed Vault has probably become Norway's internationally most well-known building and, by that, contributed significantly to increased public awareness about the importance of PGR conservation and use. As one of the preconditions for storing seeds in the Seed Vault is that the safety-duplicated genetic material should be available for plant breeding and research from the depositing genebank, the Seed Vault





**Figure 4.** This model drawing shows the different parts of the Seed Vault, from the concrete Portal Building, visible from the outside, via the tunnel, transverse hall and three seed chambers. (Drawing: Norwegian Ministry of Agriculture and Food.)



**Figure 5.** Deposited seed samples are conserved in ordinary warehouse shelves in three seed chambers. The first chamber was filled up in 2020, and the second chamber was taken into use. The third chamber has not been taken into use yet. (Photo: Riccardo Gangale/The Norwegian Ministry of Agriculture and Food)

mission may also indirectly contribute to keeping genetic resources in the public domain.

### Seed deposit management

The Svalbard Global Seed Vault offers a free-of-charge service to store duplicate samples of seeds that are conserved in genebanks worldwide. The Seed Vault is normally unstaffed, but NordGen organizes three so-called Seed Vault opening weeks each year to receive seed shipments for deposit in the Vault. Current and potential depositor genebanks are informed about the dates and encouraged to ship backup seed samples in advance of these occasions. On average, during the last few years around 12–15 genebanks have shipped seeds to the Seed Vault on each of these occasions.

The seed samples deposited in the Vault remain the property of the genebank. Only the owner genebank has

access to the seeds and can require seed boxes to be returned if needed. All genebanks making their genetic resources available for research, breeding and education are eligible to conserve backup copies of their seed collections in the Seed Vault. In addition, conditions for depositing seeds in the Seed Vault are that the genetic resource should be under long-term storage in a genebank and backed up in a second suitable genebank at another location, making the Seed Vault the second security backup (NordGen, 2024).

Guidelines for depositing seeds in the Vault recommend that a safety duplicate should contain at least 500 viable seeds for outbreeders and heterogeneous accessions with high diversity and a minimum of 300 seeds for genetically uniform accessions. Seeds for long-term storage must be well-dried and packed in watertight aluminium pouches according to the FAO Genebank Standards (FAO, 2014). Seed pouches are packed in sealed

boxes or sent in packages that are put in standard plastic boxes upon arrival. Different box materials are accepted as long as they are robust enough to remain solid through the shipment process.

Genebanks should compile and submit an accession list of the seed material to be deposited 4–6 weeks ahead of the opening date and then ship the seeds, ensuring that the seed boxes or packages are received in Longyearbyen, Svalbard before the announced seed deposit event takes place. Before deploying seed boxes in the Seed Vault, all sealed seed boxes are X-rayed for any unwanted and potentially harmful items inside the box.

Seed boxes are sealed and not opened when conserved in the Seed Vault. The depositing genebank is responsible for monitoring the quality of the seeds over time, normally by performing germination tests on seed samples of the same yield that are conserved in their home genebanks. Depositors are also allowed to deposit boxes with test samples that can be returned if a genebank wants to test seeds that actually have been conserved in the Seed Vault for a shorter or longer time. When germination falls under the genebanks' own threshold, new seeds must be produced and sent to Svalbard.

Some genebanks have started to send newer samples of previously deposited accessions, coordinated with their plans for the multiplication of accessions for their own needs. When all accessions in a previously deposited box have been re-deposited, the whole box in question will be removed from the Seed Vault.

### Seed Vault holdings

Between the opening in 2008 and November 2024, 123 institutes located in 87 different countries have deposited 1,331,458 seed samples of 6,297 crop and crop wild relative species in the Seed Vault (Figures 6 and 7).

Depositor genebank and institutes are International Agricultural Centres (IARCs), two regional genebanks (NordGen and SPGRC in Southern Africa), many national genebanks and universities, a few NGOs and so far, one private company. Information about all depositing institutes is publicly available on the Seed Portal search page <https://seedvault.nordgen.org/Search>. About two-thirds of deposited samples are owned by IARCs and one-third by national genebanks including university collections (Figure 8).

As most genebanks conserve seeds originating from many countries, there are seeds originating from 229 different countries and territories in the Seed Vault. As genebanks tend to keep the original country name as the country of origin in their databases – even when countries split, merge or change names – former countries are also included in this large number.

European depositing institutes are mainly national genebanks of different sizes and with different responsibilities regarding crop conservation. Some countries

have one centralized seed genebank while others have a more decentralized genebank system.

As of November 2024, 193,660 seed samples of 1,914 different species originating from 53 countries and territories in Europe were deposited in the Seed Vault. Thirty-eight (38) institutes/genebanks located in 30 different European countries have deposited 178,999 seed samples in the Seed Vault.

### The Seed Portal database

As mentioned, information about seed samples conserved in the Seed Vault is saved in a specially developed and designed database called the Seed Portal. Genebanks provide accession lists with some basic data about all seed samples they want to deposit before each Seed Vault opening occasion. A template has been developed for this purpose, requiring information about accession number, scientific species name, country of origin, year of regeneration and number of seeds for each sample. Accession lists are uploaded to the database, and a validation process detects typos, errors and invalid information. Basic data is publicly available at the Seed Portal website <https://seedvault.nordgen.org/>.

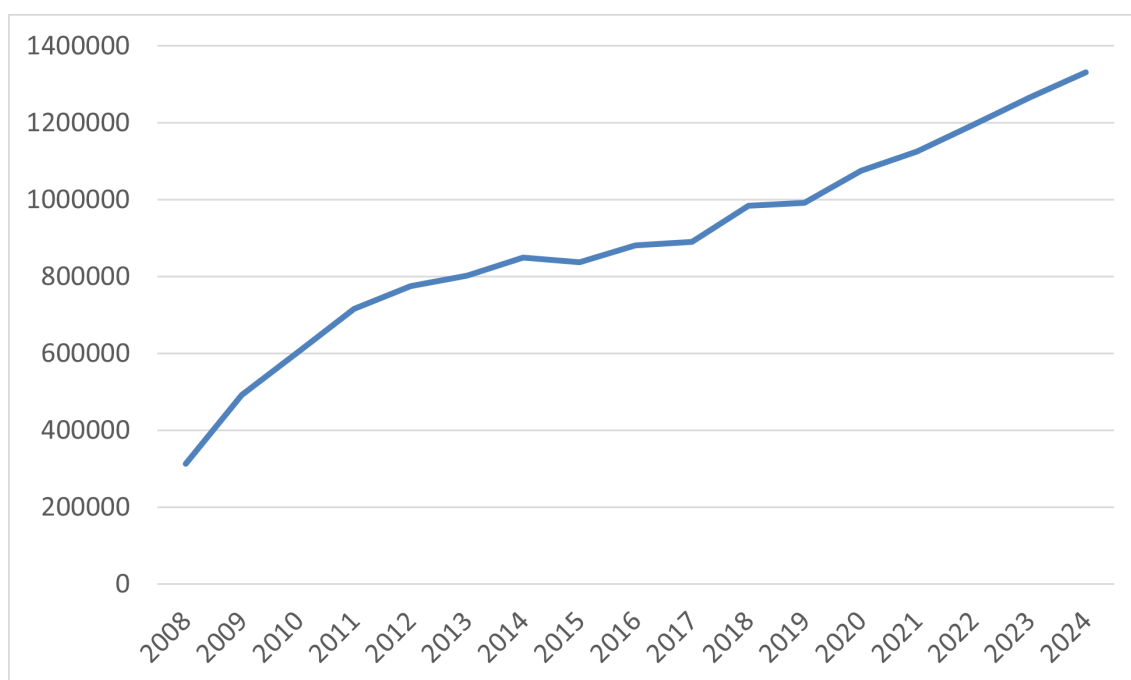
### The first and only withdrawal of seeds

The Seed Vault has already proven its value and importance for securing PGR when the International Center for Agricultural Research in the Dry Areas (ICARDA), formerly having its headquarters and genebank in Aleppo, Syria had to relocate its genebank activities, due to the Syrian Civil War. Seeds deposited from Aleppo between 2008 and 2014 were withdrawn from the Seed Vault in 2015, 2017 and 2019 and formed the basis for establishing new genebank functions at ICARDA units in Morocco and Lebanon (Westengen *et al*, 2020).

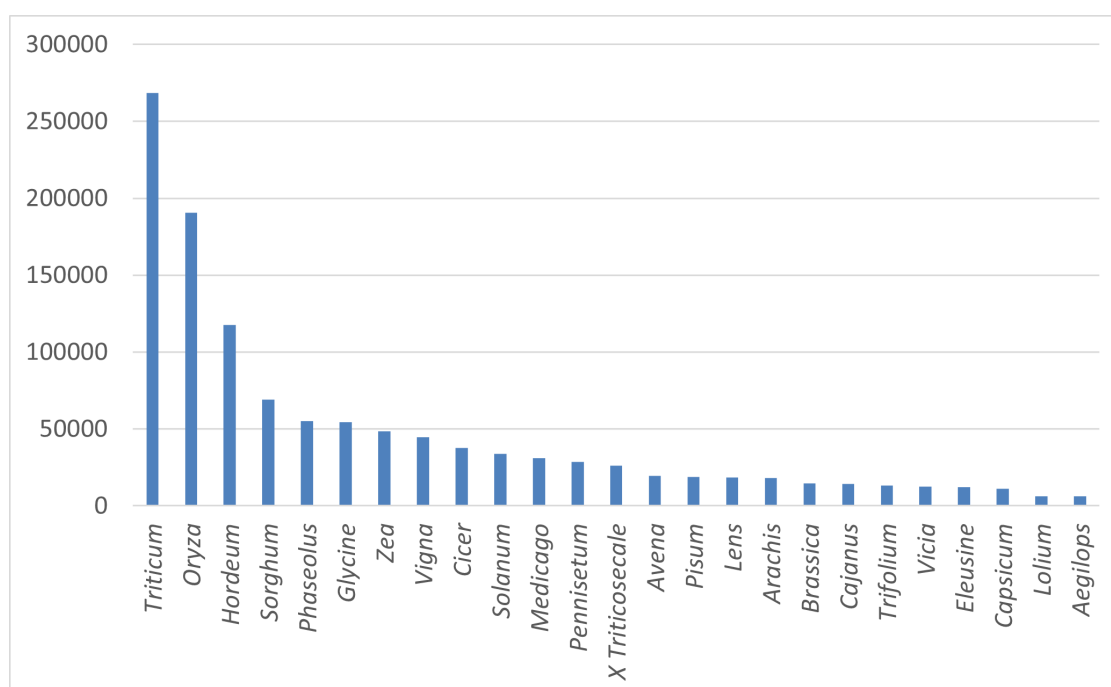
### Concluding remarks

The Svalbard Global Seed Vault is welcoming all genebanks to deposit backup samples of the collection in this very safe place in the Arctic, and the ultimate goal is that all unique plant genetic material of orthodox-seeded species conserved in genebanks is secured and copied in the Seed Vault. In a troubled world, genebanks should implement safety measures for securing their genetic resources and conserving samples in more than one place is an easy solution.

The ICARDA case illustrated that genebanks in relatively stable countries, which was the common assumption for Syria up to the Arabic Spring, should ensure that backup samples are saved at one or more other locations. In addition, natural disasters and lack of resources can cause the loss of seeds and genetic resources in genebanks. The ICARDA case also showed that the concept of the Seed Vault set up for conservation and withdrawals of duplicate seed samples works well and meets the need for extra security for PGR. However, like any other insurance policy, the hope is that no

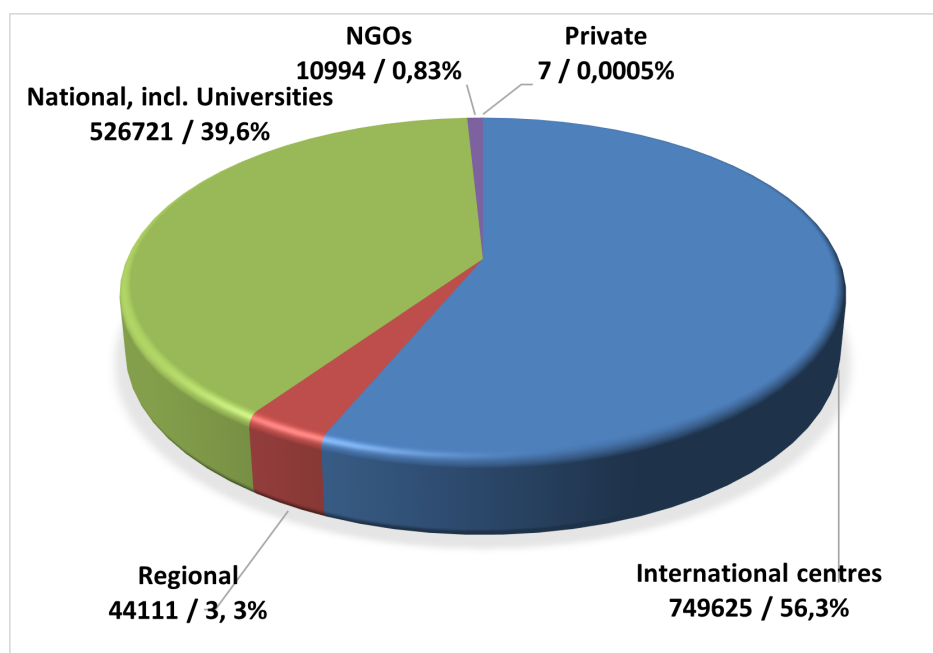


**Figure 6.** Graph showing the increase of Seed Vault holdings year by year from the opening in 2008 until the end of 2024. ICARDA withdrawals took place in 2015, 2017 and 2019, explaining a reduction or low increase in conserved numbers these years.



**Figure 7.** By the end of 2024, seed samples belonging to 6,297 species were conserved in the Svalbard Global Seed Vault. The graph shows the 25 genera having the highest numbers of samples conserved in the Seed Vault. Three genera are represented with more than 100,000 samples; *Triticum* (wheat): 268,353 samples, *Oryza* (rice): 190,758 samples and *Hordeum* (barley): 117,551 samples.





**Figure 8.** Diagram showing proportions of seed samples deposited by five different categories of genebanks and institutes.

further seed withdrawals from the Seed Vault will be needed.

Luckily, the 17-year story of the Seed Vault shows that many genebanks, along with international organizations and partners, acknowledge both the need for security measures for their genetic resources and appreciate the option provided by the Seed Vault. It is evident that the Seed Vault enjoys a high level of confidence among genebanks, ministries, scientists and other stakeholders. From the Seed Vault partners' point of view, it is highly appreciated that the number of genebanks that deposit their valuable genetic material in the Seed Vault is increasing year by year. It is also very satisfying to observe that the global support of the Seed Vault mission is quite unanimous, also in the media. Positive media coverage has undoubtedly contributed to increased public awareness about the importance of genetic resources and encouraged new genebanks to make plans for depositing seeds in the Seed Vault.

It is also inspiring to see that, in a world marked by conflicts, securing PGR in the Seed Vault is still an international effort where countries in different parts of the world cooperate despite disagreements in other fields.

The author of this article hopes that the Seed Vault mission can inspire and pave the way for peaceful international cooperation also in other sectors.

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### Conflict of interest statement

The author declares that there are no conflicts of interest

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# VIR: from a small Bureau in the Russian Empire to the present-day National Center for Plant Genetic Resources

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**Abstract:** In 2024, the All-Russian Institute of Plant Genetic Resources (VIR), the world's oldest scientific institution dedicated to plant genetic resources (PGR), celebrates its 130th anniversary. Founded as the Bureau of Applied Botany in the late 19th century, VIR has evolved into a globally recognized institute, currently also known as the National Center for Plant Genetic Resources. It preserves a collection of more than 320,000 unique accessions from 64 botanical families, 376 genera and 2,169 species. The Institute's mission includes the systematic collection, comprehensive study, long-term conservation and sustainable utilization of PGR. The foundation for most of VIR's research endeavours was laid by Nikolay Vavilov, a prominent botanist, geneticist, plant breeder and scientist with an encyclopedic scope of knowledge. A key strength of the Institute has always been its extensive cooperation with global, regional and international organizations, as well as research institutes, universities and breeding centres in many countries around the world. Today, as the National Center for PGR, VIR continues to build on this legacy. Its efforts include the development of a legislative platform for genetic resources in Russia and fostering international scientific cooperation on PGR, continuing the vision of Nikolay Vavilov.

The purpose of this review is to show the milestones that marked the development of PGR studies and utilization in Russia as well as to highlight some of the Institute's achievements since several conceptual approaches to these issues remain highly relevant.

**Keywords:** Bureau of Applied Botany, VIR, Vavilov, collection, history, study, conservation, genetic resources, plants

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## Introduction and historical events

The Bureau of Applied Botany was founded in 1894 in St. Petersburg under the auspices of the Scientific Committee of the Ministry of Agriculture and State Property, intended as an institution for collecting and studying the diversity of cultivated plants in the Russian Empire. All the measures towards its establishment were initiated and accomplished by Prof. A.F. Batalin and Prof. I.P. Borodin, who would both become the Bureau's first heads. The Bureau made significant progress starting in 1900 with the arrival of R.E. Regel, an expert in botany and agriculture. The most significant changes

occurred in 1905, when Regel was elected as the new head of the Bureau (Loskutov, 1999).

The main concrete outcome of the Bureau's activities under Regel's leadership was the collection, identification and description of the varietal diversity of cultivated plants grown in the Russian Empire. Those efforts helped to restore the lost diversity of cereal crop varieties and populations – particularly malting barley – following the devastating droughts in the Volga region. The racial, varietal and specific composition of local cultivars and populations was documented for wheat, barley, oats, rye, some legumes, vegetable crops and others (Regel and Proceedings on Applied Botany and Breeding, 1915). The Bureau's plant studies resulted in identifying hereditary morphological and agronomic traits, performing successful crosses, and clarifying the genetic nature of some of those, following Gregor

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Mendel's principles of genetic analysis. Comprehensive research on plant collections data enabled the development of the Bureau's own original botanical classification systems for a number of priority crops, based on the studied morphological, anatomical, cytological, biochemical, immunological and agronomic plant characters (Regel and *Proceedings on Applied Botany and Breeding*, 1915). In 1906, the Bureau of Applied Botany received the highest award (Diploma d'Onore) at the World Exhibition in Milan (Esposizione Internazionale di Milano) for the presentation of the barley collection and the results of its study, later summarized by Regel in his publication *Les orges cultivées de l'Empire Russe* in French (Loskutov, 1999).

Regel laid the foundations for collecting missions, both within the Empire and beyond, to supply the collections with new accessions. In 1908, he launched the publication of *Proceedings on Applied Botany*, the first scientific journal dedicated to the use of botany in agricultural practice (Loskutov, 2009). In the following years, the exchange of publications gained significant momentum: by 1928, the *Proceedings on Applied Botany* were sent to 175 research institutions worldwide, while in exchange the local scientific library received publications from 136 foreign research centres. In 2023, the journal founded by Regel (currently named *Proceedings on Applied Botany, Genetics and Breeding*) celebrated its 115th anniversary. Today, the journal is indexed in the Web of Science, the Russian Science Citation Index (RSCI), Scopus, and DOAJ (Supplements, 2023).

When Regel unexpectedly passed away from typhus in 1920, a talented young researcher, Nikolay Ivanovich Vavilov, was elected in his place. His works on the immunity of cereal crops were highly valued by leading Russian scientists at that time.

From those days onward, the Bureau's activities expanded to a completely different scale: as early as 1924, it was raised to the status of an Institute (the All-Union Institute of Applied Botany and New Crops). Vavilov established a network of experiment stations across the USSR, where all the collected crop materials were studied under various environmental conditions (VIR's current experimental network consists of 15 branches in 12 regions of Russia, from the southernmost site in Dagestan to the Arctic). He organized collecting missions abroad, to all the world's continents, as well as to the remotest nooks over the vast territory of the USSR. Vavilov enhanced the international exchange of plant germplasm among leading plant breeders and botanists on a global scale. While collecting plant resources and studying them, he continued to work on his scientific theories and fundamental principles. Based on these research endeavours, he promoted plans for himself and his associates to conduct further plant explorations across the Soviet Union and in foreign countries. He developed a scientific framework to support the expansion of the cultivation area for individual crops and the introduction of new cultivars and plant species

into the USSR territory. Unfortunately, those ambitious plans failed to come to life to their fullest (Loskutov, 2020).

In 1930, the Institute was renamed the All-Union Research Institute of Plant Industry, and during this time it adopted its world-famous acronym, 'VIR'. However, the new name did not fully encompass the Institute's principal activities and objectives. Looking ahead, it would be only at the end of 2014, when VIR gained the status of one of the first federal research centres in Russia, that the concept of 'plant genetic resources' (PGR) entered the Institute's official name (see a photo of VIR's headquarters in Figure 1).

The Great Patriotic War (World War II) of 1941–1945 was a dramatic period for the survival of the Institute's staff members and the safeguarding of its unique collections. With the outbreak of the war, even before the city was beleaguered by the Nazis, the government decided to evacuate a number of factories and institutes from Leningrad, including VIR. However, it was not until the winter of 1942 that the Institute started a partial evacuation of its employees and holdings, although the arrangements to prepare the move had been underway for a long time. Eventually, the largest and most important part of the collections was left in the besieged city. The remaining staff members had to toil under the harshest conditions of the siege, in unheated premises. The severe winter of 1941–1942 brought drastic reductions in the daily bread allowance, and the city faced starvation. Hundreds of thousands of people died from hunger, including VIR's employees, who kept seeds and tubers in the collections safe and intact. Only their heroic efforts saved the Institute's collection from destruction and loss of viability. Such heroism cost the lives of more than 20 scientists and specialists, but as irreparable as that cost might be, VIR managed to survive the most dangerous period in its existence. Suffering extreme physical exhaustion in frozen rooms, without water or electricity, under continuous shelling, the Institute's staff members secured the collection of cultivated plants and their wild relatives, the herbarium and the scientific library for future generations, often paying with their own lives (Loskutov, 2021).

## The current situation

Vavilov paid special attention to the conservation of the genetic diversity of cultivated plants and their wild relatives, collected from all over the world, for future generations. The need for safe preservation was triggered by the concern that, over time, the valuable global collection could lose its qualities or genetic homogeneity due to frequent regenerations of its accessions, which were necessary to restore high germination rates. To minimize the frequency of regenerations while ensuring the seed viability of accessions, it became essential to maintain them under controlled conditions in specialized low-temperature storage facilities.



**Figure 1.** VIR's headquarters, St. Petersburg. Photo: VIR Archives

The geographic approach to plant germplasm conservation at the Institute's experimental stations has remained predominant since Vavilov's times. All accessions, mostly those from the larger cereal collections, have been distributed among the duplicate collections maintained at VIR's experimental stations for regeneration and conservation, following Vavilov's agroecological classification of crops (Vavilov, 1957). By combining long-term storage in the national genebank with short-term storage in working and duplicate collections at experimental stations and active collections at the Institute (Figure 2), VIR has ensured integrated and highly secure germplasm conservation. In addition, the Institute's collection management is based on a thoroughly developed (to the level of varieties) botanical classification of each genus and species. While managing the preserved biodiversity, much attention is paid to the passport data of each incoming accession, especially to the true geographic origin and its correct (original) name, which are important for the timely identification of duplicate accessions in the collection (Loskutov, 2009).

### Storage facilities

Since 1946, VIR has been experimenting with the long-term storage of various crop accessions from its global collection under controlled conditions. The results of such experiments made it possible to find the optimal storage conditions for germplasm accessions, preserved as dried seed samples in hermetically sealed containers. Long-term and medium-term storage of seed samples at low temperatures is a safe and relatively inexpensive method for PGR conservation (Khoroshailov, 1978; Filipenko, 2007). PGR storage at low temperatures began at VIR in the mid-1950s with systems under various tem-

perature conditions. In 1969, the storage facility was reconstructed and updated. Special premises were allocated in the Institute's headquarters for this purpose, where a temperature of  $+5/7^{\circ}\text{C}$  was maintained, and where a significant part of the VIR collection was preserved until the late 1980s (Khoroshailov, 1978).

In 1976, the National Seed Store was built at the Kuban Experiment Station of the Institute (Krasnodar Territory) to house the VIR core collection under controlled conditions. The design of the National Seed Store, occupying the underground section of the building, allowed for the storage of seed samples in hermetically sealed glass containers at  $+4^{\circ}\text{C}$  in 24 rooms without relative humidity control, with a total estimated capacity of 400,000 accessions. For a long time, the VIR collection was successfully stored in the National Seed Store. By the mid-1990s, about 70% of the accessions held within the base seed collection had been placed for storage in the Kuban Experiment Station facility. In 1994–1997, the international community (International Plant Genetic Resources Institute (IPGRI) and United States Department of Agriculture (USDA)) helped to renovate, including the sealing of underground floors, the building and equipment of the National Seed Store, so that constant and stable storage could be maintained there. This programme included the delivery of modern refrigeration equipment and computer hardware, the latter serving as the platform whereupon the electronic databases of the VIR collections were developed (Loskutov, 2009).

In 2008, the Kuban Seed Genebank (as the National Seed Store had been renamed), a branch of VIR, was supplied with new technological equipment and larger refrigeration chambers for PGR conservation, designed to perform effective operations at a higher scientific





**Figure 2.** Working collection at VIR, St. Petersburg. Photo: VIR Archives.

and methodological level. Today, the repositories of the Kuban Seed Genebank hold 336,396 storage seed samples of various crop seed samples, including about 17,000 accessions from other scientific institutions, at low positive (+4.5°C) and negative temperatures (−5.0°C and −20.0°C) in a ratio of 10 to 1.

Modern low-temperature storage facilities were installed in the VIR headquarters (St. Petersburg) in 2000, with support from the international community (IPGRI, USDA). The temperature regime of +4°C is maintained in two seed storage rooms (437m<sup>3</sup> in volume), and −10°C in three others (434m<sup>3</sup>). The research conducted at the VIR genebank resulted in partial development and introduction of more advanced technologies (lamination, sealing) for long-term PGR conservation, which led to a significant decrease in labour costs and funding, required to maintain the collections in a viable state, reducing the need for periodic seed regeneration. Of late, significant efforts have been made to transfer seed accessions under controlled storage conditions. The seed material is now hermetically packed in laminated foil bags or glass containers in all types of storage.

As of early 2024, 438,951 seed accessions from the VIR PGR collection were stored in the Institute's low-temperature seed storage facilities in St. Petersburg. Information about these accessions is available in the database of stored materials: 308,481 accessions

are placed under medium-term storage (−10°C), and 130,470 under long-term storage (−20°C) conditions (Table 1). At present, a total of over 750,000 storage units (including safety duplicates) with genetic resources accessions from the unique global collection of VIR are placed for safe conservation in one or the other type of temperature-controlled storage facilities within the Institute's genebank network (Loskutov, 2025).

In 2023, as part of the instrumentation upgrade programme under the National Project 'Science and Universities', VIR installed new updated seed storage facilities, with a temperature regime of −18°C and a capacity to store over 300,000 units.

## Plant exploration

The revival of Vavilov's name and ideas in the second half of the 20th century catalyzed a thorough exploration of plant resources all over the globe. Not only Soviet and Russian researchers were credited with this endeavour, but also scientists and experts from many foreign countries (Zeven and Zhukovsky, 1975; Sazonova *et al*, 1994; Frankel *et al*, 1995; Harlan, 1995; Pistorius, 1997; Pistorius and Van Wijk, 1999; Loskutova and Ozerskaya, 2018, 2019, 2020, 2021; Solberg *et al*, 2023).

**Table 1.** Structure of preservation of the world collection of VIR, 2024. \*, seed samples.

№	Type of storage	Place of storage	Number of accessions
1	<i>Ex situ</i>	VIR, St. Petersburg, working collection	290,000
2		Network of VIR stations, field genebank	30,000
3		VIR, St. Petersburg, medium-term storage	308,481*
4		VIR, St. Petersburg, long-term storage	130,470*
5		Kuban Seed Genebank, medium-term storage	290,692*
6		Kuban Seed Genebank, long-term storage	45,704
7	<i>In vitro</i>	St. Petersburg	1,134
8	Cryo	St. Petersburg	3,677

Besides, Vavilov's life and work, the activities of the Institute he led, and the importance of the VIR collections for the global community have been a subject of interest for Russian and foreign researchers (Rokityansky *et al*, 1999; Pringle, 2008; Nabhan, 2011; Cohen and Loskutov, 2016; Goncharov, 2017; Reznik, 2017, 2021; Loskutov *et al*, 2023).

The new knowledge obtained from contemporary plant explorations inspired the Institute's scientists to develop and expand Vavilov's concept of the centres of crop origin. The data accumulated through comprehensive research on the enormous diversity of crop species and their wild relatives helped to develop new botanical classifications or adjust the existing taxonomies for the most important crops, published in new volumes of the Cultivated Flora, a series of publications started by Vavilov. An in-depth study of intraspecies diversity made it possible to identify or artificially develop the plant forms predicted by Vavilov's law of homologous series in hereditary variation (Loskutov, 2025).

A crucial part of VIR's international activities in the 1960s–1980s was organizing and implementing the work on collecting, studying and conserving PGR within the framework of the Council for Mutual Economic Assistance (COMECON), which bound together the USSR, the socialist countries of Eastern Europe, and Mongolia. Their joint collecting missions and seed exchange efforts enlarged the genebank collections of such countries as Czechoslovakia (1.5 times), Hungary (by twofold), Bulgaria and East Germany (threefold), and Poland (sevenfold). In addition, the collections of Mongolia and Romania were also expanded (Alexanian, 2002).

After the dissolution of the USSR in 1991, independent PGR genebanks emerged in the former Soviet Republics. In some cases, national genebanks were established based on agricultural, plant breeding or botanical research institutes that had been closely cooperating with VIR for decades in various areas, including plant germplasm exchange, such as those in Estonia, Latvia, Lithuania, Moldova, Georgia, Armenia, Tajikistan and Kyrgyzstan. In other cases, national genebanks developed from collections previously maintained at experimental stations or base sites within the VIR network before 1991. These genebanks were established

in Ukraine, Belarus, Azerbaijan, Uzbekistan, Kazakhstan and Turkmenistan (Loskutov, 2025).

Only in the past ten years, 110 collecting missions have explored the Russian Federation and foreign territories to monitor PGR *in situ* and enrich VIR's *ex situ* collections (State Research Centers, 2023).

### Composition of the collections and research on PGR diversity

Thanks to the large-scale activities undertaken by Vavilov and his associates, VIR is now the holder of two unique biological collections:

- The VIR Collection, a global collection of genetic resources of cultivated plants and their wild relatives. It is one of the world's largest in terms of the botanical, genetic, geographic and ecological diversity, and includes samples from 64 botanical families, 376 genera and 2,169 species. In terms of crop groups, the collection is composed of cereals (137,500 samples), legumes (46,500), forage crops (32,000), vegetable and melon crops (52,000), industrial crops (28,500), tuber crops (8,300), fruit crops (23,000), totalling 327,800 samples. As of 1 November 2024, the volume of VIR's world collection amounted to more than 320,000 unique samples (Loskutov, 2025);
- The VIR Herbarium (acronym 'VIR'), a collection known as the Herbarium of Cultivated Plants of the World, Their Wild Relatives and Weeds of the N.I. Vavilov All-Russian Institute of Plant Genetic Resources. It is one of the world's richest herbaria, specializing in cultivated plants, with a status corresponding to a specialized herbarium of global significance. As of 1 November 2024, it includes 141,293 herbarium specimens and 379,292 herbarium sheets (Khlestkina *et al*, 2022).

Currently, VIR employs over 1,200 people, of which about 500 conduct research in St. Petersburg and over 700 work at experimental stations – the VIR branches. The Institute's structure includes several components. In St. Petersburg, the departments of genetic resources focus on collecting, studying in the field, propagating, preserving and distributing samples of the collection. Additionally, methodological departments and laboratories conduct in-depth research



on the collection samples from the point of view of genetics, physiology, biochemistry and molecular biology. The structure of VIR also includes a genebank, represented by the Laboratory for Long-Term Storage of PGR, collective-use centres and a network consisting of 15 VIR experimental stations located in different parts of the Russian Federation. Every year, VIR distributes more than 5,000 collection samples free of charge to more than 200 requests from state research, breeding institutes and universities of the Russian Federation.

Vavilov's plans for genetic research into plant species diversity, which he was unable to fully realize during his lifetime, became one of the main priorities of the Institute. This mission formed the basis of a programme aimed at selecting and developing sources and donors for important agronomic traits. The resulting data underpinned the establishment of trait-specific and genetic collections with identified genes (Merezhko, 1994; Mitrofanova, 1994; VIR, 2005; Porokhovina *et al*, 2013). In the 2000s, modern methods of molecular biology started to be widely introduced for the in-depth study of the genetic diversity preserved in the VIR global collection (Anisimova *et al*, 2011; Antonova *et al*, 2011, 2016; Artemyeva *et al*, 2012, 2017; Zlotina *et al*, 2013; Burlyaeva, 2014; Gavrilenko *et al*, 2014; Teplyakova *et al*, 2017; Novakazi *et al*, 2019; Sallam *et al*, 2021). Today, the Institute is engaged in extensive genomic and postgenomic research activities, including those within the framework of projects under the auspices of the Federal Scientific and Technical Program for the Development of Genetic Technologies for 2019–2030, and the National Project 'Science and Universities', targeted at different crop groups: cereals (Antonova *et al*, 2022; Porotnikov *et al*, 2022; Khlestkina *et al*, 2022; Chikida *et al*, 2023; Gnutikov *et al*, 2023; Loskutov *et al*, 2023; Rozanova *et al*, 2023; Semilet *et al*, 2023; Lukina *et al*, 2024; Shvachko *et al*, 2024), grain legumes (Krylova *et al*, 2023, 2024a,b), oilseeds and industrial crops (Mikhailova *et al*, 2024, 2022; Anisimova *et al*, 2023), vegetables (Strygina and Khlestkina, 2022; Berensen *et al*, 2023; Fateev *et al*, 2023), forage crops (Malysheva *et al*, 2023), fruit crops (Kamnev *et al*, 2023; Razgonova *et al*, 2023a,b), potato (Antonova *et al*, 2020; Fomina *et al*, 2020; Rybakov *et al*, 2020; Gavrilenko *et al*, 2023; Gurina *et al*, 2022), and many others.

Following the implementation of those programmes and projects, new knowledge was obtained in the field of plant genetics, physiology, biochemistry and taxonomy, including the identification, mapping and/or labelling of more than 100 target genes and loci of quantitative traits. Only from 2018 to 2022, VIR's scientists identified 8,566 sources of important agronomic traits and identified 32 donors of valuable genes. Twelve edited lines of various crops have been released and are being studied using genetic technologies under VIR's projects jointly with the Institute's partners.

## Sustainable use of PGR

From 2007 to 2023, based on the assessment of more than 100,000 accessions of cereals, grain legumes, oilseeds, fruit and berry, and vegetable crops for the most important economically valuable and biochemical quality traits, more than 6,700 sources for various breeding areas were identified. More than 26,000 accessions of various crops were studied for resistance to diseases and pests against harsh infectious backgrounds. More than 970 sources of high resistance were identified. The effectiveness of resistance sources of various crops to populations of harmful organisms was also studied – causative agents of leaf rust, dark brown leaf spot and Fusarium head blight in wheat, barley and oats; net spot, loose and stone smut in barley; coccomycosis in cherry; common cereal aphid in sorghum. More than 5,250 accessions of the gene pool of cultivated plants were screened for resistance to abiotic environmental factors, and more than 500 sources of valuable physiological traits were identified (weak photoperiod response and early maturity, tolerance to excess mobile aluminium, cold and drought resistance). Over the past 5 years (2019–2023), VIR identified 6,820 sources of economically valuable traits and created 19 donors of valuable genes, which were sent to the leading breeding centres of the Russian Federation. Since 2013, VIR has registered almost 130 patents (varieties and technologies for implementation in production in the agro-industrial complex). All selected and created genetic sources and donors were sent to leading breeding institutions of the Russian Federation.

During the same period, over 300 new varieties of major agricultural crops were created and approved for use in agricultural production of the Russian Federation based on VIR sources and donors in breeding centres and research institutes. VIR employees created 98 varieties of economically significant crops included in the State Register of Breeding Achievements of the Russian Federation, and received 47 patents and 102 author's certificates for varieties.

## Implementation of modern technologies

In 2004, the genebank of VIR was equipped with cryogenic systems (Figure 3), and the Institute was able to start cryopreservation activities. Presently, pollen samples and cuttings of fruit and berry crops are preserved in liquid nitrogen vapours, and cuttings are also placed under liquid nitrogen (Filipenko, 2007).

Currently, biotech and digital approaches are widely applied at VIR to develop methodologies for maintaining *ex situ* PGR collections, including their conservation and two- or three-fold safety duplications under controlled conditions (short-, medium- and long-term storage of seed accessions in specialized low-temperature facilities, cryopreservation, and *in vitro* conservation of vegetatively propagated crop samples), safe maintenance of perennial crop accessions in the field, regeneration of accessions to ensure seed



**Figure 3.** Storage facilities, cryopreservation, St. Petersburg. Photo: VIR Archives.

germination and obtain fresh material (Figure 4), etc. (Khlestkina *et al*, 2022).

By now, VIR has used biotechnologies to maintain 1,134 accessions *in vitro*, including 1,081 accessions of vegetatively propagated temperate-climate crops (656 potato, 21 onions, 348 berry and fruit plants, and 53 ornamentals), as well as 2,045 pollen samples, 1,184 cuttings of various fruit plants and grapes, and 448 samples of apical meristems (415 of potato, and 33 of raspberry) for a total of 3,677 accessions under cryopreservation (Table 1).

The methods developed at the Institute to preserve and evaluate PGR are published in the format of guidelines (Filipenko, 2007; Loskutov *et al*, 2012).

## International events

VIR has organized more than ten major international events in recent years, including the International Wheat Conference in 2010, the International Oat Conference in 2016, regular International Vavilov Conferences (the last one in 2022; Khlestkina *et al* (2022) ) held every five years, and major international conferences dedicated to the Institute's birthday, the last of which was successfully held in November 2024. Besides, VIR was the initiator and co-organizer of two major fora 'Genetic Resources of Russia', bringing together the holders of biological collections of all specializations (Tikhonovich *et al*, 2022, 2023). Partly owing to this initiative and following broad multidisciplinary discussions at those forums, the National Law on Bioresource Centers and Biological Collections was drafted in Russia (Tikhonovich *et al*, 2024).

## Sustainable future and an intensified research programme

Over the past five years, the Institute has significantly intensified comprehensive research on the VIR collection through the implementation of large-scale research programmes, including collaboration with partner institutions participating in programmes coordinated by VIR, as well as through VIR's active involvement in consortia. Examples of such partnerships are the 'National Network Collection of Plant Genetic Resources for Effective Scientific and Technological Development of the Russian Federation in the Field of Genetic Technologies', and 'Breads of Russia', supported in the framework of the Federal Scientific and Technical Program for the Development of Genetic Technologies for 2019–2030. An example of participation in a consortium is the programme of the World-Class Scientific Center, 'Agrotechnologies for the Future'.

In 2022, two Decrees were issued by the President of the Russian Federation. The first founded the National Center for Plant Genetic Resources based on VIR, while the second established the Interdepartmental Commission on the Formation, Preservation and Use of Plant Genetic Resources Collections (Khlestkina *et al*, 2022). In 2023, the Government of the Russian Federation approved the Program for the Development of the National Center for Plant Genetic Resources for 2023–2030. This programme provides conducive conditions for the development of scientific, research and development activities in the field of PGR to ensure scientific and technological development of the Russian Federation, finding integrated solutions to the questions





**Figure 4.** Regeneration field, harvest time, Pushkin, St. Petersburg. Photo: VIR Archives.

associated with the accelerated progress of genetic technologies.

The Law On Bioresource Centers and Biological (Bioresource) Collections was adopted by the State Duma of the Russian Federation on 26 November 2024, and on 30 November 2024, Russian President Vladimir Putin signed the Federal Law.

Within the framework of the Program for the Development of the National Center for Plant Genetic Resources, annual collecting missions are planned to be conducted abroad from 2024 to 2030. The first mission was carried out in 2024, jointly with the National Research Institute of Plant Genetic Resources of the Republic of Uzbekistan.

In 2023, VIR signed an agreement on scientific cooperation with the National Council of Humanities, Sciences, and Technologies (CONAHCYT) of the United Mexican States; among other objectives of the agreement, a joint collecting mission is planned to be organized in Mexico.

VIR is open to discussions on undertaking important international collecting missions together with other countries within the framework of planning for the period up to 2030.

The VIR World Collection, which began as the Russian Collection of Cultivated Plants, has grown into one of the most systematically collected, comprehensively studied and representative collections of PGR in the world. At present, the activities of VIR as the National Center for Plant Genetic Resources, and the development of the legislative system on genetic resources in Russia will be conducive to fostering effective international scientific cooperation in the field of PGR, thus keeping Vavilov's legacy alive. The National Center will provide

effective solutions to new challenges in the systematic collection, comprehensive study, reliable preservation and sustainable use of PGR in the Russian Federation at a new level.

### Authors contributions

Conceptualization, I.G.L., Yu.V.U. and E.K. Kh.; writing—original draft preparation, I.G.L., Yu.V.U. and E.K. Kh; writing—review and editing, I.G.L., Yu.V.U. and E.K. Kh. All authors have read and agreed to the published version of the manuscript.

### Conflict of interest statement

The authors declare no conflict of interest

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# The Versailles Arabidopsis Stock Center (VASC): original genetic resources exploiting both induced and natural diversity to investigate gene functions and analyze the impact of variation on plant biology

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**Abstract:** *Arabidopsis thaliana* is a powerful plant model for functional biology, genetics and, more recently, population genomics. The Versailles Arabidopsis Stock Center collects, produces, preserves, characterizes and distributes various Arabidopsis biological resources. Besides large collections of mutants, including homozygous mutant lines, this stock centre offers numerous natural genotypes collected worldwide, as well as resources resulting from crosses between these variants. Most of the resources are unique and can be useful to a wide range of users, ensuring cumulative characterization of the same material over time. They are accompanied by molecular characterization, genotyping or sequencing data, enabling the analysis of diversity's impact, particularly on complex plant traits. The collections are made easily and reliably available through an information system comprising a database and a web portal for description and distribution (<https://publiclines.versailles.inrae.fr/>). Several thousand seed lots are provided each year to the international scientific community.

**Keywords:** Plant genetic resources, *Arabidopsis thaliana*, natural variation, mutants

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## Introduction

*Arabidopsis thaliana* (L.) Heynh. is a small wild plant belonging to the Brassicaceae family, like rapeseed, cabbages, radish or mustard. It is easy to grow, has a short life cycle in greenhouse conditions, is mainly self-pollinating but can undergo crosses, and produces many seeds. Thanks to these biological characteristics, it became a plant model species in the 1980s (Meinke *et al*, 1998). In 2000, it was the first plant whose genome was completely sequenced (Arabidopsis Genome Initiative, 2000). As the international scientific community working on Arabidopsis has grown, numerous shared molecular tools, data and genetic resources have emerged and developed, making Arabidopsis the model

system of choice in plant functional biology. In addition to enabling the understanding of many biological questions in this species, findings or biotechnological methods developed in Arabidopsis have also been transposed into crops or other organisms, and to more applied scientific fields such as plant breeding or even medicine (Yaschenko *et al*, 2024). Community-driven databases and stock centres have been created and have played a major role in the advancement of many research programmes. The Arabidopsis Information Resource (Reiser *et al*, 2024) maintains an extensive database, with links to other Arabidopsis resources. Besides the historical stock centres – the Arabidopsis Biological Resource Center (ABRC), Ohio, USA and Nottingham Arabidopsis Stock Centre (NASC), UK, the Versailles Arabidopsis Stock Center (VASC) was developed in the early 1990s in Versailles (France), at the Institute Jean-Pierre Bourgin for Plant Sciences (IJPB) of the

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National Research Institute for Agriculture, Food and Environment (INRAE), with a first collection of T-DNA insertion mutants to explore gene function. Since then, VASC has produced many specific resources exploiting both induced and natural diversity. Except for natural genotypes collected worldwide, these resources are unique, they are not distributed elsewhere, so VASC is complementary to other existing Arabidopsis stock centers. In addition to T-DNA insertion mutant lines and homozygous EMS mutant lines, the collections include worldwide natural genotypes and segregating populations or cytelines derived from crosses between these genotypes, to analyze the impact of natural diversity particularly on complex plant traits such as growth, development, reproduction or stress tolerance. The resources are molecularly characterized and provided to the Arabidopsis community all over the world.

## Mutant collections

### T-DNA insertion lines

The earliest collection was a set of 55,000 T-DNA insertion mutant lines, generated in the Ws (Wassilewskija) background (Bechtold *et al*, 1993), in which T-DNA was inserted randomly in the genome. This collection has been extensively used in numerous studies of forward genetics, based on the screening of mutated lines affected in diverse phenotypes, and the subsequent cloning of the tagged genes. Then, genomic sequences flanking the T-DNA insertions (Flanking Sequence Tags, FST) have been determined for all the T-DNA lines. A total of 46,236 FST have been systematically sequenced (Balzergue *et al*, 2001). They are available in the databases SIGnAL (<http://signal.salk.edu/cg-i-bin/tdnaexpress>) and TAIR (<https://www.arabidopsis.org/>), allowing reverse genetics approaches which consist in looking for a line with an insertion in a candidate gene and then analyzing the mutant phenotype.

Genetic screens have played a major role in deciphering the genetic basis of many biological processes. Both forward and reverse genetics have been used for example to get insight into plant meiosis (Mercier *et al*, 2001). Many genes involved in meiosis were identified in *A. thaliana* by using a phenotypic screen on reduced fertility in the greenhouse, and, in parallel, by searching mutants in homologs of genes that play a role in meiosis in non-plant organisms, for example *Saccharomyces cerevisiae* (Couteau *et al*, 1999; Gallego *et al*, 2001).

Today, these T-DNA insertion mutants are still used to validate candidate genes involved in numerous biological processes.

### Homozygous EMS mutant lines (HEMs)

After a while, forward genetic screens had identified most of the meiotic genes which, when mutated, cause a dramatic reduction in fertility in *A. thaliana*. However, an increasing number of genes that play a role in meiosis without causing marked phenotypes when mutated

were being identified by reverse genetics, suggesting that many genes with a meiotic function remained to be discovered. To this end, VASC, together with the IJPB team working on meiosis, produced about 900 lines randomly mutagenized by EMS (Ethyl Methyl Sulfonate), which were then made homozygous or nearly-homozygous through either haplodiploidization or four generations of selfing by single seed descent (Capilla-Perez *et al*, 2018). In both cases, each line is composed of identical or nearly identical plants. In addition to mutations in promoters and untranslated regions (UTRs) that can impact gene expression, each line contains between 100 and 500 homozygous mutations that affect the sequence of protein-coding genes (e.g. amino-acid change, stop codon, loss of splicing sites). These resources can be used for forward genetic screening, examining either a single plant per line, or several plants to observe a more quantitative phenotype, and enable subtle and repeated phenotyping.

In the HEM collection, 43 lines with meiotic defects were phenotypically identified, of which 21 lines had a mutation in a gene whose role in meiosis had already been demonstrated in another organism. For six of these genes, this was the first time they were identified in a direct screen in Arabidopsis (Capilla-Perez *et al*, 2018). These results show the value of the HEM population and illustrate its potential to screen for any qualitative or quantitative phenotype.

In addition, the whole-genome sequences of all the HEM lines were recently made available (Carrère *et al*, 2024), enabling reverse genetics approaches. On average, three mutations affecting protein sequences are found per gene in the collection. The ATHEM web interface (<https://lipm-browsers.toulouse.inra.fr/pub/ATHEM/>) provides the community with the raw sequences, SNP calling results, and an interface to search for SNPs in given HEM lines or genes. Reverse genetic screens for various functions show the power of this resource to obtain different types of mutant alleles (Carrère *et al*, 2024). In addition, the knowledge of mutations greatly accelerates the search for causal genes in forward genetic screens.

Since 2020, this resource has been the most widely distributed by VASC.

## Collections exploiting natural diversity

### Natural variants (accessions)

Arabidopsis grows naturally throughout the northern hemisphere, in a wide variety of ecological conditions. This makes it an excellent model for studying natural diversity and adaptation, either directly in association studies using natural genotypes, or through segregating populations (Bazakos *et al*, 2017). At present, over 600 natural accessions – individuals collected worldwide in diverse environments – are available at VASC. Most of these genotypes exist in other stock centres or laboratories under the same name, but correspond to different batches of seeds. Due to possible mislabeling or



sequence divergence across time between lineages, these seed batches can be genetically different and should not be mixed to avoid affecting genetic analyses. Each seed batch of the VASC accessions was identified by genotyping with a set of 384 SNP markers (Simon *et al*, 2012), and the genotyping data are available on the dedicated web interface ANATOOL (<https://www.versailles.inra.fr/ijpb/crb/anatool/index.html>). This interface also provides tools that offer a simple and efficient means to verify or determine the identity of the accessions in any laboratory, without the need for any specific or expensive technology.

Recently, chromosome-level genome assemblies were generated from long-read de novo sequencing for 69 natural accessions, using the DNA of plants issued from the VASC seed batches (Simon *et al*, 2022; Lian *et al*, 2024). These data provide insight into the overall genetic variation of the species and add value to our collection of natural accessions. All the parental lines of the VASC recombinant inbred lines (RILs), heterogeneous inbred families (HIFs), and cytolines (see below) are part of these 66 sequenced accessions.

### Mapping populations: F2s, RILs and HIFs

The most important plant traits are quantitative traits, controlled by several genes at different loci and their interactions. To characterize the genetic architecture and identify the molecular basis of such traits, segregating populations dedicated to quantitative trait loci (QTL) analyses have been developed. The VASC generated 262 F2 families and 16 RIL populations from crosses between natural accessions. RILs are particularly interesting because they are nearly homozygous and can be propagated as genetically identical individuals, enabling the phenotyping of many traits on the same material genotyped only once. The VASC RIL populations (Simon *et al*, 2008) have been generated from genetically and phenotypically distant accessions, covering a wide range of diversity (Mckhann *et al*, 2004). They are composed of large numbers of individuals (343 on average per population) to enhance the statistical power of QTL detection. In addition, an optimal subset of 164 lines (core population) was determined for each RIL population, allowing users to phenotype a reduced number of lines with limited QTL detection power loss. The genetic maps rely on common markers, enabling the localization of QTLs mapped with different RIL populations to be compared. A very large number of studies have been published using this resource to decipher the genetic basis of various traits (for example Brachi *et al* (2010); Gravot *et al* (2011); Poque *et al* (2015); Shahzad *et al* (2016); Wuest and Niklaus (2018); Brock *et al* (2020); Hanemian *et al* (2020)).

HIFs are nearly isogenic lines used as a complement to the RIL populations to confirm QTLs (Loudet *et al*, 2005). They were selected in the progeny of RILs that show a single residual heterozygous region. Three complete HIF populations covering the whole genome are currently available.

### Cytolines

Because the functioning of organelles (mitochondria and plastids) involves the interaction of proteins encoded by the nuclear and cytoplasmic genomes, these genomes are coadapted at the species level. To assess the impact of cytoplasmic variation and nucleo-cytoplasmic interactions on plant phenotypes, we created a unique series of 56 cytolines, whose cytoplasmic and nuclear genomes come from two different natural accessions (Roux *et al*, 2016). The cytolines were generated from reciprocal crosses between eight natural accessions representative of the species diversity, followed by recurrent backcrossing with the nuclear genome donor. Cytonuclear interactions were shown to affect several phenotypic traits, 1) indicating that cytoplasmic and nuclear genomes can interact to shape integrative traits that contribute to adaptation, and 2) highlighting a possible role for these interactions in the evolutionary dynamics of the species (Roux *et al*, 2016).

### Epigenetic recombinant inbred lines

In addition to genetic variation, epigenetic variation can affect plant phenotype. Epigenetic modifications, such as DNA methylation, do not alter the DNA sequence but can be transmitted from one generation to the next. DNA methylation is a source of heritable phenotypic variation notably because it can affect gene expression. A set of 500 epigenetic recombinant inbred lines (epiRILs) was generated to study the impact of DNA methylation on phenotypic variation (Johannes *et al*, 2009). These epiRILs are derived from two closely related parents that have few DNA sequence differences but contrasting DNA methylation profiles. One parent is the accession Col-0, and the other is a homozygous mutant in Col-0 for the *DDM1* gene, involved in the maintenance of DNA methylation (Vongs *et al*, 1993). These epiRILs enable the analysis of epigenetic variation and the mapping of epigenetic QTL associating epialleles with phenotypic traits (Zhang *et al*, 2021; Petitpas *et al*, 2024).

## Management

### Staff and partnership

VASC is run by two permanent INRAE staff members, a scientific manager (Research Engineer) and an operating manager (Technician), for its scientific and technical activities. Since 2022, these two members have been supported by two additional staff, each for 20% of their time, in charge of the quality and certification procedures. Governance includes a steering committee comprising these four persons plus the head of the IJPB, a user committee comprising the steering committee plus IJPB researchers and an external scientist, and a scientific advisory board made up of the user committee plus a foreign scientist.

Despite its limited staff, VASC manages to continue the development and characterization of new genetic resources, such as the HEM collection recently, through

projects carried out in partnership with other research teams, at IJPB or more widely. VASC is always open to developing new collaborations. We can provide our expertise in producing resources dedicated to specific approaches that can subsequently be useful to a wide audience. We can also maintain, host and distribute resources collected or generated by other laboratories. To this end, VASC benefits from IJPB's infrastructures (large-scale greenhouses, growth chambers, seed conservatories) and a skilled workforce for plant growing. Within the IJPB Plant Observatory, VASC also interacts closely with the Phenoscope high-throughput phenotyping platform (Tisné *et al*, 2013). A large proportion of our genetic resources (accessions, RIL, HIF, cytolines) have been phenotyped using this tool under homogeneous and highly controlled conditions (for example, Marchadier *et al* (2019)).

VASC is part of the National Research Infrastructure of Agronomic Biological Resource Centers RARe (Agronomic Resources for Research). This enables us to share experiences with other resource centres, particularly in terms of management, regulation and quality.

### Information system, distribution and funding model

VASC has established its own information system comprising a database and a web portal for data and distribution (<https://publiclines.versailles.inrae.fr/>). The online catalogue presents all resources and their descriptions. Collections are systematically characterized and molecular data are made easily available to the scientific community via downloadable files or hypertext links. Seeds can be ordered directly from the catalogue pages of the website. The price of orders is calculated automatically, and seeds are paid for online at the time of ordering. An invoice is issued and sent automatically to the client. An e-mail is automatically sent when the seeds are shipped, on average within four working days. The website enables the VASC staff to track all orders and clients.

Over the past five years, an average of more than 5,000 seed samples were distributed annually. More than 200 customers, from 26 countries, have placed orders, of which around one-third in France and two-thirds abroad. The most represented foreign countries were Germany, the USA, the Netherlands, Belgium, the United Kingdom, Italy, Switzerland and China. The most widely distributed resources are always the most recent. The EMS collection, which is the most recent, has been the most widely distributed since 2020. This motivates us to acquire new resources.

Seed sales represent a total income of about €20,000 per year. VASC is part of IJPB and has no funding of its own: VASC revenues are pooled at the institute level and operating costs are covered by IJPB funds.

### Quality

Multiplication of seed stocks is conducted according to defined protocols designed especially to avoid seed con-

tamination. Seeds are kept in a seed conservatory under controlled conditions at a low temperature (4°C) with 12% hygrometry. Security duplicates are maintained at -20°C to ensure preservation of the resources in the long term. Germination rates are regularly evaluated on samples from the different collections, testing 100 seeds per sample. To regenerate seed stocks, propagation is carried out by self-fertilization in insect-proof greenhouses. An identification number is assigned to each seed batch and is associated with a barcode that enables computerized tracking from sowing to harvesting and distribution. These procedures guarantee traceability and reliability during the production and distribution of the resources. Under these high-quality standards, VASC obtained the IBISA<sup>1</sup> label in 2023. It has also implemented a Quality Management System based on the ISO9001:2015 standard, and achieved certification in 2024. Our efforts in the production, conservation and characterization of resources, as well as in the establishment of an efficient information and distribution system, have already earned us worldwide recognition for the interest and quality of our collections, our prompt distribution and the support we provide to our customers.

### Past and present research projects

The resources produced have always been exploited by the VASC team in research projects. This enables us to anticipate the needs in terms of genetic resources, to obtain funding and gain recognition. Our research focuses on genomes, both their expression and their evolution, particularly from the point of view of genomic conflicts that can lead to the establishment of reproductive barriers.

A transcriptome study of two RIL populations has revealed, in each population, several thousands of expression QTLs (eQTLs; Cubillos *et al* (2012)) providing a basis for identifying the gene networks involved in different pathways (Xue *et al*, 2024).

We have observed genetic incompatibilities in the progenies of certain crosses, where particular combinations of alleles at different loci lead to lethality (e.g. at the embryonic stage) or to total or partial sterility. We have found in our RIL populations several different pairs of loci that lead to this type of situation, and we have identified the partner genes and elucidated the mechanisms involved, some of which are epigenetic in origin (Bikard *et al*, 2009; Durand *et al*, 2012; Agorio *et al*, 2017; Jiao *et al*, 2021). These phenomena can explain the lethality observed in hybridizations between varieties or species, which can have major implications for plant breeding and introgression programmes. Studying the reproductive barriers they create can also help us understand the mechanisms that lead to the formation of new species, an overarching goal in biology.

<sup>1</sup> <https://www.ibisa.net/annuaire-crb/versailles-arabidopsis-stock-center-vasc-306.html>

We uncovered a cryptic cytoplasmic male sterility (CMS) in *A. thaliana*. CMS, which is a source of reproductive polymorphism in angiosperms and of major relevance in hybrid breeding, is genetically determined by both mitochondrial and nuclear factors. A new mitochondrial gene causing sterility (Gobron et al, 2013) as well as a nuclear gene restorer of fertility (Durand et al, 2021) were identified, and the process of pollen abortion in this CMS system was characterized (Dehaene et al, 2024). This CMS participates in the hybrid sterility phenotypes observed in some crosses, together with segregation distorter loci responsible for pollen lethality (Simon et al, 2016). We characterized one of these pollen killers, identifying three genes involved in its functioning and exploring the high locus diversity at the species level (Simon et al, 2022; Ricou et al, 2025). We found both sensitive and killer plants coexisting in local French populations, which constitutes an invaluable resource for studying pollen killer evolution in the wild. Indeed, understanding how gamete killers appear and propagate in populations remains a major issue in evolutionary biology, and Arabidopsis proved to be a powerful model for investigating evolutionary dynamics at complementary geographical scales.

### Conclusion and perspectives

We are determined to continue our commitment to proposing high-quality genetic resources, guaranteeing their long-term conservation, and generating knowledge on these resources to increase their value for research.

Our recent results (Ricou et al, 2025) underline that Arabidopsis, originally mainly a functional biology model, is also a valuable model for conducting studies in population biology, thanks to tens or hundreds of genotypes collected in many local populations (Brachi et al, 2013; Frachon et al, 2017). In this framework, our upcoming resources coming soon will consist of 458 whole-genome sequenced accessions collected from 168 natural sites located in the southwest of France and characterized for a unique set of ecological factors, including climate, edaphic properties, bacterial communities (soil, root and leaf), plant communities and human activities including urbanization (Bartoli et al, 2018; Frachon et al, 2019; Roux et al, 2023). Both whole-genome sequences and deep ecological characterization of their native habitats represent a strong added value to these resources.

We wish the Arabidopsis community to keep using the VASC resources. Citing this article when publishing your results that use these resources will enable us to list the studies based on our collections, attest their usefulness, and therefore ensure the continuity of VASC funding.

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### Author contributions

CC wrote the manuscript and AR, CG, CH and OL reviewed it

### Conflict of interest statement

The authors have declared that no conflicts of interest exist.

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# Organization of plant Biological Resource Centers for research in France: History, evolution and current status

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**Abstract:** Since their inception, the French academic organizations dedicated to agricultural research have developed plant collections in genebanks, often within a public–private framework, to support the study of plant traits and the development of new improved varieties. In addition, since the 2000s, a centre for genomic resources has also been established in France. Over the last 20 years, this decentralized system, consisting of the academic genebanks and the centre for genomic resources, has been supported by a national coordination structure. The objectives were to align the network activities with the framework proposed by the Organisation for Economic Co-operation and Development (OECD) for Biological Resource Centres and to foster collaboration with other national stakeholders involved in the conservation and characterization of plant genetic resources (PGR). In 2015, the network was named BRC4Plants and become part of the French National Research Infrastructure RARE ([www.agrobrc-rare.org](http://www.agrobrc-rare.org)), supported by the French Ministry of Research. This paper describes BRC4Plants, its users, services and cross-cutting activities. We also highlight its relations with its national and international stakeholders involved in the conservation and characterization of PGRs. BRC4Plants aims to be a key player in addressing societal and research challenges regarding agroecology, climate change mitigation and healthy food systems.

**Keywords:** Plant genetic resources, genomic resources, BRC, Research Infrastructure, ECPGR, genebanks

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## Origins of BRC4Plants, the French network of Plant Biological Resource Centers (BRC) for research

Since their inception, the French academic organizations for research in agriculture have built plant collections to support the study of plant traits and the development of new improved varieties. Among the oldest academic French collections, the grapevine collection began to be established in the late 19<sup>th</sup> century by the College of Agriculture of Montpellier, to tackle the challenges of mildews and phylloxera that were devastating French viticulture (Pouget, 1990). Another emblematic example is the creation in the 1960s of the PRO-MAIS French non-governmental organization grouping all the French maize breeders to facilitate collaborations with the academic sector, in particular to collect and conserve maize genetic resources (Dallard *et al.*, 2000). Today, the conservation of biodiversity – particularly cultivated biodiversity or agrobiodiversity – is more crucial than ever and is key for adaptation to the accelerating global changes impacting the planet, human health and nutrition (Pilling *et al.*, 2020; ECPGR, 2021; Lefèvre *et al.*, 2024).

The organization and evolution of the French activities dedicated to genetic resource conservation are described in Roux-Cuvelier *et al.* (2021). Here, we summarize the main steps and provide additional insights.

In 1983, the French Ministry of Agriculture set up a coordination office for the conservation of genetic resources (plants and domestic animals) which operated until 2008 – the *Bureau de Ressources Génétiques* (BRG). The BRG was a *Groupeement d'Intérêt Scientifique*, a French associative structure gathering under a memorandum of understanding the Ministries of Research, Agriculture and Environment and six French Research Institutes. It had three main mandates: the coordination of genetic resource management, the representation of France in international bodies, and the facilitation of genetic resource research. The BRG was in particular mandated to implement a decentralized system for PGR conservation and to facilitate the establishment of commodity-specific and multi-stakeholders networks – including academic institutions, non-governmental organizations, regional organizations and private companies – to carry out this conservation. After 2008, the French Government decided to merge BRG with a newly created foundation, the *Fondation pour la Recherche sur la Biodiversité*. In reality, coordination and facilitation – in particular for basic genetic resource conservation activities – have not been taken over by the *Fondation pour la Recherche sur la Biodiversité*, which has focused on supporting the development of biodiversity knowledge. To address this gap, the managers of the plant collections from the main French research

institutes developed their own inter-institute networking and coordination activities. Since the end of the 1990s, the management of plant biological resources dedicated to research and development activities (R&D) has been professionalized using the concept of Biological Resource Center (BRC, OECD - Organisation for Economic Co-operation and Development, OECD (2001)) as a reference. In particular, academic BRC managers actively contributed to the development of a French quality management norm dedicated to BRCs, S96-900:2011. Later on, they contributed to the international norm dedicated to Biobanking ISO 20387:2018 and its technical specification (ISO/TS 23105:2021) in order to improve the quality of their services, which include, among others, the conservation, documentation and provision to users of plant biological resources. A still growing number of French BRCs dedicated to R&D are certified based on these norms and/or ISO9001. These developments were supported by a French inter-research institute organization, *Infrastructures en Biologie, Santé et Agronomie* (IBiSA, <https://www.ibisa.net/>), aiming at labelling research facilities and funding their developments.

In 2015, the informal network of plant BRCs supported by French academic organizations joined four other BRC networks on domestic animals, microbes, forest trees and environmental samples to set up a national infrastructure of resources for research in agriculture, RARE (<https://www.agrobrc-rare.org>; Figure 1) (Tixier-Boichard *et al.*, 2024). RARE is included in the French Ministry of Research's roadmap and will soon need to reapply for this label, which provides access to funding and recognition. The plant network was named BRC4Plants: it currently gathers 21 BRCs, including the French National Center of Genomic Resources (CNRGV, Table 1). The network spans the entire French territory, including overseas departments and regions, and conserves species cultivated in a wide range of climates (Figure 2).

The main missions of BRC4Plants's BRCs are to 1) ensure the proper maintenance of crop diversity in relation to the challenges faced by both biodiversity and agriculture (Lefèvre *et al.*, 2024) and 2) provide access to high-quality biological resources and associated data to the scientific community of plant biologists and breeders. BRC4Plants coordination activities aim to effectively support these initiatives through strategic discussions and decisions, training and facilitation.

BRC4Plants has developed a formal governance structure that supports strategic decisions through a committee comprising representatives of the main funding institutes (INRAE, the National Research Institute for Agriculture, Food and Environment; CIRAD, the French agricultural research and cooperation organization working for the sustainable development of tropical and Mediterranean regions, and IRD, the French National Research Institute for Sustainable Development) and the coordinator of RARE who meet twice a year. In addition, coordinated cross-cutting activities are organized

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both at the plant network level and the RARE level. For instance, all BRCs are supported by such internal transversal activities for their compliance with access and benefit sharing (ABS) rules, the General Data Protection Regulation and for their capacity to become certified ISO9001. In 2020, RARE established its international scientific advisory board comprising experts of all domains, which has met annually since then, along with an annual thematic scientific workshop. These two new annual events foster scientific strategic discussions across domains. RARE facilitated the contribution of several networks to common projects such as the Coordination Support Action of the H2020 EC programme, ‘GenRes Bridge’ (grant agreement No 817580). The GenRes Bridge project partners developed a Genetic Resources Strategy for Europe ([GenRes Bridge Project Consortium, ECPGR, ERFP and EUFORGEN, 2021](#)) in collaboration with the three European networks for the conservation of genetic resources – the European Cooperative Programme for Plant Genetic Resources (ECPGR), the European Forest Genetic Resources Programme (EUFORGEN) and the Regional Focal Point for Animal Genetic Resources (ERFP). BRC4Plants coordinated a strategic document on data management ([Adam-Blondon et al, 2021](#)).

Finally, RARE has clarified the conditions necessary for a BRC to join or leave the infrastructure. As a result, within some of the RARE’s networks such as BRC4Plants, BRCs are divided into two groups: full members of RARE and those in progress towards membership. BRC4Plants is currently composed of 18 RARE member BRCs and three in-progress BRCs ([Table 1](#)). The difference between these two statuses is not yet significant as the only benefits obtained by BRCs from RARE derive from low-cost networking activities and it is not in the global interest to restrict access. However, the criteria of differentiation (certification of the quality management and/or IBiSA label) might soon be important for getting access to some funding dedicated to BRCs. After almost

ten years of operation, this framework has reached its full maturity and its renewal on the roadmap is planned for 2025.

### BRC4Plants collections and services for the research community

Crop genetic and genomic resources have always been instrumental for research in plant genetics, plant biology and for fostering academic and socio-economic research and development partnerships. They have been intensively used to characterize genetic diversity, explore the evolutionary history of crops, develop advanced genomic tools, select panels of genetic resources for association studies, carry out pan-genome studies, develop prediction models and breeding strategies, screen for adaptive traits, and decipher molecular mechanisms with mutant collections ([Jourdan et al, 2015](#); [Allier et al, 2020](#); [Leuenberger et al, 2024](#); [Healey et al, 2024](#)).

Most BRCs and their collections are managed or co-managed by INRAE, CIRAD and IRD in partnership with technical institutes and higher education institutions ([Roux-Cuvelier et al, 2021](#)).

As a whole, BRC4Plants maintains 214,918 accessions of a large range of model species, field crops, vegetables and fruit trees ([Table 1](#)). An accession is defined as an entry in the collection and the unit of conservation. A crop variety can be represented by several accessions. Different types of material (samples) can be conserved and distributed from a given accession: reproductive (e.g. seeds, in vitro plants, pollen, cuttings, etc.) or non-reproductive (e.g. leaf, DNA, etc.). The collections are usually very diverse, in terms of accessions’ country of origin, phenotypes or genetic diversity ([Salinier et al, 2022](#)). They include varieties, landraces and wild relatives but also material derived from breeding or research programmes ([Roux-Cuvelier et al, 2021](#); [Salinier et al, 2022](#); [Esnault et al, 2025](#)).



**Figure 1.** Organization of the RARE National Research infrastructure ([Tixier-Boichard et al, 2024](#)), in five domain-specific networks of Biological Resource Centres (BRCs): CRB-Anim manages biological resources for domestic animals, BRC4Env manages biological resources and specimens in relation with various types of terrestrial environments (soils, lakes, pathogens, etc.), FBRC manages forest trees biological resources dedicated to research purposes, BRC4Microb manages microbial resources and BRC4Plants manages model and cultivated plant biological resources.



**Table 1.** List of the Biological Resource Centers that are members of BRC4Plants or with a status of candidate, their supporting institutes, the type of resources that they manage, the state of their contribution to national public–private networks dedicated to the conservation of genetic resources and to international working groups (2024).

Name	Main genera or species	Institute(s)	French public–private networks	International networks	Rare	Reference
French Plant Genomic Center (CNRGV)		INRAE			Member	<a href="#">Roux-Cuvelier et al (2021)</a>
Versailles Arabidopsis Stock Center (VASC)	<i>Arabidopsis thaliana</i>	INRAE			Member	<a href="#">Ricou et al (2025)</a>
BrACySol	<i>Brassica oleracea</i> , <i>Brassica napus</i> , <i>Allium cepa</i> Aggregatum group, <i>Allium sativum</i> , <i>Solanum tuberosum</i>	INRAE	Vegetable crucifers, Oilseed crucifers and potato networks	ECPGR <i>Allium</i> , <i>Brassica</i> , Potato	Member	<a href="#">Roux-Cuvelier et al (2021)</a> ; <a href="#">Esnault et al (2025)</a>
Coffea	<i>Coffea</i> spp.	IRD, CIRAD		ECPGR Cryopreservation	Member	<a href="#">Joët et al (2021)</a>
Carrot and other vegetable Apiaceae (Carpia)	<i>Daucus</i> spp., <i>Chaerophyllum bulbosum</i> , other Apiaceae	Institut Agro	Carrot and other <i>Daucus</i> network (coordination)	ECPGR Umbellifer crops EVA ISHS Carrot and other Apiaceae	Member	<a href="#">Roux-Cuvelier et al (2021)</a>
Small grain cereals (Cereales)	<i>Triticum</i> spp., <i>Hordeum</i> spp., <i>Secale</i> spp., <i>Triticosecale</i> spp., <i>Avena</i> spp.	INRAE	Small grain cereals network	ECPGR Wheat, <i>Avena</i> , Barley	Member	<a href="#">Debiton (2021)</a>
Citrus	<i>Citrus</i> spp., <i>Poncirus</i> spp., <i>Fortunella</i> spp., <i>Clausena</i> spp.	INRAE, CIRAD			Member	<a href="#">Roux-Cuvelier et al (2021)</a>
Forage and turf species (Prairies)	<i>Lolium</i> spp., <i>Festuca</i> spp., <i>Dactylis</i> spp., <i>Medicago</i> spp., <i>Trifolium</i> spp.	INRAE	Forage and turf network	ECPGR Forages	Member	<a href="#">Sampoux et al (2025)</a>
Pip fruit and rose (RosePom)	<i>Malus</i> spp., <i>Pyrus</i> spp., <i>Cydonia</i> spp., <i>Rosa</i> spp.	INRAE	Pip fruits and Rose networks	ECPGR <i>Malus</i> / <i>Pyrus</i>	Member	<a href="#">Roux-Cuvelier et al (2021)</a> ; <a href="#">Feugey et al (2025)</a> submitted
Seeds adapted to Mediterranean and tropical conditions (GAMÉT)	<i>Oryza</i> spp., <i>Sorghum</i> spp., <i>Digitaria</i> spp., <i>Gossypium</i> spp., <i>Arachis</i> spp., <i>Vigna unguiculata</i> , <i>Zea</i> spp., <i>Medicago</i> spp.	INRAE, CIRAD	Promais network	ECPGR Maize	Member	<a href="#">Maghnaoui and Proserpi (2017)</a>

Continued on next page

Table 1 continued

Name	Main genera or species	Institute(s)	French public-private networks	International networks	Rare	Reference
Vegetable germplasm (Leg)	<i>Solanum melongena</i> , <i>Capsicum annuum</i> , <i>Solanum lycopersicum</i> , <i>Cucumis melo</i> , <i>Lactuca sativa</i>	INRAE	Solanaceae, <i>Lactuca</i> and melon networks	ECPGR Cucurbits, Solanaceae, Leafy Vegetable	Member	<a href="#">Salinier et al (2022)</a>
French Maize Inbred Lines Genebank (Mais-Lig)	<i>Zea</i> spp.	INRAE	Promais network	ECPGR Maize	Candidate	<a href="#">Diaw et al (2017)</a>
Olive trees (Olivier)	<i>Olea europaea</i>	Conservatoire Botanique National Méditerranéen (CBNMED), INRAE		International Olive Council	Candidate	<a href="#">Marchal et al (2017)</a> ; <a href="#">Roux-Cuvelier et al (2021)</a>
Perennial plants in Guyana (PPG)	<i>Coffea</i> spp., <i>Theobroma</i> spp., <i>Hevea</i> spp., <i>Dalbergia</i> spp.	CIRAD			Member	
Tropical plants (PlanTrop)	<i>Musa</i> spp., <i>Ananas</i> spp., <i>Saccharum</i> spp., <i>Mangifera</i> spp., <i>Dioscorea</i> spp.	CIRAD, INRAE			Member	<a href="#">Roux-Cuvelier et al (2021)</a>
Grain legumes (Protea)	<i>Vicia faba</i> , <i>Lupinus</i> spp., <i>Pisum</i> spp.	INRAE		ECPGR Grain Legumes	Member	<a href="#">Aubert et al (2023)</a> ; <a href="#">Carrillo-Perdomo et al (1914)</a>
Prunus-Juglans (Pru-Ju)	<i>Prunus armeniaca</i> , <i>Prunus dulcis</i> , <i>Prunus persica</i> , <i>Prunus cerasus</i> , <i>Prunus domestica</i> , <i>Juglans</i> spp.	INRAE	<i>Prunus</i> (in progress), <i>Juglans</i>	ECPGR <i>Prunus</i>	Member	<a href="#">Roux-Cuvelier et al (2021)</a>
Sunflower and soy (Helia-Soja)	<i>Heliantus</i> spp., <i>Glycine max</i>	INRAE	<i>Helianthus</i> and Soya		Candidate	<a href="#">Terzić et al (2020)</a>
Vanilla of Tahiti (Vanira)	<i>Vanilla</i> spp.	Etablissement Vanille de Tahiti			Member	<a href="#">Roux-Cuvelier et al (2021)</a>
VATEL	<i>Vanilla</i> spp., tropical <i>Allium</i> spp., roots and tubers, neglected vegetables	CIRAD		Germination International Network	Member	<a href="#">Roux-Cuvelier et al (2021)</a>
Grapevine Biological Resources Center (Vigne)	<i>Vitis</i> spp.	INRAE	<i>Vitis</i> network	ECPGR <i>Vitis</i>	Member	<a href="#">Roux-Cuvelier et al (2021)</a>

In addition, CNRGV develops and maintains plant genomic resources with a catalogue of more than 300 bacterial artificial chromosome (BAC) libraries (around 35 million samples) from more than 60 different plant species. CNRGV provides the research community with a range of services to characterize genetic resources at the genomic level with cutting-edge technologies, in collaboration with other French national genomic facilities that together allow the production of high-quality reference genomes required for diversity analysis and association studies, and the detailed analysis of intraspecific diversity at loci carrying key genes.

In 2022, BRC4Plants provided around 13,000 genetic resources accessions, over 100 BAC clones or libraries and globally billed an important volume of genomic services to a diversified panel of users: 49% to French public institutes, 37% to foreign public institutes, 13% to private companies or producers and 1% to schools, regional public structures, NGOs etc. The introduction and distribution activities follow national and international regulations concerning sanitary issues and ABS. The terms of access to BRC's services are available on the CNRGV website for genomic services (<http://cnrgv.toulouse.inra.fr/fr/Services>) and on the web portal of BRC4Plants for the other BRCs (e.g. <https://florilege.arcad-project.org/fr/crb/bracysol/conditions-generales>). The Standard Material Transfer Agreement (SMTA) of the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) is used for species on Annex 1 of ITPGRFA. An INRAE standard material transfer agreement, compliant with the Convention on Biological Diversity (CBD) Nagoya Protocol is used for other species or for material developed by INRAE (e.g. segregating population, pre-breeding material, etc.). In case of scarcity of the material for distribution, the priority has been so far to support research, development and training.

BRC4Plants activities (of individual BRCs as well as those coordinated by the network) are supported by academic organizations, within the framework of their research priorities, with permanent positions dedicated to the BRC missions and a wide range of infrastructures, including robots, refrigerators, freezers (-20°C and -80°C), cryotanks, seed drying rooms, greenhouses, field plots for seed regeneration or plant conservation (vegetatively-propagated or perennial plants).

The network as a whole also provides an important volume of communication activities that contribute to raising awareness of genetic resources to a large audience (e.g. Pic *et al* (2017), New York Times (Poll, 2021), Le Monde (Rosier, 2021)). Finally, the network contributes to training and education on biodiversity conservation as well as on genomic approaches.

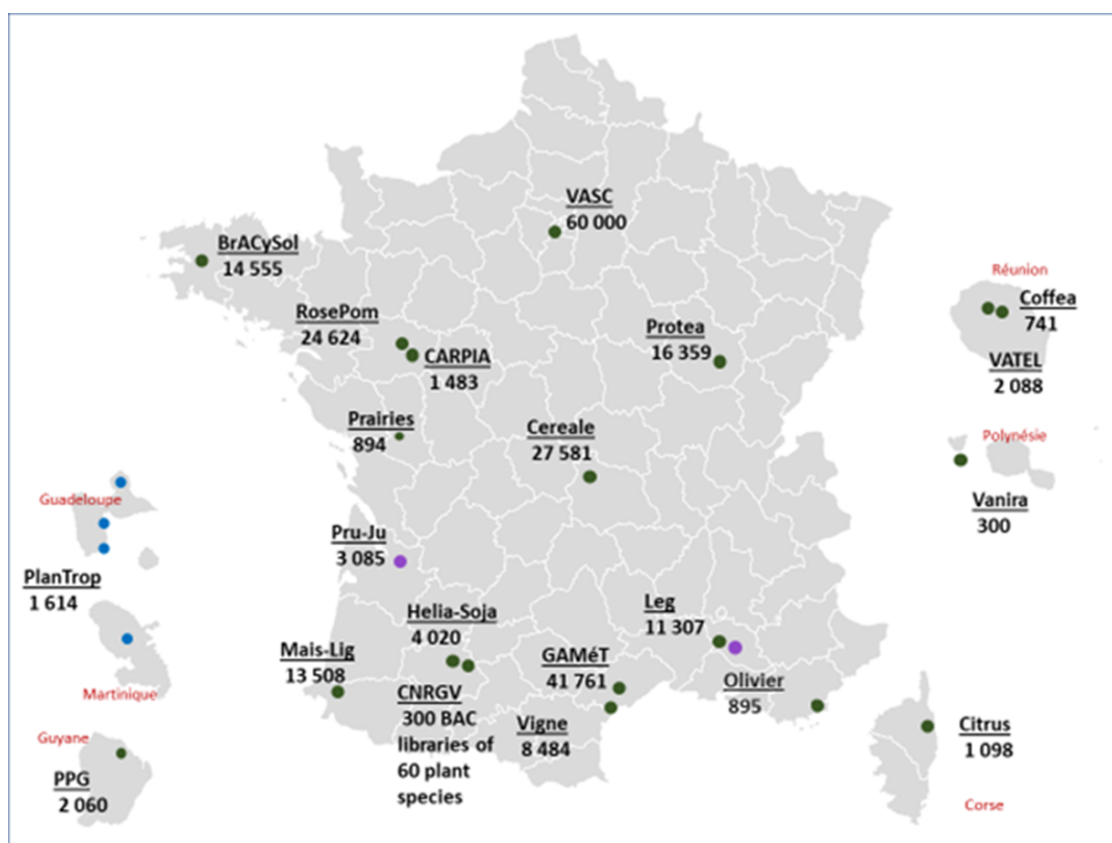
### BRC4Plants R&D activities

BRC4Plants regularly updates its priorities in terms of R&D, which can be seen at two levels.

First, R&D activities that aim at improving the efficiency of conservation and distribution of accessions. Key areas include transversal issues such as management and legal compliance regarding phytosanitary and ABS issues (Tixier-Boichard *et al*, 2024), FAIR (data are findable, accessible, interoperable and reusable) data management and automated data workflows between local databases and central information systems, and improved cryopreservation methods (Markovic *et al*, 2015). These activities also include local improvements of processes and equipment in BRCs and are mainly funded by French research organizations, the French National Research Agency or sometimes European Funds for Regional Development (FEDER).

The second level includes R&D activities aiming at improving services dedicated to research projects using genetic resources to achieve their goals: development of new services, such as the development of new populations and core collections (Esnault *et al*, 2017; Terzić *et al*, 2020; Salinier *et al*, 2022), new knowledge on biological resources, e.g. through their genomic or phenotypic characterization (Antoine *et al*, 2023; Martínez-Flores *et al*, 2020), and improved breeding strategies (Sanchez *et al*, 2023). These R&D activities are supported by a diverse set of national and European calls and the applications are frequently driven by public and/or private researchers who are not BRC members. Calls dedicated to infrastructures at the national or international level can be an opportunity to work with other infrastructures. For instance, BRC4Plants has actively liaised with the European infrastructures EMPHASIS (plant phenotyping) and ELIXIR (bioinformatics for life sciences) to contribute to the development of a suite of guidelines and resources supporting FAIR-compliant management of plant genotyping and phenotyping data. This work was supported by several EU programme Horizon 2020-funded projects: ELIXIR-Excelerate, grant agreement no. 676559 <https://elixir-europe.org/about-us/how-funded/eu-projects/excelerate>; AGENT, grant agreement no. 862613 <https://www.agent-project.eu/> and ELIXIR-CONVERGE, grant agreement no. 871075 <https://elixir-europe.org/about-us/how-funded/eu-projects/converge>, and all the results can be accessed through the ELIXIR portal of resources for data management (<https://rdmkit.elixir-europe.org/>). Building on these resources, a national project currently gathers partners from three French national infrastructures, RARE, the French Institute of Bioinformatics (<https://www.france-bioinformatique.fr/>) and INRAE Genomics (<https://inrae-genomics.hub.inrae.fr/>) to develop a comprehensive service of genomic data management from data production to data submission to the European archives maintained by the European Bioinformatics Institute (EMBL-EBI). Projects including the characterization of PGR or the development of pre-breeding populations can be funded by national calls dedicated to public-private partnerships. They often build on the trust developed in the public-private networks in which many of the BRCs are involved (see below and Table 1).





**Figure 2.** Localization of the 21 BRCs facilitated by BRC4Plants together with the number of accessions they manage. In general, they are single-located (green points) but two have several locations, Pru-Ju (purple points) and PlanTrop (blue points).

They can also be funded by the European Commission (e.g. H2020 G2P-Sol project, grant agreement no. 677379, <http://www.g2p-sol.eu/>).

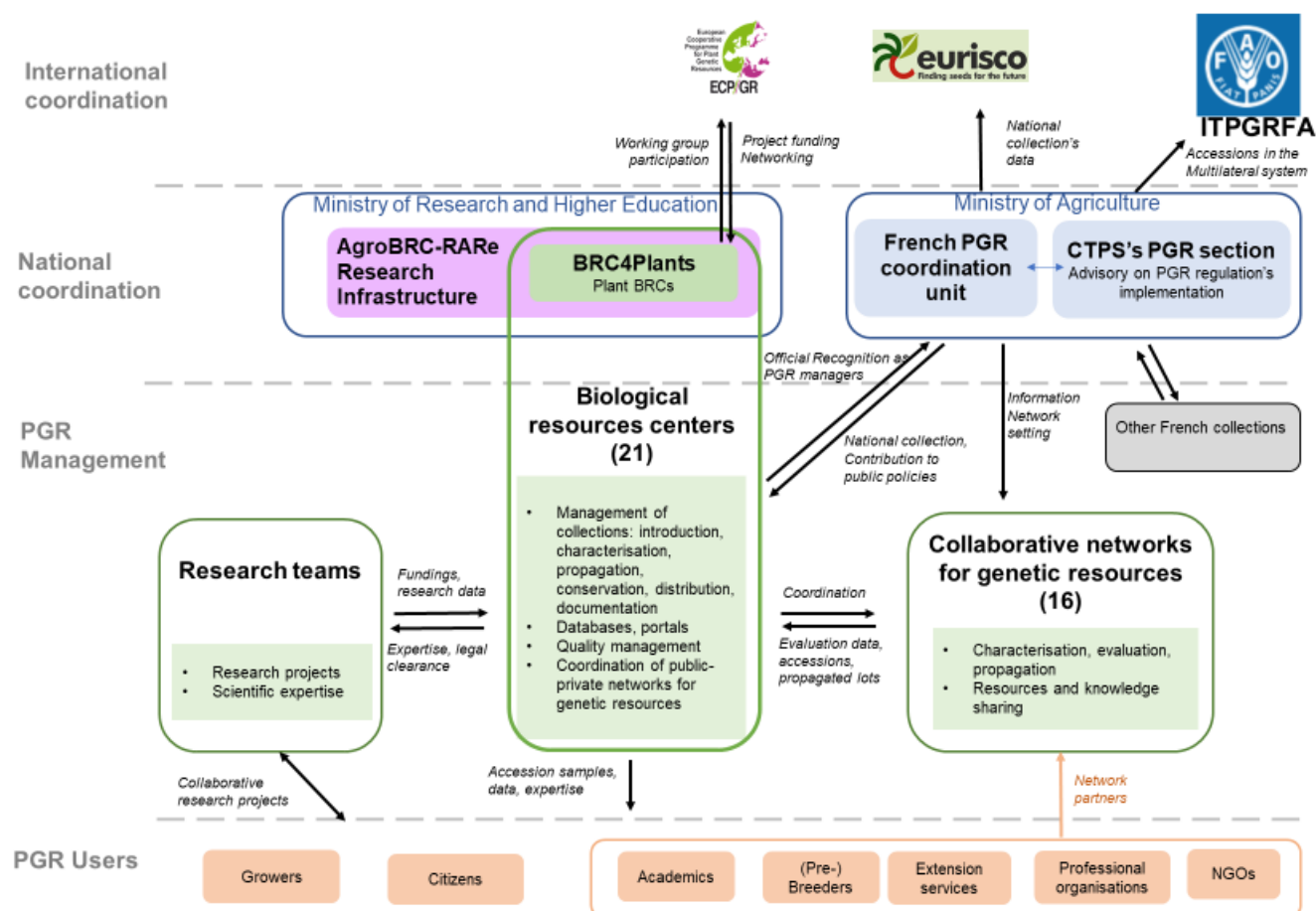
BRC4Plants organizes training and dissemination activities to ensure that the outputs of the projects can be leveraged by all BRCs.

### Public-private partnerships

Half of the BRCs collaborate with networks gathering different types of partners (seed companies, breeders, extension institutes, regional organizations for genetic resource conservation, NGOs; Table 1, Figure 3 ). These collaborative networks, established in the 1990s, aim to pool efforts to manage and study mostly traditional genetic resources of a species or a related group of species. A model charter for these ‘genetic resources networks’ was developed in 2018 and updated in 2021 by BRC4Plants, INRAE central services, the French Seed Association and the French Ministry of Agriculture to define the rules for material and data exchange within and out of the network. Some of these networks have formal organizations (e.g. Promaïs for maize, <http://pro-maize-corn.com/>, the *Federation Franc* for the rose or a joint unit of INRAE with the *Institut Français de la Vigne et du Vin*, IFV, for grapevine).

The existence of such partnerships has made it possible to inventory and secure old French genetic material (e.g. Dallard et al (2000); Terzić et al (2020)).

Additionally, the grapevine-dedicated joint unit has been instrumental in exchanging technical expertise. IFV and INRAE have also brought together all the other French regional extension services for viticulture and oenology to formally organize the development of grapevine varieties resistant to diseases and adapted to each French terroir (INRA, 2018). INRAE and IFV are promoting both traditional genetic resources (a renewal of interest for old varieties was observed in many places) and new resistant varieties under the brand ENTAV-INRA ([www.entav-inra.fr](http://www.entav-inra.fr)). Similarly, a convention between INRAE and the four French potato breeders gathered within the association named ACVNPT (*Association des Créateurs de Variétés Nouvelles de Pomme de Terre*) was signed in 1995. ACVNPT provides financial support to INRAE for the conservation and characterization of potato genetic resources maintained within the BrACySol BRC, and in return gets free access to the pre-breeding material generated by INRAE within the framework of its research activities (Kerlan et al, 2017; Esnault et al, 2025). Since 1995, INRAE has proposed 994 pre-breeding clones, improved mainly for resistance to biotic stress. Forty-one potato varieties, originating from these pre-breeding clones, have been registered.



**Figure 3.** BRC4Plants in its ‘ecosystem’ of stakeholders at different levels. At the international level, BRC4Plants contributes to the activities of the European Cooperative Programme for Plant Genetic Resources (ECPGR), deposits data in the catalogue of ECPGR, EURISCO, and contributes to the deposition of collections of accessions in the multilateral system of the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA). At the national level, BRC4Plants: 1) is funded by the French Ministry of Research and follows its recommendations related to research infrastructures (enforced by BRC4Plants main funding institutes, INRAE, CIRAD and IRD) and 2) interacts with the French coordination on plant genetic resources conservation and contributes to the section of the technical committee for breeding (CTPS) dedicated to plant genetic resources that are both under the governance of the French Ministry of Agriculture. The daily management of BRC4Plants collection is made in collaboration on one side with research units that were often at the origin of the collections and often on the other side with national public-private networks of partners that may also represent important users of those collections. The interactions with BRC4Plants stakeholders are represented by arrows and their results or purposes are specified in italics.

### BRC4Plants contribution to the French National Coordination for Plant Genetic Resources activities

BRC4Plants is a self-organized, research-driven network of BRCs. Many other organizations are contributing to the conservation of PGR in France (Duval *et al.*, 2023): regional centres, NGOs, the Network of Botanical Conservatories, private companies, etc. The French Ministry of Agriculture is in charge of developing the French strategy for the conservation and use of PGR for food and agriculture. In particular, this strategy aims to contribute to FAO’s global plans of action on genetic resources and to the ITPGRFA (Duval *et al.*, 2023). To support this endeavour, the Ministry

established the French National Coordination for PGR, which comprises a national support structure hosted within GEVES (Group for the Study and Control of Varieties and Seeds) and a section of the French multi-stakeholder organization CTPS (Permanent Technical Selection Committee). This coordination is responsible for implementing French policies on genetic resources (Duval *et al.* (2023); Figure 3). BRC4Plants contributes to the CTPS plant genetic resources section and facilitates, in collaboration with its funding bodies, the official recognition of BRCs through ministerial acknowledgement. This recognition identifies them as managers of genetic resources and acknowledges their contribution to the French National Collection of Genetic Resources (Duval *et al.*, 2023). So far, INRAE and

the *Etablissement Vanille de Tahiti* have been officially recognized as managers of genetic resources and INRAE is yearly increasing the official collection set under its responsibility in collaboration with its BRCs (MAA, 2022, 2024).

The French National Coordinator for Plant Genetic Resources is also the French National Coordinator for ECPGR (Duval et al (2023); Figure 3). BRC4Plants' coordination and its BRCs are actively contributing to ECPGR Working Groups (Table 1) dedicated to crops as well as to cross-cutting themes. In collaboration with the National Coordination, BRC4Plants deposits data in the European Search Catalogue for Plant Genetic Resources (EURISCO), managed by ECPGR, and contributes to the inclusion of accession collections in the ITPGRFA multilateral system. In 2019, ECPGR launched the European Evaluation Network (EVA) which leverages public–private partnerships at the pre-competitive stage, to jointly generate standardized characterization and evaluation data on crop accessions present in European genebanks. These accessions are often poorly characterized and, consequently, underutilized. ECPGR provides the EVA networks with standards and platforms for documentation and results sharing (Kumar et al, 2024). So far, four French BRCs are contributing to EVA networks: CRB GAMÉT for maize (Balconi et al, 2024), CRB CARPIA for carrot (Goritschnig et al, 2023) and CRB-Leg for pepper and lettuce.

## Conclusions and perspectives

The overarching goal of BRC4Plants is to facilitate the mobilization of its resources and services for research and innovation, with the aim of enhancing genetic diversity and beneficial interactions within agroecosystems. This supports the agroecological transition and the development of sustainable and healthy food systems. The network of BRCs plays an important role as a source of genetic diversity to be screened for new traits (e.g. plant-plant or plant-microbe favourable interactions). BRCs can also support the development of new plant materials for research on new breeding or cropping strategies. In this context, BRC4Plants faces several challenges that will need to be addressed through a mix of social, technical and scientific approaches:

- How should BRC4Plants be organized to address new needs regarding species, material development or introduction of accessions, while ensuring these efforts remain feasible within a realistic level of funding and support?
- How to increase *in situ* dynamic conservation activities and their integration with *ex situ* conservation?
- How to deal with the threats associated with climate change, including the spread of new diseases, in particular for the conservation of perennial plants in orchards?
- How to ensure that the variety of jobs necessary for the management and dissemination of

genetic resources remains attractive and rewarding despite increasing legal and administrative complexity and responsibility?

Tackling these challenges requires the co-construction of strategies with BRC4Plants supporting research organizations, as well as with other stakeholders involved in the conservation and use of genetic resources, in particular with the National Coordination on PGR. The current lack of full alignment or integration between research, agriculture and environment-driven national governance on PGR conservation is reducing the efficiency of efforts to coordinate the different stakeholders. It should be noted that this lack of integration is also a challenge at the European level and was identified as a major issue in the Genetic Resources Strategy for Europe developed in the frame of the GenRes Bridge project (GenRes Bridge Project Consortium, ECPGR, ERF and EUFORGEN, 2021).

Nevertheless, at the national level, RARE, as a national research infrastructure, is an effective platform for conveying messages through its participation in ministry-driven committees and initiatives, its governance committee, which includes representatives of its main supporting institutes, and its advisory committee, which includes members of the research community and other international infrastructures. RARE can develop and present coordinated perspectives on issues common to all genetic resources domains (e.g. implementation of the French law on ABS or phytosanitary risk management). In addition, BRC4Plants operates at the interface of a significant variety of stakeholders as illustrated in Figure 3. This position enables it to co-develop responses and solutions with research organizations, different types of genetic resources users, and policymakers, such as national decision support systems dedicated to ABS or diseases under regulation. Another important benefit of these multi-stakeholder approaches is a more realistic distribution of responsibilities among research organization central offices, official agencies and individual BRC managers.

The contribution of BRC4Plants BRCs to multi-stakeholder networks facilitates the development of species-specific collaborations aiming at characterizing and enhancing the use of PGR. However, a challenge now is to expand their diversity to include new players such as farmers, citizens, or industries, in order to develop integrated *in situ* and *ex situ* approaches for the management of genetic resources. This will also require an evolution in networking practices, including the structure of governing and advisory bodies (Louafi et al, 2021). Some BRCs are currently involved in projects aiming at developing a common understanding of different stakeholders and expectations, and co-developing common objectives in the context of the conservation and use of genetic resources.

The French choice to organize PGR conservation in a fully decentralized way has some disadvantages in terms of visibility and requires a layer of coordination to avoid redundancy and identify and support common devel-



opments and resources. However, it should be noted that nationally centralized genebanks in other countries primarily hold seed-propagated genetic resources and only a few vegetatively propagated species, whereas BRC4Plants manages many of the latter. Moreover, the French academic decentralized network has a historical origin: BRC collections were initially developed by individual researchers for their own breeding and research objectives, and were later centralized by crops by Research Institutes. Since then, they have remained closely linked to research units that contribute to their development, characterization, funding and reputation within their specific species communities of interest. Given the very low level of long-term funding for the basic operation and maintenance of collections, these tight links with research teams, various regional bodies and other stakeholders such as industry have been instrumental in raising funds for BRCs. The decentralized organization also offers significant flexibility for including new partners with additional collections or new expertise and tools. Additionally, it can provide backup solutions should any of the BRCs face problems due for example to climatic and environmental changes (perennials) or changes in the local scientific and technical teams (all types of BRCs).

Many of the challenges described above are also relevant in other countries (Smith *et al.*, 2021). On the other hand, the international level can provide cooperation and solutions. At the European level, the conservation of genetic resources is organized in a decentralized model by ECPGR, which fosters cooperation under the ITPGRFA principles. BRC4Plants is already well involved in this framework. An ongoing project, PRO-GRACE (<https://www.grace-ri.eu/pro-grace>), funded by the EU programme Horizon Europe (grant agreement no. 101094738) aims at extending or completing this framework through the creation of a pan-European Research Infrastructure on PGR. BRC4Plants is keen to contribute to such an infrastructure, including through its links with other national (e.g. METABOHUB for metabolomics, PROFI for proteomics and France Genomics for genomics) and European infrastructures (EMPHASIS for phenotyping and ELIXIR for life science data) that could be leveraged in the context of the characterization and use of genetic resources. Such a Research Infrastructure would strengthen the links of BRCs with public and private researchers in to foster their contributions to addressing the current challenges of agriculture<sup>1</sup>.

<sup>1</sup> See for instance the publications derived from three projects aiming at facilitating adaptation of different crops to climate change: SUNRISE for Sunflower, [https://anr.hal.science/search/index/?q=\\*&anrProjectReference\\_s=ANR-11-BTBR-0005](https://anr.hal.science/search/index/?q=*&anrProjectReference_s=ANR-11-BTBR-0005); the AMAIZING for maize, [https://anr.hal.science/search/index/?q=\\*&rows=30&anrProjectReference\\_s=ANR-10-BTBR-0001](https://anr.hal.science/search/index/?q=*&rows=30&anrProjectReference_s=ANR-10-BTBR-0001) or BREEDWHEAT for wheat, [https://anr.hal.science/search/index/?q=\\*&rows=30&anrProjectReference\\_s=ANR-10-BTBR-0003](https://anr.hal.science/search/index/?q=*&rows=30&anrProjectReference_s=ANR-10-BTBR-0003)

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## Authors contributions

VB and AFAB drafted the paper, JMA, AB, AD, JD, SD, FE, EG, BG, CJ, AL, PL, NM, CM, FN, NP de la R, AP, VR and PT revised it. AP and EG contributed to the design of the figures.

## Conflict of interest statement

The authors declare that there are no conflicts of interest

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# The German Federal Ex Situ Genebank for Agricultural and Horticultural Crops – Conservation, exploitation and steps towards a bio-digital resource centre

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**Abstract:** Over more than 80 years, the collections of the German Federal Ex Situ Genebank for Agricultural and Horticultural Crops have grown to around 152,000 accessions of 3,000 species preserved at three locations: Gatersleben, Groß Lüsewitz and Malchow/Poel. More than 96% of the material is stored as desiccation-tolerant orthodox seeds according to the active–base–safety (A-B-S) replicate approach at -18°C. Almost 70,000 freshly regenerated safety replicates are stored in the Svalbard Global Seed Vault. However, 4% of the material (2,000 field, 3,000 *in vitro* and 2,500 cryopreserved accessions) can only be maintained vegetatively, as no or few seeds or no true-breeding seeds are available.

Most of the accessions are provided via the standard material transfer agreement (SMTA) and more than 1.2 million samples have been distributed since the genebank was founded. To guarantee the identity of the living plant material, reference samples comprising about 450,000 voucher specimens, 110,000 seed and fruit samples and 57,000 cereal spikes are used for comparisons.

Genebank workflows are supported by the Genebank Information System (GBIS), which also manages workflow-independent data to describe the genebank accessions by passport, phenotypic and taxonomic data, thus allowing users to make targeted selections of material. The genebank-related processes, including acquisition, preservation, regeneration, documentation and material distribution, are certified for quality management in accordance with ISO 9001.

Nowadays, the genebank is undergoing a transformation process to become a bio-digital resource centre to improve utilization of the genetic resources in research and breeding to address future challenges.

**Keywords:** German crop genebank, plant genetic resources, conservation, *ex situ*, *in vitro*, cryo, documentation, exploitation

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## Introduction and historical background

The availability, accessibility and diversity of plant genetic resources (PGR) are the basis for the adaptation of our crops to environmental challenges and human needs. PGR are pivotal for breeding towards increased biotic and abiotic stress tolerance, optimizing human and animal nutrition and efficient use of renewable resources, including for the energy, chemical and pharmaceutical industries (Grusak and Dellapenna, 1999; Hoisington et al, 1999; Metzger and Bornscheuer, 2006; Tilman et al, 2006; Qian et al, 2018). However, since the beginning of industrialization and the introduction of the targeted selection of advantageous local plant varieties – so-called landraces – PGR have steadily disappeared (Tanksley and McCouch, 1997). This effect was already recognized by various researchers at the turn of the 20<sup>th</sup> century and led to the first collecting missions, e.g. those organized by Nikolai Ivanovich Vavilov and Frank Nicholas Meyer (Hammer and Diederichsen, 2009; Baranski, 2013). Against this background, the Seed and Plant Introduction Office (Beltsville, USA) and the Office for Agricultural Crops (St. Petersburg, Russia) were established in 1893 and 1894, respectively (Hammer, 2020), and are considered the two most important forerunners of today's genebanks. A first organized seed-bank was established in the predecessor institution of today's N.I. Vavilov Institute of Plant Genetic Resources (VIR) in St. Petersburg (then Petrograd) and stimulated the worldwide movement to preserve the diversity of agricultural and horticultural plants as a basis for food security. Vavilov's postulation of geographical centres of origin, defining assumed regions where the domestication of cultivated plants began, played an important role in the guidance of early collecting trips (Vavilov, 1926). These narrowly defined geographical areas were characterized by a great diversity of cultivated and wild forms of domesticated species. Although only some of Vavilov's centres of origin turned out to be areas for crop domestication, the high genetic diversity in these regions is still present today.

In Germany, the latest findings on genetic mechanisms stimulated researchers such as Fritz von Wettstein and Erwin Baur to argue for the preservation and exploitation of the diversity of crops. At a seed breeding conference organized in Berlin in February 1914, Erwin Baur stated: "It is very urgent now to become active to save and maintain the quickly disappearing old and primitive varieties of our cultivated crops" (Baur, 1914). Since that time, efforts were initiated to establish an institute for research on crops which was finally founded in 1943 on the Tutenhof estate near Vienna as Kaiser Wilhelm Institute for Crop Plant Research (Kaiser-Wilhelm-Institut für Kulturpflanzenforschung). The first collections included mainly materials from expeditions carried out before the institute was founded. After the

Second World War, the first director, the geneticist Hans Stubbe, successfully re-established the institute in Gatersleben and initiated a period of systematically planned collecting trips all over the world (Müntz and Wobus, 2013). Larger collecting trips were made to southern Italy, Afghanistan, China and Mongolia, among others (Supplemental Table 1). From 1948 onwards, there was also an intensive exchange of seeds with botanical gardens, agricultural and horticultural institutes and breeders. While the collections comprised approximately 3,500 accessions at the time of the transfer to Gatersleben, by 1962 they had already grown to 23,000 (Lehmann, 1963).

For the first years, the seeds of genebank accessions could only be stored at ambient conditions and thus had to be regenerated every 3–5 years (Lehmann and Mansfeld, 1957). The construction of a seed cold-storage facility, completed in 1976 (Anon, 1978), led to a drastic change in conservation management. The increased storage capacity and storage temperatures of -15 to -18°C extended the storage periods of the seeds, resulting in fewer regeneration cycles and lower costs (Figure 1). However, systematic large-scale screening on various crops for raw protein content and the essential amino acid lysine began in the late 1960s, see e.g. Lehmann et al (1978) and Grebenščikov (1985), and led to a sharp increase in seed regeneration in some years.

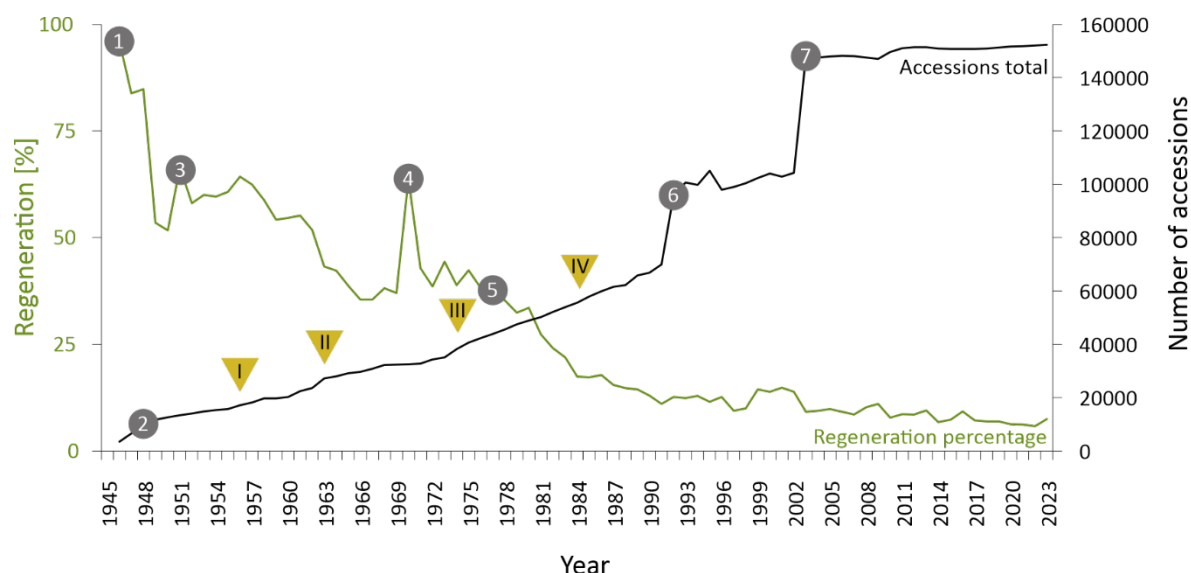
The Gatersleben genebank collections had grown to more than 65,000 accessions by the end of the 1980s. However, with the German reunification in 1990 and the desire to consolidate the PGR for agriculture and horticulture in one institute, the collections in Pillnitz (fruit genetic resources), Gülzow (rye and triticale), Malchow (oil and forage crops) and Groß Lüsewitz (potato) were integrated. The total collection size thus increased to almost 96,000 accessions by 1992. The institute was now renamed the Institute of Plant Genetics and Crop Plant Research (IPK). Between 2001 and 2003, around 50,000 accessions from the former West German genebank were transferred to the IPK genebank. Originally, the West German genebank was established at the Research Centre for Agriculture (Forschungsanstalt für Landwirtschaft, FAL) in Braunschweig in 1970 (Hammer, 1998). It was later assigned to the Federal Centre for Breeding Research (Bundesanstalt für Züchtungsforschung, BAZ) now part of the Julius Kühn Institute (JKI). In this context, the collection of fruit genetic resources in Pillnitz was transferred to the BAZ by the end of 2002 and the IPK genebank was renamed the 'German Federal Ex Situ Genebank for Agricultural and Horticultural Crops'.

## The composition and conservation of the genebank collections

### Composition

The IPK genebank collections today comprise almost 152,000 accessions of 3,000 species from 750 genera (Table 1). They are actively managed by eight curator

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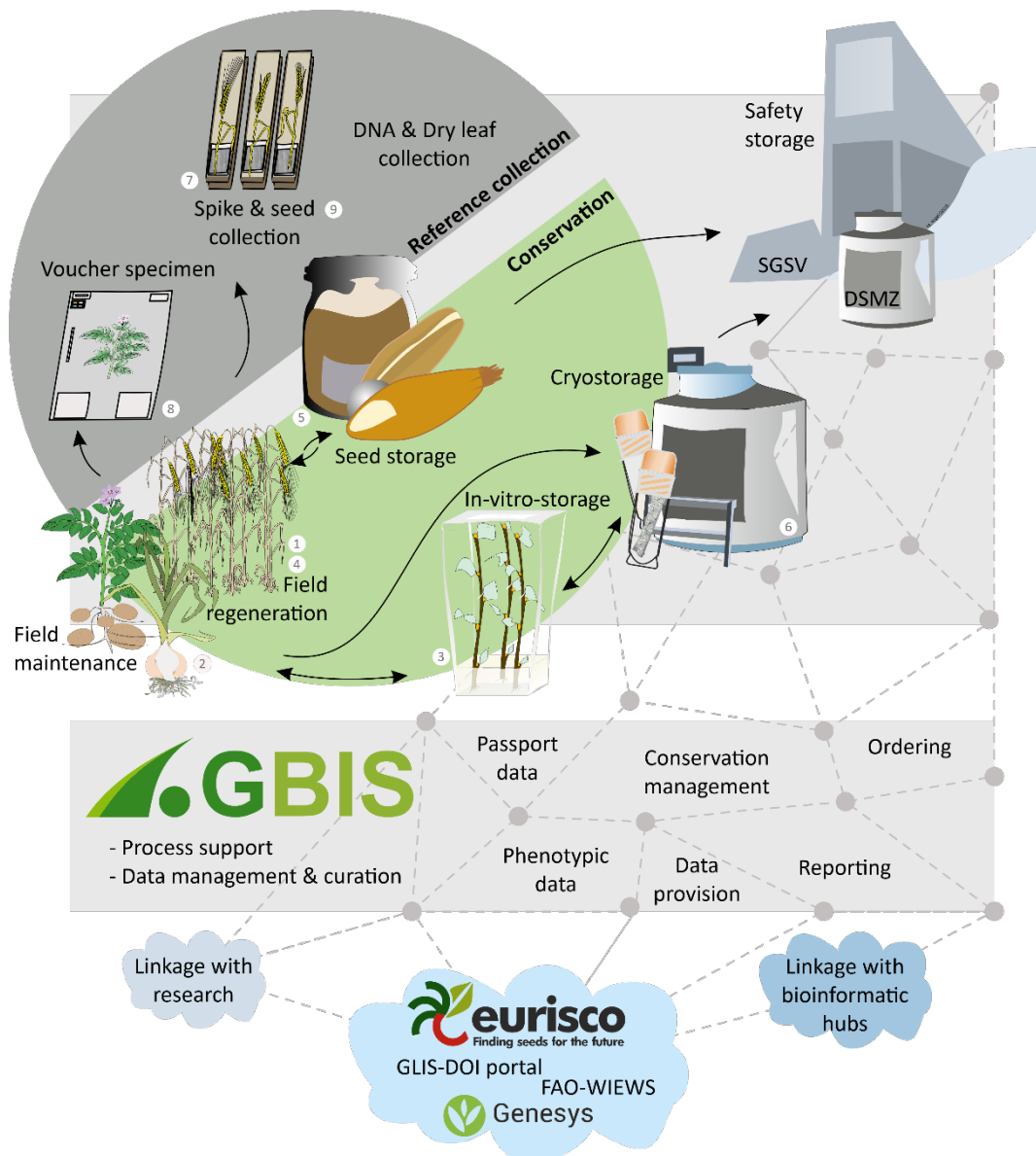
**Figure 1.** Development of the number of accessions and the regeneration percentage of the IPK genebank collections since the relocation to Gatersleben in 1945/1946. Selected events that can be seen from the curves are: 1) the regeneration rate of almost 100% due to the transfer of 3,500 accessions to Gatersleben, 2) the increase in the number of accessions due to the establishment of a lively exchange of seeds between botanical gardens, research institutes and breeders from 1948, 3) the renewed increase in the regeneration rate due to the incorporation of material from the first major collecting trips, 4) the start of large-scale screening of the raw protein content and the essential amino acid lysine in various cultivated plants, 5) the introduction of seed cold storage, which led to longer storage times and thus to a reduction in regeneration cycles, 6) the integration of the collections from Pillnitz, Gülzow, Malchow and Groß Lüsewitz, 7) the integration of the West German genebank collections and transfer of the fruit genetic resources in Pillnitz to BAZ. Supplemental Table 1 provides an overview of collecting missions of genetic resources worldwide that have been at least partially incorporated into the IPK genebank and have contributed to the continuous increase in the number of accessions. Four of these are mentioned here as examples: I) integration of material from the FAO collecting missions to Iran under H. Kuckuck (1952–1954) from 1956, II) integration of E. Mayr's alpine landrace collection (1922–1932) from 1964, III) start of various landrace collections in Slovakia and Moravia and integration into the genebank from 1974, IV) various collecting missions to Italy and continuous integration into the genebank (1980–1992).

groups – cereals, vegetables, tomatoes and beans, legumes, medicinal plants, potatoes, oil and forage crops, and *in vitro* and cryopreservation – organized in three research groups at three different locations. All groups collaborate intensively and contribute to the reference collection (Figures 2 and 3). About 86% of the material is maintained at the main site in Gatersleben (DEU146), the remainder at two satellite stations in Groß Lüsewitz (DEU159, 4%) and Malchow/Poel (DEU271, 10%).

Overall, the largest collections comprise accessions of wheat (18%), barley (15%), *Phaseolus* bean (6%) and potato (4%), which are among the largest global genebank collections. For example, IPK holds 6% of the total accessions of barley, 5% of *Phaseolus* bean and 11% of the potato held in the global genebanks (WIEWS, 2025). About 37% of the accessions are classified as traditional cultivars/landraces, 28% as advanced or improved cultivars, 15% are wild or weedy and 10% are breeding/research material. The remainder is not specified. The country of provenance is known for almost 125,000 accessions in the collections. Most accessions originated in Europe (66,400 accessions), followed by Asia (32,200), the Americas (13,800), Africa (12,000) and Oceania (500) (Figure 4).

## Seedbank

About 96% of the material is preserved as orthodox, desiccation-tolerant seed and maintained according to the genebank standards for plant genetic resources for food and agriculture (FAO, 2014). Every year, about 8,000 to 10,000 accessions are regenerated or multiplied in the fields, following best agricultural practices concerning fertilizer supply, pest/weed control and crop rotation. Self-pollinating species are grown side by side on areas of 10–15 hectares. Most accessions are separated by a different crop, e.g. wheat accessions by barley or forage grasses by rye. Cross-pollinators such as rye are grown in separation strips with larger distances or in more than 170 isolation greenhouses of 5–10m<sup>2</sup>. The latter are mainly used for insect-pollinated accessions and are equipped with solitary bees, bumblebees or flies. Biennial accessions are often grown in the open field and transferred to isolation greenhouses or cages after evaluation in the second year. During the growing season, crop-specific descriptors based on IPGRI/Bioversity descriptor lists (Bioversity, 2024) are used for characterization. Extended morphological and physiological information about adaptation and resistances towards environmental stresses and diseases are often obtained during targeted projects, e.g. on



**Figure 2.** Overview of genebank management for conservation of seed and clonal accessions including safety storage of seeds at the Svalbard Global Seed Vault (SGSV) and of cryosamples at the German Collection of Microorganisms and Cell Cultures (DSMZ). Arrows indicate the direction of the main workflows. The Genebank Information System (GBIS) provides process support including management and curation of data, allowing users to specifically select and order material, and is linked to international information systems, bioinformatics hubs and research projects. 1-9, Numbers in circles indicate steps visualized in Figure 3.

legumes, forage grasses (Supplemental Table 2) and support data complementation and breeders to select and utilize PGR.

Maturity of seeds is crucial for the development of optimal desiccation tolerance and seed longevity (Lep-rince *et al*, 2017). When full seed maturity is reached, the plants are cut manually. Most of the material is placed in drying cabinets at 20% relative humidity (RH) and a temperature of 20°C. Depending on the workload, the material is further threshed and cleaned. Clean seeds or separately harvested spikes are compared with reference material and then transferred to drying cabinets at 15% RH and 20°C to reach a final seed moisture content of 5–7% depending on the species. In parallel, the initial germination capacity and moisture content of

the seeds are tested. If germination of cultivated species reaches more than 80%, the material is further processed and separated into active–base–safety replicates. Active replicates are mainly kept in sealed glass jars with silica gel tops and stored at -18°C, in some cases also at -8°C or 4°C. Base and safety replicates are vacuum sealed and stored at -18°C. Once per year, safety replicates are transferred to the Svalbard Global Seed Vault, Spitsbergen. Within the last 16 years, freshly reproduced seeds of almost 70,000 IPK accessions have been deposited at the global backup storage, providing an important level of security against the loss of seeds due to human-caused or natural disasters.

Highly vigorous seed material is the basis for the long-term availability of genetic resources (Ellis and Roberts,





**Figure 3.** Various steps during conservation of seed and clonal genebank accessions. 1, Regeneration of cereal accessions in the Gatersleben fields (Photo: Michael Grau, 2008); 2, *Allium* field genebank in Gatersleben (Photo: Manuela Nagel, 2020); 3, *In vitro* slow-growth storage of potato (*Solanum tuberosum* L.) in Groß Lüsewitz (Photo: Manuela Nagel, 2019); 4, Regeneration of red clover (*Trifolium pratense* L.) accessions in Malchow/Poel (Photo: Daniela Impe, 2019); 5, Active storage of runner beans (*Phaseolus coccineus* L.) in Gatersleben (Photo: Heike Müller, 2014); 6, Long-term cryostorage of clonal accessions (Photo: Lynne Main, 2016); 7, Spike reference collection (Photo: Sam Rey, 2012); 8, Voucher specimen (IPK Herbarium); 9, Seed reference collection in Gatersleben (Photo: Sam Rey, 2012).

**Table 1.** Composition of the IPK genebank collections shown by species groups by June 2024.

Species groups	Accessions	Species groups	Accessions
<b>Cereals and grasses</b>	<b>66,434</b>	<b>Vegetables</b>	<b>17,861</b>
Wheat	28,307	Tomatoes	3,910
Barley	23,839	Pepper	1,533
Oat	4,863	Eggplants	113
Rye	2,582	Beta beets	2,376
Triticale	1,619	<i>Raphanus</i>	766
<i>Aegilops</i>	1,513	Carrots	505
Millets	841	Chicory	673
Maize	1,532	<i>Allium</i>	1,974
Others	1,338	<i>Brassica</i>	2,178
		Lettuce	1,145
<b>Legumes</b>	<b>27,862</b>	Spinach	215
<i>Phaseolus</i>	9,013	Celery	254
Field beans	3,038	Quinoa	953
Soybeans	1,491	Others	1,296
Other beans	615		
Pea	5,392	<b>Medicinal and spice plants</b>	<b>8,244</b>
Chickpea	527	Poppy	1,135
Vetchling	514	Tobacco	590
Vetches	1,845	Others	6,519
Lupines	2,712		
Lentils	473	<b>Mutants</b>	<b>1,684</b>
Clover	1,970	Tomato mutants	743
Others	272	Soybean mutants	527
		<i>Antirrhinum</i> mutants	414
<b>Cucurbitaceae</b>	<b>2,668</b>		
Pumpkins	1,054	<b>Potatoes</b>	<b>6,357</b>
Melons	728		
Cucumbers	738	<b>Small-grained oil and forage crops</b>	<b>15,157</b>
Others	148	Oilseed rape and forage kale	2,645
		Grasses	11,157
<b>Larger-grain oil, fibre and dye plants</b>	<b>5,470</b>	Red clover and alfalfa	1,344
Flax	2,324		
Sunflower	677		
Dye plants	458		
Fibre plants	191		
Oil plants	548		
Others	1,272	<b>Total</b>	<b>151,737</b>

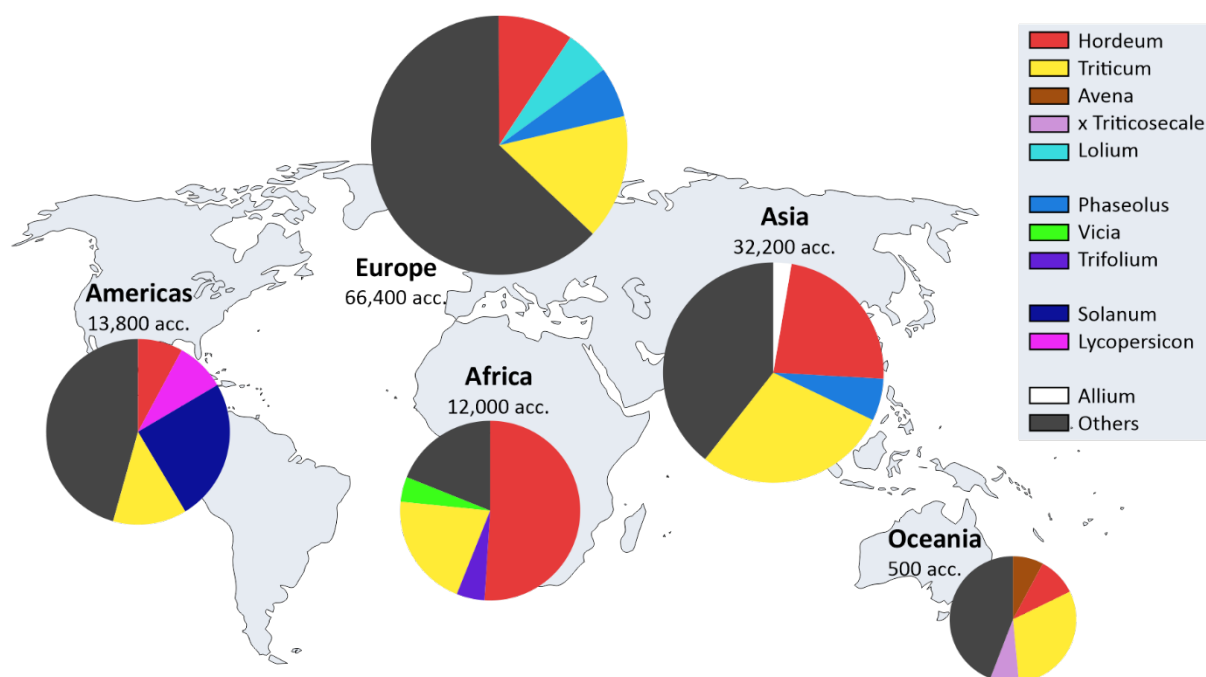
1980). A lower number of regeneration cycles increases the cost-efficiency of the genebank and lowers the risk of loss of genetic integrity. Therefore, all accessions stored at -18°C are regularly checked for seed germination after 8–20 years and are considered for regeneration when seed germination has dropped to less than 70% of the initial germination. Regeneration is also considered when the number of actively stored seeds is reduced due to the distribution of seed samples. Depending on the species, most seeds have been regenerated after 20 to 40 years. However, seed storability depends on the genetic background, the environmental conditions during growth and the storage conditions (Nagel et al,

2015). In future, advances in sensor technology may allow the individual control of e.g. seed moisture content and temperature in storage to optimize survival periods.

### Field genebank

Maintaining clonal plants in the field is the most traditional conservation method. It allows characterization and evaluation on site and immediate distribution of material (Engels and Visser, 2003; Panis et al, 2020). At IPK, about 4% of the accessions are preserved vegetatively, because no or little seeds or no true breeding seeds are available. Of these, about 2,000 accessions





**Figure 4.** Overview of main genera presented by continent. Approximately 750 genera have been summarized as ‘Others’.

of various *Allium* species, changing accessions of potato landraces, as well as mint and other species are grown in a field genebank.

The management of the field genebank varies in terms of growth requirements, propagation cycle and field design depending on the species. For example, the present *Allium* collection, merged from the Taxonomic Reference Collection and the *Allium* Crop Collection (Keller and Kik, 2018), comprises 1,080 accessions of 189 species and has been located at the main site for 5–6 years. Although best field management practices are applied, *Allium* accessions lose vigour over time due to soil exhaustion and need to be transplanted to another site to minimize the risk of infections and diseases. In the case of 392 garlic (*Allium sativum* L.) accessions, for example, cloves and bulbils are harvested in July after full senescence of leaves and stems. The material is then cleaned, prepared for planting and kept at 7°C. In autumn, the cloves are planted in plots of 1.5×1.5m and develop adventitious roots and flat leaves before winter. Some accessions bolt and develop inflorescences with flower buds and bulbils in May. Other materials, i.e. 82 shallot accessions and approximately 200 potato accessions are grown annually. At the IPK site in Groß Lüsewitz, the potato collections (GLKS) comprise 2,800 accessions from Europe and North America and approx. 650 native landraces from the Andes that are maintained clonally, most of them *in vitro*. For field reproduction and characterization, 10 tubers are pre-germinated and planted in the field between March and April. Over the vegetation period, various phenotypic traits are recorded following Huaman *et al* (1977), and tubers are harvested after 4–5 months before or at maturity. On average, about 400 field accessions have

been distributed annually to 122 users, mainly private individuals, since 2017. The accessions are available for distribution but require phytosanitary certificates for shipment abroad and systematic evaluation of quarantinable diseases. Recent projects, i.e. ‘ECPGR Garli-CCS’ and ‘ObiVonKnobi’ (see Supplemental Table 2) intensively evaluate the morphology, composition and genetic architecture to provide more comprehensive data to breeders and support identification of unique and duplicate genotypes for further decision-making processes.

Major challenges for field collections depend on the year’s climate and are the potential exposure of accessions to unfavourable conditions or threats such as pests and diseases. The *Allium* collection, for example, was exposed to an infestation of larval stage click beetles (Elateridae family, known as wireworms) in 2013. As a consequence, 52 *Allium* accessions were lost while 73 could be rescued by replanting (Panis *et al*, 2020). In addition, material that is maintained permanently in the field accumulates viruses, bacteria, fungi and mutations (McKey *et al*, 2010). This increases the necessity for careful evaluation and selection, besides frequent weeding, and seed or propagule harvest to avoid mixing of different accessions. Due to this high workload in field collections, *in vitro* slow-growth storage and cryopreservation were established at the Gatersleben genebank in the 1980s and 1990s, respectively.

### *In vitro* slow-growth storage

*In vitro* slow-growth storage is an essential tool for the conservation of accessions that are permanently propagated clonally, as they fail to produce seeds due to sub-optimal field/greenhouse conditions. The

storage of *in vitro* cultures allows the preservation of disinfected, pathogen-free material under precisely controlled environmental conditions, which is available for distribution. If plant physiology permits, lower temperatures and light intensities are used to reduce the metabolic activity, which extends the storage period and reduces the workload (Panis et al, 2020). At IPK, 2,900 potato, 150 mint, 30 *Dioscorea* and 50 accessions from other species are preserved in *in vitro* slow-growth storage. Here, 967 samples of *in vitro* potato accessions have been distributed to breeders and researchers since 2017.

Similar to the field genebank, the conservation practices vary between species, specifically regarding media composition and growth conditions. In the case of potato, apices from sprouting potato tubers are excised, surface-sterilized and grown on a Murashige and Skoog (1962) medium (MS). Accessions are tested for the common virus strains, such as Potato Virus A (PVA), Potato Leaf Roll Virus (PLRV), Potato Viruses M, S, X, Y (PVM, PVS, PVX, PVY), and quarantine pests, e.g. bacterial ring rot (BRR), among others (Nagel et al, 2022). If plants test positive for six common potato viruses, they are subjected to chemo- or thermotherapy (depending on the virus) followed by meristem isolation. The procedure is repeated until the viruses are eliminated. The meristems are then grown on MS media supplemented with 6% sucrose and exposed to a combination of warm (20°C for 1–2 months) and cold phases (10°C for 2–4 months, low light intensity). Under cold conditions, *in vitro* potato plants develop microtubers, which can be kept in a dormant state at 4°C and low light intensity for 12–15 months. When microtubers begin to sprout, either these or the nodal segments are transferred to fresh media and the cycle is initiated again. For other *in vitro* cultures, nodal segments of young plants grown in the field or greenhouse are surface-sterilized and grown on MS media supplemented with 3% sucrose and species-specific phytohormone compositions (Senula and Nagel, 2021). Most mint accessions, but also 18 *Antirrhinum* and 17 *Brassica* accessions, are kept for 12–20 months under two different cold regimes at 2°C and 6°C, and 16h light before they need to be sub-cultured. Warm-adapted mint, *Dioscorea*, but also eight *Artemisia*, three *Salvia*, three *Sechium*, three *Orthosiphon* and two *Plectranthus* accessions are kept at 25/20°C and 16/8h light/dark and need to be sub-cultured after 2–5 months.

Although *in vitro* slow-growth storage has been established for a number of species, some plant species fail to grow and develop (Benson, 2000). This phenomenon, also called *in vitro* recalcitrance, was observed in *Allium* species. In 1995, IPK maintained 645 *Allium* accessions *in vitro*. After some sub-cultures, the plants failed to grow and were contaminated indicating that growth conditions were not optimal and favoured growth of endophytic microorganisms. Unfortunately, efforts to adapt the media and conditions failed, and

hence, plants rejuvenated in the greenhouse were used for immediate cryopreservation. For the remaining accessions, field material, i.e. bulbs, cloves and bulbils, was collected and used to introduce *Allium* species directly into cryopreservation. For potato and mint, *in vitro* propagation is an essential step to achieve year-round cryopreservation and long-term preservation of clonal plants with minimal workload and costs.

## Cryobank

Cryopreservation is the storage of biological material at ultralow temperatures, usually below -130°C. This is realized in liquid nitrogen (LN, -196°C), in its vapour phase (between -165°C and -190°C) or in electric freezers (-150°C). Under these conditions, molecular movements cease, which increases the possibility of storing biological material indefinitely. However, the cryopreservation of plants was only established in the 1980s, when particular challenges, such as uncontrolled ice crystallization due to the presence of stiff plant cell walls and vacuoles, had to be overcome (Panis et al, 2020; Nagel et al, 2024). At IPK, international progress in cryopreservation triggered the start of safety duplication of the clonal potato collection stored in Groß Lüsewitz and led to the cryopreservation of the first potato accessions in 1997. Later, as a part of the restructuring of the German Federal Ex Situ Genebank, 578 accessions were transferred from BAZ Braunschweig to Gatersleben, resulting in a collection of 900 potato accessions in 2002 (Keller and Dreiling, 2003). Over the next two decades, a range of methods, i.e. DMSO droplet freezing, PVS2 and PVS3 vitrification, were tested and adapted (Keller et al, 2014) and form the basis for about 2,100 potato, 250 *Allium* and 160 *Mentha* accessions cryopreserved by 2024 (Nagel et al, 2024).

Nowadays, IPK routine cryopreservation of potato, *Allium* and *Mentha* is based on a vitrification approach using the cryoprotectant PVS3. This method has been applied to a range of clonal species preserved at the IPK genebank and proven the most convenient, successful, rapid and reproducible for these accessions. In brief, 1–2mm shoot tips are excised, precultured on MS media with 3% sucrose and exposed first to a loading solution with 13.7% sucrose and 18.4% glycerol and then to PVS3 solution containing 50% sucrose and 50% glycerol (Senula and Nagel, 2021). The increased sucrose concentrations facilitate osmotic dehydration and stabilize proteins and membranes (Lerbret et al, 2011). Glycerol permeates quickly into cells, replaces hydrogen bonds and prevents ice formation by separating water molecules (Towey et al, 2012). Shoot tips treated with a combined solution are transferred to vials or aluminium foil strips containing fresh PVS3 droplets and submerged to LN. The rapid temperature drop of ~130 K/s results in vitrification of the cytoplasm which reduces the potential to develop lethal intracellular ice. Based on statistics of Dussert et al (2003) and availability of propagules, 300 shoot tips for potato and *Mentha*, and 150 for *Allium* species are cryopreserved, of which 90 and 50,



respectively, are thawed to evaluate the cryopreservation success. If more than 30 shoot tips regrow, they are considered safely cryopreserved. On average, however, potato, *Allium* and *Mentha* regrew at higher percentages of 47%, 38%, 64%, respectively, which is a promising basis to increase the threshold to 35%, as suggested by an international team of cryoexperts (Volk *et al.*, 2017). After successful cryopreservation, the number of shoot tips is divided into triplicates; two replicates are stored in separate tanks at IPK and one in tanks at a backup storage facility at the German Collection of Microorganisms and Cell Cultures (DSMZ) in Braunschweig, Germany.

The cryopreserved material is occasionally requested for activation, comparisons and distribution, which provides information about their status of viability. However, activating the material is time-consuming and costly. Therefore, permanent conservation in cryo is only considered at IPK if the accessions are not actively used, such as duplicates or non-requested accessions, or material which does not survive in the field or *in vitro*. For the *Allium* collection, 60 accessions exist only in cryo due to unfavourable field conditions.

## Reference collection

The IPK genebank has been operating reference collections of preserved plants and plant parts since 1946 (Anon, 1953). Some reference materials even date back to the early 19<sup>th</sup> century (e.g. *Allium angulosum*, GAT0011009, from 1809). Today, the reference collections comprise more than 450,000 herbarium voucher specimens, 110,000 reference seed and fruit samples and 57,000 cereal spikes, which serve as important sources to guarantee the identity of the reproduced genebank material. Besides the genebank reference collection, the herbarium contains a representative specimen collection of cultivated plants and their wild relatives which provided the basis for Mansfeld's Encyclopedia of Agricultural and Horticultural Crops (Hanelt, 2001). Moreover, the herbarium stores important types, i.e. the specimens of organisms to which newly described taxonomical units such as species or subspecies refer, and also functions as a repository for physical references of plants used in molecular systematics studies.

To prepare herbarium vouchers, entire plants or plant parts important for determination and differentiation are collected during the vegetation period, pressed, dried and mounted as voucher herbarium specimens. A label including taxonomic and collection information is attached to the voucher, which is then stored in the IPK herbarium. Plant parts that cannot be prepared such as tubers or fruit clusters were preserved dry or wet (in alcohol) (Anon, 1953). However, due to the high workload, the latter activity had not been continued and only the available reference material is refreshed occasionally. To ensure long-term preservation of the reference collections, they are protected by separate quarantine areas where the vouchers are prepared and

frozen at -20 °C for one week to kill parasites before they are introduced into the collection. Insects are prevented by mosquito meshing at the windows and annual fumigations with phosphine (PH<sub>3</sub>) help to keep museum beetles (*Anthrenus museorum* L.) in particular out of the collections.

The herbarium collection is continuously processed and digitized in high resolution and currently provides about 53,000 digital images of the vouchers that can be accessed online in the joint herbarium management system JACQ (<https://www.jacq.org/>, herbarium code: GAT) and, hence, via the Global Biodiversity Information Facility (GBIF). Further scans are currently being processed. This reduces shipping of the valuable specimens among herbaria, thus minimizing the danger of losing materials. Moreover, researchers who work on taxonomic revisions of specific plant groups have fast access to digital collections, which very much speeds up taxonomic procedures, as high-resolution scans provide the most important details. The availability of digitized vouchers will support emerging machine learning approaches for species determination, help in understanding the geographic distribution and ecological settings of certain species, and allow easier search for and compilation of datasets of developmental and anatomical features of the taxa.

## Documentation

Documentation plays an important role in both conservation and exploitation, and thus, utilization of PGR. The more information is available about a resource, the more precise statements can be made about its value for breeding and research. Furthermore, a genebank collection can only be developed further in a meaningful way if its composition is well documented. This makes it possible, for example, to identify species or geographic regions that are underrepresented in the collection via gap analysis. Moreover, the management of information is essential both for the physical management of the collection and for the fulfilment of legal obligations (Weise *et al.*, 2020).

There are three categories of data: 1) pure management data, 2) data of legal significance and 3) data that allows the assessment of PGR value. The first category includes data like germination percentage, age of samples, storage quantities and locations, results of health tests and responsibilities for conservation. This data needs to be stored in a structured way. The second category comprises the documentation of collecting permits, correspondence with other institutions or documentation of receipt. The third category can be further subdivided into different kinds of data. Passport data comprise the basic information on PGR, in particular they facilitate the identification of the material. Stable and unique identifiers, such as Digital Object Identifiers (DOIs), are of great importance in this context (Garrity *et al.*, 2009; Alercia *et al.*, 2018). In addition, passport data contains, among others, the scientific name, information on origin and acquisition as well as the type of material and

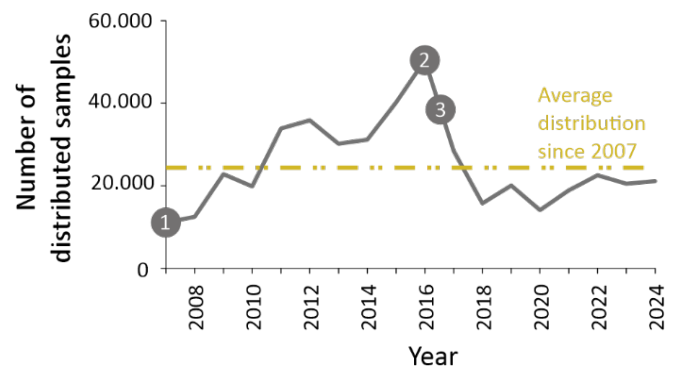
is based on the Multi-Crop Passport Descriptors (MCPD) data standard (Alercia *et al*, 2001, 2015). Other important data that help to assess the potential value of an accession for research and breeding are phenotypic characterizations, including morphological and agronomic traits. At IPK, this information is initially collected during the first cultivation of each accession and checked during each subsequent regeneration.

The first system for the management of the IPK genebank data was established in the 1980s and has been continuously developed thereafter. As part of the fusion of the former Eastern and Western German genebank collections (see above), resources were also made available to develop an integrated information and management system, the Genebank Information System (GBIS) (Oppermann *et al*, 2015). In 2006, GBIS started to operate and has been managing the above-mentioned data. In parallel, GBIS supports the processes for maintaining genebank accessions. For this purpose, it is made up of three components. The GBIS/M management module is primarily used to support the daily work processes in the genebank and enables the management and curation of data on the preserved material. The GBIS/B evaluation module is used for the electronic recording of phenotypic data with mobile devices during the regeneration, and the GBIS/I internet module provides potential users of genebank material with relevant information via a public web interface, thus allowing them to specifically select and order material (Figure 5). GBIS also documents genebank-related processes including acquisition, preservation, regeneration, documentation and material provision under the regulations of the standard material transfer agreement (SMTA) for quality management. Furthermore, it supports the fulfilment of reporting obligations at national and international levels, e.g. with regard to the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA). As a result, all of the genebanks' accessions are also listed in international aggregator systems such as EURISCO, Genesys and FAO-WIEWS.

The active curation of data on PGR is becoming increasingly important (Shaw *et al*, 2023). The genebank's information pool is therefore continuously updated. This includes comparing and supplementing existing data sets with those from external sources, e.g. from information systems of other collections. This significantly increases the quality and quantity of information on genebank accessions. Furthermore, historical data has also been explored and stepwise added. Even unbalanced data, i.e. phenotypic data recorded during reproduction in different years, can provide added value, for example by being used to predict the phenotypic performance of genebank accessions (Philipp *et al*, 2018; Berkner *et al*, 2024).

### Quality management

The IPK genebank aims to efficiently use the available economic, human and technical resources to ensure



**Figure 5.** Development of the number of samples distributed. In 2007, the Genebank Information System (1, GBIS) was introduced allowing scientists, breeders and private persons to order accessions free of charge. This led to a continuous increase in the number of samples reaching over 50,000 samples in 2016 (2). Due to the financial burden and workload, a processing fee was introduced in mid-2016 (3), which has limited the annual distributions to a manageable level of 25,000 samples on average (yellow line). Overall, the IPK genebank has distributed more than 1.2 million samples over the last 80 years.

the permanent availability of collection material and to offer the users a high-quality service. Therefore, the IPK genebank introduced a quality management (QM) system according to ISO 9001 in 2007. Quality management is a tool for monitoring all activities, tasks and processes required to maintain a desired level of quality in products and/or services. An effective QM system involves clear organizational strategies and goals, efficient and transparent processes, measurable results and continuous process improvements. The establishment of a QM system and certification according to ISO 9001 is a measure to increase the satisfaction of the stakeholders (service quality) and to improve the internal genebank management. Moreover, the documentation of the individual processes is a key issue to perpetuate the long-standing experience of the employees and their knowledge for a sustainable continuation of PGR conservation. Finally, the transparency of the genebank processes ensures that they are aligned with agreed genebank quality standards.

Since 2007, all relevant key processes have been visualized in 51 procedure instructions and described in detail in 72 working instructions. A quality management handbook and an operational genebank manual, available on the ECPGR website (<https://www.ecpgr.org/egis/aquas/genebank-manuals/>), describe the QM system. Internal and external quality audits are planned and carried out annually, and a certification company recertifies the genebank every three years. The continuous improvement is pursued through the development and implementation of state-of-the-art knowledge and research conducted at IPK. These collaborative activities guarantee high-quality services and progress in the field of preservation, propagation, conservation, taxonomic classification as well as information technologies.

## Development of a bio-digital resource centre

Progress in life sciences is increasingly centred around data availability, quality and management. In line with this, the genebank is undergoing a transformation process to become a bio-digital resource centre (Mascher *et al*, 2019). This means describing PGR on an ever-better scale in order to optimize their use for research and breeding. The aims are to successively raise existing data to a higher level through curation and complementation, and to obtain additional data. The latter pursues two goals: on the one hand, additional data from domains that are already being used will be tapped. This includes, among other things, further phenotypic data integrated from additional sources, e.g. from high-throughput phenotyping. On the other hand, data from domains that have not yet been used in the past will be harnessed, in particular genetic characterizations. Genomic data can help to decipher genetic diversity and provide insights into geographical origin, row type, growth habit or domestication status, for example. It can also help with the identification of duplicates and enables applications such as genome-wide association studies. Entire sub-collections are increasingly being genotyped, for example barley (Milner *et al*, 2019) and wheat (Schulthess *et al*, 2022), and their data are made available via crop portals.

The above-mentioned processing of historical data from the last 80 years, particularly from seed regenerations, also plays an important role for the bio-digital resource centre as it helps to assess the value of PGR accessions for breeding and research purposes. This data is extensively curated and published in accordance with the Findability-Accessibility-Interoperability-Reusability principles (FAIR; (Wilkinson *et al*, 2016)). In addition, this data is also analyzed together with genotyping data.

A cooperation with the DSMZ in Braunschweig has been established with regard to a safety backup for cryomaterial (see above). To store valuable resources together with their most important data, a pilot project together with the Norwegian company GenEver was initiated and special cryoboxes developed. The boxes combine cryovials with data on a roll of film (piql film). This technology is extremely robust and promises to last for centuries. Until the end of 2024, all cryo backup samples stored at the DSMZ will be supplemented with data on film strips.

In recent years, a great deal of energy has been invested in establishing efficient data management at IPK, and previously isolated information systems have been and are being successively interlinked. In addition, IPK is also involved in the establishment and further development of data standards such as ‘Minimum Information About a Plant Phenotyping Experiment’ (MIAPPE; Krajewski *et al* (2015); Papoutsoglou *et al* (2020)) and is embedded in national and international networks for PGR. For example, the European Search Catalogue for Plant Genetic Resources (EURISCO)

has been operated and further developed by an IPK genebank working group on behalf of the European Cooperative Programme for Plant Genetic Resources (ECPGR) since 2014 (Weise *et al*, 2017; Kotni *et al*, 2023).

## Challenges and future plans

As in any genebank, there are a number of challenges associated with the various activities; maintenance and regeneration in particular are labour-intensive and costly. In order to utilize the available resources as efficiently as possible, one of the options currently being discussed is to rely on a higher degree of automation and digitization. Furthermore, cryopreservation of heterozygous, short-lived and hybrid seeds might also be a backup solution for material which cannot be maintained adequately by conventional long-term storage.

The identification of duplicates also plays an important role in the more efficient use of resources. In large collections comprising hundreds or even thousands of accessions of a species (such as wheat and barley in the German genebank), duplicates within the collection are unavoidable. In addition, there is a large percentage of duplicates between genebanks (van Hintum and Visser, 1995). Unfortunately, the identification of duplicates is not a trivial task; reliable statements can only be made by jointly analyzing passport data, phenotypic data and genotyping data in combination with comparative cultivations. In addition, the definition of threshold values is useful here. Such approaches have been tested as examples, but have not yet been carried out on a larger scale. However, duplicates, both within and between collections, open up possibilities for normalizing data, especially historical phenotypic data. This is an approach that is currently being pursued in the AGENT project (<https://www.agent-project.eu/>).

Despite progress, at least in the large sub-collections (see e.g. González *et al* (2018); Philipp *et al* (2018)), there is still a great need for the digitization and curation of historical data. However, consistent recording, storage and curation of data also require continuous maintenance and further development of the Genebank Information System. This includes the regular porting of both data and software components. To facilitate the recording of phenotypic data, a new client for mobile devices was recently finalized. It is based on the PhenoApp (Röckel *et al*, 2022) and has been specially extended to meet the needs of the genebank. A particular challenge is the integration of phenotypic data that was not collected as part of the regeneration of material by the genebank staff themselves, but in the context of research projects. There are still no widely accepted standards regarding the collection of phenotypic data using standardized traits and methods (Krajewski *et al*, 2015). However, approaches such as MIAPPE facilitate description and reproducibility, at least for future data.

Not all biodiversity is secured in the world's genebanks. Especially against the backdrop of the



climate crisis, this represents a race against time. It is therefore necessary to specifically analyze existing sub-collections and to identify priority species and regions for collecting. Such an analysis has already been carried out using oilseed rape as an example (Weise et al, 2023). This allows the targeted acquisition of material from other collections and, if possible, the organization of collecting trips.

The IPK genebank is involved in various infrastructure projects and research programmes, and has genotyped entire sub-collections. However, the participation in the exploitation and utilization of (neglected) crops and crop wild relatives (e.g. Legume Generation (<https://www.legumegeneration.eu/>) and COUSIN (<https://cousinproject.eu/>) projects) as well as the participation in the establishment of a European research infrastructure for PGR (PRO-GRACE project, <https://www.grace-ri.eu/>) will continue to conserve and utilize our European PGR as efficiently as possible.

### Supplemental data

**Supplemental Table 1:** Collecting trips by German-speaking researchers

**Supplemental Table 2:** Recent third party-funded projects under participation of the IPK genebank

### Author contributions

SW and MN drafted the manuscript. All authors revised and edited the manuscript, and approved the final version.

### Conflict of interest statement

The authors declare that they have no competing interests.

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# The INRAE *Prairies* genebank for *ex situ* conservation of forage and turf species in France

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**Abstract:** In France, INRAE (Institut national de recherche pour l'agriculture, l'alimentation et l'environnement - French National Research Institute for Agriculture, Food and Environment) maintains a genebank of grass and legume perennial species for forage and turf usages in the research unit P3F located in Lusignan (region Nouvelle-Aquitaine). This genebank is a component of the plant pillar (BRC4Plants) of the French National Research Infrastructure RARE. Collections comprise natural populations collected across France and other countries, landraces, cultivars removed from registration lists and some scientific materials. 967 accessions are currently available for distribution, among which 852 are available in the frame of the Multilateral System of Access and Benefit-sharing of the UN Food and Agriculture Organization (FAO). The genebank policy has been to set up core collections of the main forage and turf species diversity and to apply a high standard of conservation and regeneration to collections of relatively small size. During past decades, genetic resources held by the genebank highly contributed to the breeding of forage and turf cultivars in France and to various scientific studies. Recently, high-throughput genotyping of accessions has provided unprecedented means to discover phylogeographic patterns and genomic adaptation in natural populations of perennial ryegrass (*Lolium perenne* L.) and to understand the breeding history of lucerne (*Medicago sativa* L.). Such approaches open promising prospects for future genetic adaptation of forage and turf species to changing environmental conditions and new usages.

**Keywords:** cultivar, grass, grassland, landrace, legume, natural population, perennial species

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## Introduction

Sown temporary grasslands cover around 2.47 million hectares in France (Huyghe *et al.*, 2014). They provide grazed or cut forage for livestock but also various other ecosystemic services such as soil fertility improvement, carbon sequestration and biodiversity shelter (Martin *et al.*, 2020). Sown grasslands are seeded with improved cultivars from various perennial grass and legume species. Most of these species commonly grow as natural populations in permanent and natural grasslands covering 9.8 million hectares in France (Huyghe *et al.*,

2014) and exhibit a diversity of ecotypes adapted to various conditions of soil, climate and use. Only few species, e.g. lucerne (*Medicago sativa* L.) and Italian ryegrass (*Lolium multiflorum* Lam.), were bred as landraces before the onset of modern plant breeding. Natural populations and landraces were the starting material to develop continuously improved cultivars adapted to various pedoclimatic conditions and forage systems, e.g. Sampoux *et al.* (2011) for perennial ryegrass (*Lolium perenne* L.). Grown as grass–legume binary associations, or as mixtures involving several species (up to ten or more), temporary grasslands can provide a high yield of good quality forage without artificial nitrogen and herbicide inputs (Surault *et al.*, 2024). Furthermore, some grass species have also been

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bred for small size, high density and slow growth to create turf cultivars improved for either sport or lawn requirements (e.g. [Sampoux et al \(2012\)](#)). The breeding of turf-type genotypes of legume species, e.g. white clover (*Trifolium repens* L.), lucerne (*M. sativa*), birdsfoot trefoil (*Lotus corniculatus* L.), has also recently begun. Finally, all grassland and lawn areas contribute to providing pleasant landscapes for recreational activities.

The INRAE multidisciplinary research unit for grasslands and forage species (UR P3F) in Lusignan hosts the *Prairies* genebank targeted to the conservation of genetic resources of the main grass and legume species sown in France for either forage or turf usage ([Figure 1](#)).

### Genetic materials maintained by the *Prairies* genebank

Collections of natural populations from grass and legume species of the *Prairies* genebank are the outcome of collection trips undertaken by scientists of INRA (Institut National de la Recherche Agronomique, former acronym of INRAE) in France and other countries. Some of these collection trips were undertaken by INRA staff alone and others together with academic research institutes from other countries (e.g. Spain, Portugal, Morocco, Algeria, USA) or breeding companies. Most of the seed-collecting trips were carried out from 1970 to 1990 to gather samples of genetic resources necessary to start or diversify breeding programmes. Since then, the long-term conservation and characterization of collected accessions have been progressively implemented ([Prospéri and Sampoux, 2005](#)). A more recent collecting campaign carried out in the southern part of France in 2014 gave the opportunity to collect new accessions from the natural diversity of grass and legume species that were afterwards included in the genebank besides already existing collections.

The genebank also maintains some lucerne landraces, as well as some grass and legume historical cultivars formerly bred by INRAE or other breeders, all removed from national lists. As a matter of fact, in France and other European countries, the commercialization of new cultivars is allowed only after their registration on national lists, which depends on sufficient value for cultivation and use (VCU) and distinctiveness, uniformity and stability (DUS) requirements. After a certain duration (usually ten years), cultivars are removed from national lists unless they have not been outpaced by more recent cultivars (see for examples rules in France at [www.geves.fr/variety-seed-expertise/field-forage/registration-of-field-and-forage-varieties-in-the-french-catalogue](http://www.geves.fr/variety-seed-expertise/field-forage/registration-of-field-and-forage-varieties-in-the-french-catalogue)).

### Collection of orchard grass

#### Natural populations

A breeding programme for orchard grass (*Dactylis glomerata* L.) started in 1962 at INRA in Lusignan. The programme focused on the improvement of forage performances in the temperate climate areas of Europe.

An early start of vegetative growth in spring and a relatively late spike emergence date were targeted, as they were expected to provide a long annual period of forage production. Thus, collection campaigns from 1962 to 1982 were directed towards oceanic regions of Europe with mild winter and cool summer conditions, i.e. Brittany and Cotentin in France, northwestern Spain and northern Portugal ([Mousset, 2000](#)). Populations from the widespread autotetraploid ( $4x = 28$ ) taxon *D. glomerata* subsp. *glomerata* were collected in France, Spain and Portugal. Populations of the rare diploid taxon ( $2x = 14$ ) *D. glomerata* subsp. *lusitanica* Stebbins & D. Zohary were found in Portugal and populations of Galician type ([Ortiz and Rodríguez-Oubiña, 1993](#)), either diploid or tetraploid, were found in Spain and Portugal. The autotetraploid materials thus collected were used by the INRA plant breeding unit of Lusignan to create several successive innovative cultivars: ‘Lully’ (1977), ‘Lude’ and ‘Lutétia’ (1978), ‘Lupré’ (1992), ‘Ludac’ (1997), ‘Luron’ and ‘Ludovic’ (1998). Many orchard grass cultivars currently available from private breeding companies for oceanic climates in Europe still stem from these original collections.

Later, from 1987 to 1992, new collections were undertaken to collect Mediterranean types of orchard grass offering some winter growth potential and more or less substantial summer dormancy in southern France, southern Portugal, southern Spain and Algeria ([Mousset, 2000](#)). Thereby, populations were collected from the widespread autotetraploid taxon *D. glomerata* subsp. *hispanica* (Roth) Nyman and from the diploid taxa *D. glomerata* subsp. *castellata* Borrill & Parker and *D. glomerata* subsp. *mairei* Stebbins & D. Zohary. The autotetraploid collected materials were used by the INRA plant breeding unit in Montpellier to select the cultivar ‘Medly’ (1996), which presents adaption to summer drought and heat stresses of Mediterranean areas.

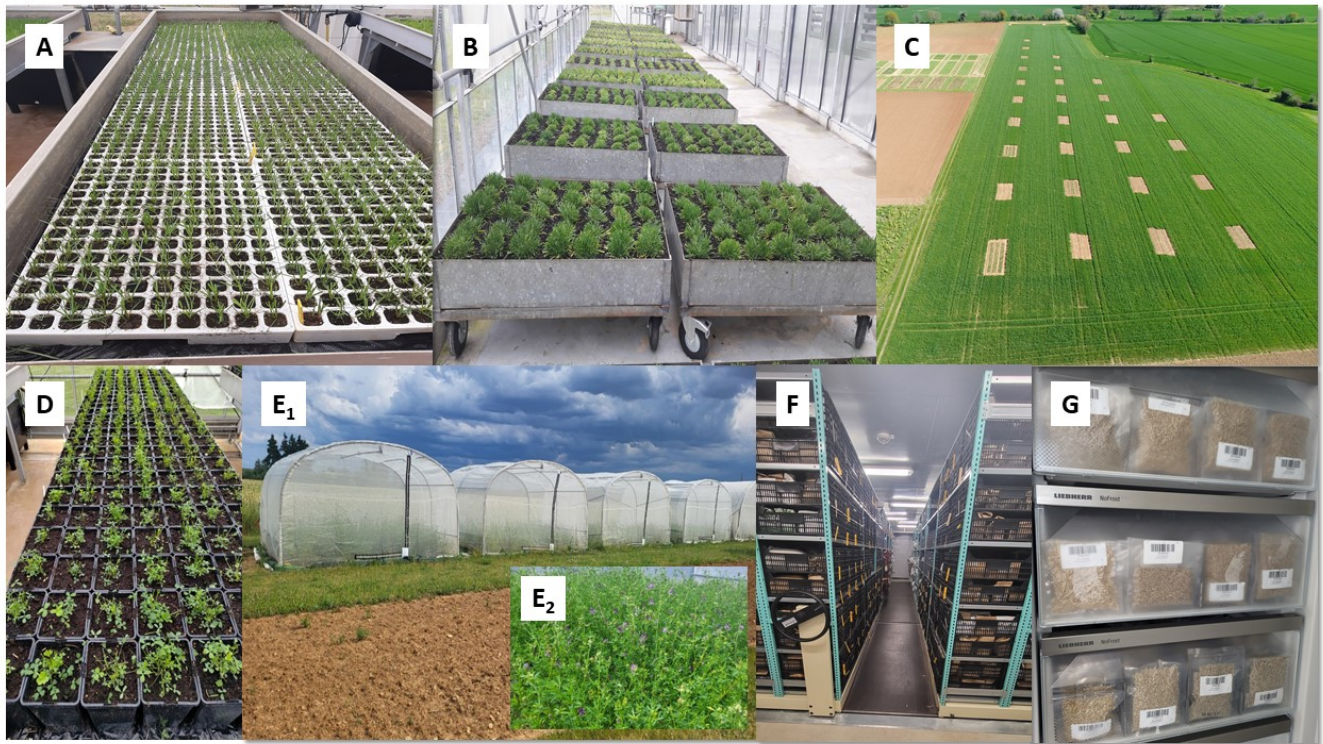
A total of 702 orchard grass natural populations were collected between 1962 and 1992. A core collection of 172 populations sampling the variability within the different taxa was extracted from this large set and is currently available from the *Prairies* genebank.

The new collection campaign undertaken in 2014 in the southern part of France enabled the collection of 39 new natural populations of the autotetraploid *D. glomerata* subsp. *glomerata*. These new entries added to the previously mentioned core collection to make the set of orchard grass natural accessions currently available from the *Prairies* genebank ([Figure 2](#) and [Table 1](#)).

#### Other genetic materials

Accessions publicly available from the genebank also include the *D. glomerata* subsp. *glomerata* cultivars ‘Floréal’ (1957), ‘Lully’ and ‘Lutetia’, formerly bred by INRA.





**Figure 1.** Implementation of different tasks in the facilities of the INRAE *Prairies* genebank. A, Growing grass plantlets in the greenhouse prior to planting accessions in spaced spots in a triticale (*x Triticosecale*)–rye (*Secale cereale* L.) field; B, Growing grass plants in trays prior to transfer in confined compartments of a partitioned greenhouse; C, Grass accessions planted in spaced spots within a triticale–rye crop which acts as a pollen barrier; D, Growing lucerne (*M. sativa*) plants in pots prior to planting under insect proof tents; E<sub>1</sub>, Insect-proof tents used to perform legume cross-pollinations; E<sub>2</sub>, A lucerne accession under an insect-proof tent (pollen transport inside the tent is ensured by bumblebees); F, Storage of active seed lots in a cold room (5°C and 30% hygrometry); G, Storage of safety seed lots in a freezer (-20°C).

## Collection of tall fescue

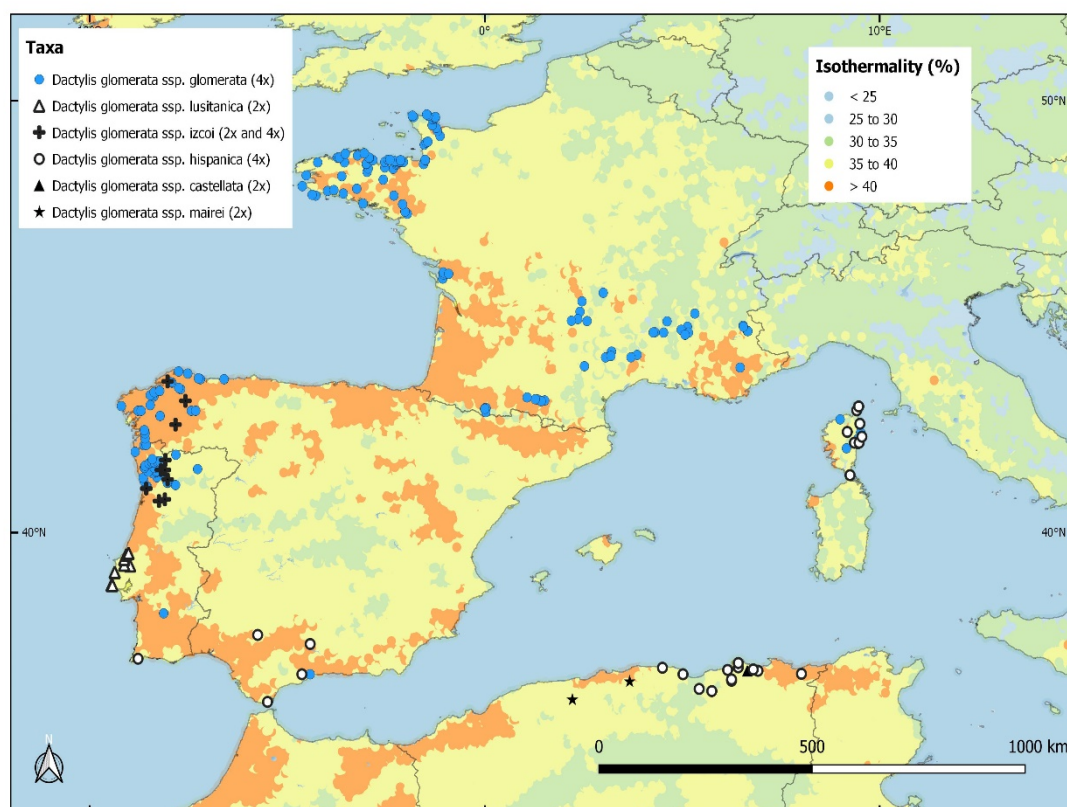
### Natural populations

Several hundred natural populations of tall fescue (*Festuca arundinacea* Schreb.) were collected by scientists of INRA Lusignan from 1969 to 1992 (Ghesquière and Jadas-Hécart, 1995). Collection campaigns focused towards southern France and the Mediterranean basin (northern Africa, Portugal). The collected populations were then characterized at INRA Lusignan from 1985 to 1995 and a core collection of 128 accessions sampling the diversity of the different taxa was drawn up. This core collection included accessions from the common allohexaploid ( $6x = 42$ ) tall fescue (*F. arundinacea* subsp. *arundinacea*) collected in France, Portugal and Switzerland (77 accessions) and in northern Africa (34 accessions). Sources of *Festuca arundinacea* subsp. *arundinacea* from temperate areas and the Mediterranean part of France were discovered to give deeply sterile hybrids with Mediterranean sources from southern Portugal, southern Spain and northern Africa (see Jadas-Hécart and Gillet (1978) for the production of temperate  $\times$  Mediterranean amphiploid cultivars). The core collection was also supplemented with accessions from two other northern African types, three from *F. arundinacea* var. *atlantigena* (St.-Yves) Auquier (octoploid,  $8x$

$= 56$ ) and 14 from *F. arundinacea* var. *letourneuxiana* (St.-Yves) Torrecilla (decaploid,  $10x = 70$ ).

Five accessions of *F. arundinacea* var. *glaucescens* Boiss. are also maintained in the genebank. They result from the pooling of various natural populations of this taxon collected around lake Embrun in the French Alps in 1982. This taxon, which is autotetraploid ( $4x = 28$ ), has been recognized as one of the two progenitors of the hexaploid tall fescue *F. arundinacea* subsp. *arundinacea* together with the diploid ( $2x = 14$ ) *Festuca pratensis* Huds. after natural amphiploidization (Humphreys et al, 1995). *F. arundinacea* var. *glaucescens* was widely used by the INRA plant breeding unit of Lusignan from the 1980s onwards in hybridization with autotetraploid ryegrasses to create the first *x Festulolium* varieties (Ghesquière et al, 2010).

The collection campaign carried out in France in 2014 provided 36 new natural populations of the tall fescue *F. arundinacea* subsp. *arundinacea*. The historical core collection of 128 accessions, supplemented by accessions from *F. arundinacea* var. *glaucescens* and accessions collected in 2014 make the set of tall fescue natural populations available from the *Prairies* genebank (Figure 3 and Table 1).



**Figure 2.** Geographical distribution of *D. glomerata* natural populations maintained by the *Prairies* genebank. In the background, isothermality is the ratio ‘temperature diurnal range/temperature annual range’ in percentage. Values are computed from 1989–2010 climate norms. High isothermality values are typical of oceanic temperate climate whereas low values are typical of continental climate. Credit: Fabien Sampoux, project ColNat\_Fourrage+ (AAC RPG 2018-2 CTPS GNIS)

## Other genetic materials

Several *x Festulolium* accessions created by the INRAE plant breeding unit of Lusignan are maintained. This includes three amphiploid cultivars created after hybridization between *F. arundinacea* var. *glaucescens* genotypes and *Lolium multiflorum* tetraploid genotypes, namely ‘Lueur’ (2007), ‘Luxane’ (2008) and ‘Lusilium’ (2008). This also includes several pools obtained from the backcross of amphiploid (*F. arundinacea* var. *glaucescens*  $\times$  *L. multiflorum*) material into tetraploid *L. multiflorum* or *Lolium perenne* materials.

Accessions publicly available from the genebank also include the *F. arundinacea* subsp. *arundinacea* cultivars ‘Gloria’ (1976), ‘Lubrette’ (1981) and ‘Ludical’ (2002) bred by INRA.

## Collection of ryegrasses

### Natural populations

In 1983 and 1984, the INRA plant breeding unit of Clermont-Ferrand, together with the breeding companies of ACVF (Association des Créateurs de Variétés Fourragères – Society of plant breeding companies involved in the breeding of forage and turf cultivars in France), undertook a dense and even collection of more than 500 natural populations (diploid,  $2x = 14$ ) of peren-

nial ryegrass (*L. perenne*) across France. These populations were thoroughly characterized at INRA Clermont-Ferrand during the following years (Charmet *et al.*, 1990), providing data to reveal informative relationships between characterization traits and ecogeographical variables at the sites of origin of the populations (Balfourier and Charmet, 1991). The collected materials provided the sources to create the cultivar ‘Clerpin’ (1996) by the INRA plant breeding unit of Clermont-Ferrand and were the root of a substantial leap in the release of perennial ryegrass cultivars with late heading date and improved crown rust resistance by private breeding companies. A core collection of 160 accessions was finally set up using a clustering based on characterization data combined with a geostatistics contiguity constraint (Charmet and Balfourier, 1995). In 2008, this core collection was transferred to the *Prairies* genebank in Lusignan which is maintaining it.

The whole set of perennial ryegrass natural populations maintained by the genebank also includes 27 other natural populations collected in France at diverse times as well as 36 natural populations collected during the 2014 collection campaign in southern France (Figure 4 and Table 1). The 2014 collection campaign also gave the opportunity to collect seven spontaneous popula-



**Table 1.** Number of accessions per species made publicly available by the *Prairies* genebank in 2024. \*, The whole set of 852 accessions proposed for notification by France as Contracting Party to the Multilateral System (MLS) of the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) comprises three different subsets: 1) a subset of 531 accessions maintained by INRAE (*Prairies* genebank) and notified to the MLS in 2014, 2) a subset of 146 accessions maintained by INRAE (*Prairies* genebank) and 3) a subset of 175 accessions maintained by INRAE (*Prairies* genebank) and ACVF (society of companies breeding forage and turf species in France), 2) and 3) proposed in 2024 to the French Ministry of Agriculture for notification to the MLS. The subsets of 531 and 175 accessions can already be browsed on the public databases EURISCO, Florilège and Siregal. The subset of 146 accessions is currently in the process of addition to these databases. <sup>a</sup>, Accessions whose occurrence data were forwarded to the Global Biodiversity Information Facility (GBIF) database are a subset of the set of accessions proposed for notification to the MLS of the ITPGRFA. <sup>b</sup>, 52 perennial ryegrass cultivars, 27 *Phalaris* accessions and 3 x *Festulolium* cultivars are currently in the process of seed regeneration and will be afterwards added to the public databases Siregal, Florilège and EURISCO.

Species in collection		Number of accessions					GBIF <sup>a</sup>
Latin name	Vernacular name	Natural pop.	Landraces	Cultivars	EURISCO Florilège Siregal	Proposed to MLS of TIRPAA*	
<i>Dactylis glomerata</i>	Orchard grass	211		3	175	214	172
<i>Lolium perenne</i>	Perennial ryegrass	223		54 <sup>b</sup>	193	206	191
<i>Lolium multiflorum</i>	Italian ryegrass	7		2	2	9	
<i>Lolium hybridum</i>				1	1	1	
<i>Festuca arundinacea</i>	Tall fescue	169		3	128	164	
<i>Festuca pratensis</i>	Meadow fescue	11				11	
x <i>Festulolium</i>				3 <sup>b</sup>			
<i>Festuca rubra</i> aggr.	Red fescues	139		3	142	142	139
<i>Festuca ovina</i> aggr.	Sheep fescues	35			35	35	35
<i>Phleum pratense</i>	Timothy			2	2	2	
<i>Phalaris arundinacea</i>	Phalaris	27 <sup>b</sup>					
<i>Phalaris aquatica</i>							
<i>Medicago sativa</i>	Lucerne	12	17	25	41	48	
<i>Trifolium pratense</i>	Red clover	2		3	3	5	
<i>Trifolium repens</i>	White clover	6		4	4	10	
<i>Onobrychis viciifolia</i>	Sainfoin			2	2	2	
<i>Vicia sativa</i>	Vetch			3	3	3	
<b>Total</b>		<b>842</b>	<b>17</b>	<b>108</b>	<b>731</b>	<b>852</b>	<b>537</b>

tions (diploid,  $2x = 14$ ) of Italian ryegrass (*L. multiflorum*) in natural grasslands.

## Other genetic materials

Accessions publicly available from the *Prairies* genebank also include the perennial ryegrass cultivars ‘Primevère’ (1957) and ‘Clerpin’ and the Italian ryegrass cultivars ‘Lutil’ (1972) and ‘Rina’ (early 1960s), all bred by INRA. Furthermore, a set of 52 perennial ryegrass cultivars from different breeding origins was gathered in 2005 to represent the history of modern breeding in this species for forage and turf usages. This set was used to experimentally assess the genetic improvement for these two usages (Sampoux et al, 2011, 2012) and it is currently in the process of seed regeneration in order to make it publicly available.

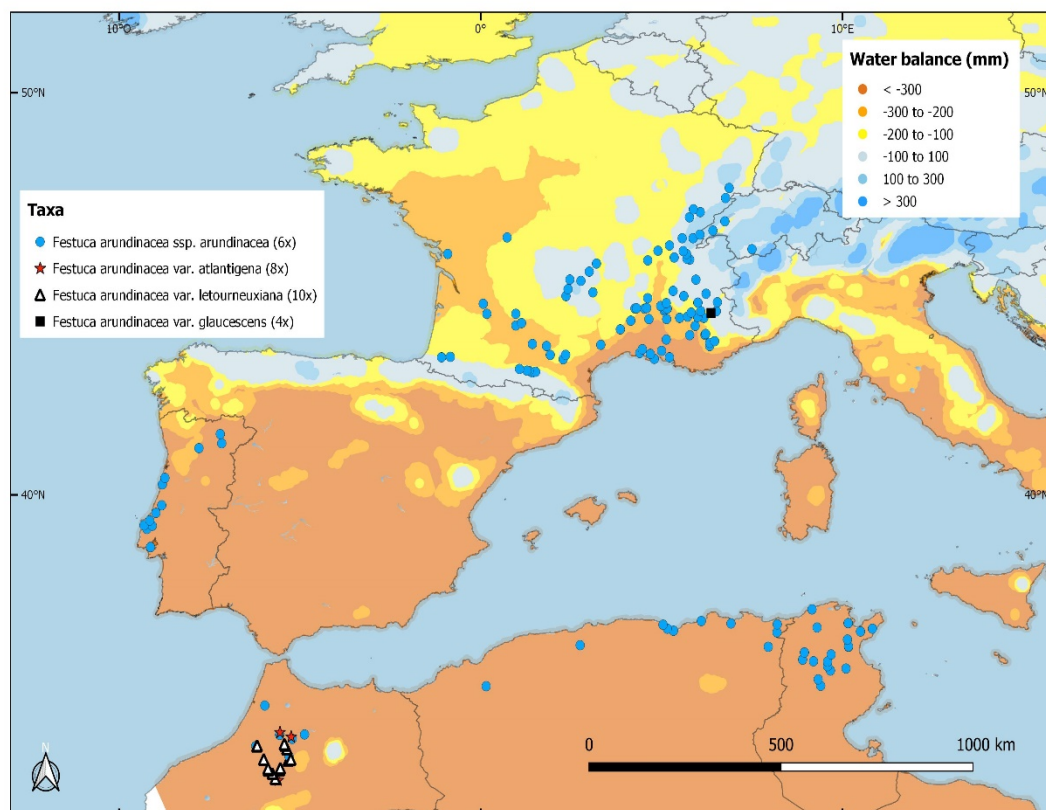
## Collection of fine-leaved fescues

### Natural populations

An extensive collection was performed for fine-leaved fescues by the INRA plant breeding unit of Lusignan and breeding companies of ACVF in 1993 and 1994. More

than 500 natural populations from red fescue (Stace, 1980) and sheep fescue (Wilkinson and Stace, 1991) taxa were collected across France. During the following years, they were characterized, evaluated for turf performances in dense swards and regenerated. Sampoux and Huyghe (2009) showed that the summer water balance, soil texture and land use were the main environmental variables differentiating the realized niches of the inland taxa, i.e. the caespitose red fescue taxon *Festuca nigrescens* Lam. (hexaploid,  $6x = 42$ ), the strong creeping (abundant long rhizomes) red fescue taxa *Festuca rubra* subsp. *fallax* (Thuill.) Nyman (octoploid,  $8x = 56$ ) and *F. rubra* L. subsp. *rubra* (hexaploid,  $6x = 42$ ) and various *Festuca ovina* L. taxa with karyotype varying from diploids ( $2x = 14$ ) to octoploids ( $8x = 56$ ). Sampoux and Huyghe (2009) also highlighted that the differentiation of inland taxa for adaptive traits contributed more than their ploidy level variation to the diversity of their realized niches (Hutchinson, 1957). Coastal populations of red fescues were also collected along the Channel coast. Hexaploid ( $6x = 42$ ) slender creeping (few short rhizomes) red fescues were found on salt marshes (green *F. rubra* subsp. *litoralis* (G.Mey.) Auquier) and





**Figure 3.** Geographical distribution of *F. arundinacea* natural populations maintained by the *Prairies* genebank. In the background, the water balance is the cumulated rainfall minus the cumulated evapotranspiration of June, July and August. Values are computed from 1989–2010 climate norms. Credit: Fabien Sampoux, project ColNat-Fourrage+ (AAC RPG 2018-2 CTPS GNIS)

on calcareous cliffs (glaucous *F. rubra* subsp. *pruinosa* (Hack.) Piper), and an octoploid (8x = 56) strong creeping red fescue (*F. rubra* subsp. *arenaria* (Osbeck) Syme) was found on sand dunes. During the years after collection, the populations of inland and coastal fine-leaved fescue taxa were extensively used by breeding companies in France to select new cultivars for turf usage. A core collection of 170 accessions was identified by selecting populations sampling the environmental range of each taxon (Figure 5) and it now makes the set of fine-leaved fescues natural populations available from the *Prairies* genebank (Table 1).

### Other genetic materials

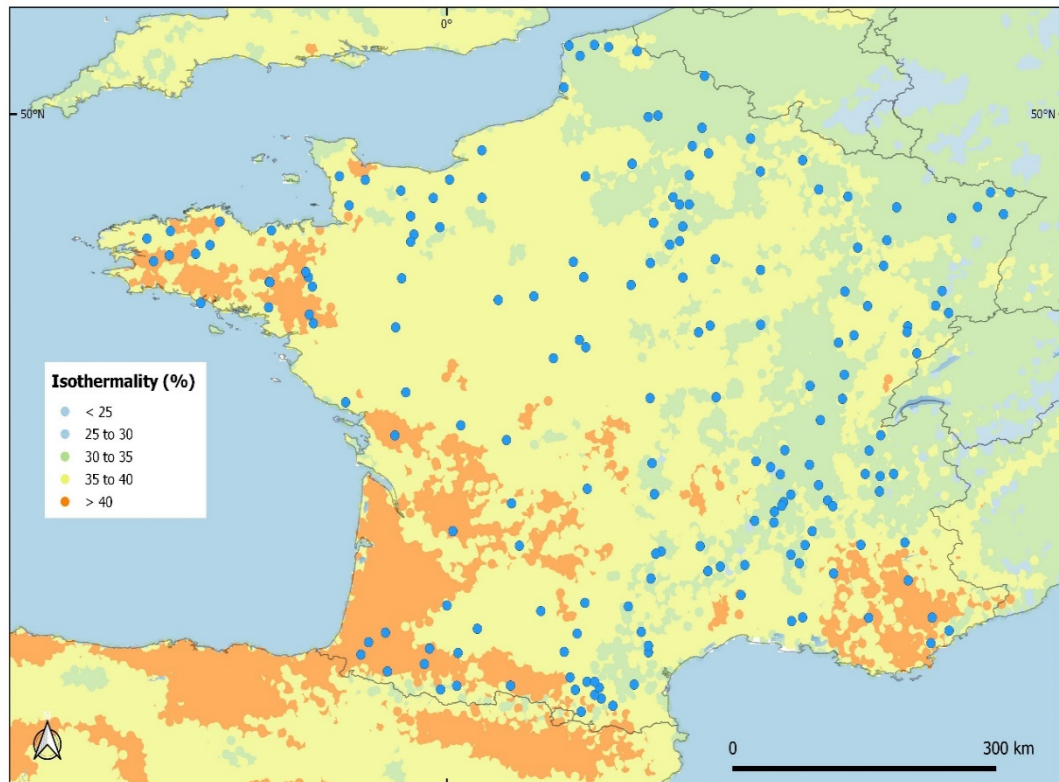
Accessions publicly available from the genebank also include the *F. rubra* subsp. *fallax* cultivars ‘Ludivine’ (1981) and ‘Milda’ (1975) and the *F. rubra* subsp. *pruinosa* cultivar ‘Luciole’ (2002), all formerly bred by INRA.

### Collection of lucerne

#### History of the collection

The cultivated lucerne (*Medicago sativa* subsp. *sativa*) is not present with natural populations in France, except some feral populations escaped from sown stands. However, some wild populations of the spontaneous sub-

species *Medicago sativa* subsp. *falcata* (L.) Arcang. and *Medicago sativa* subsp. *glomerata* (Balb.) Rouy can be found in northeastern and southeastern France, respectively (Julier, 1996). Lucerne as a forage crop (the subspecies *sativa*) was introduced in France in the 16<sup>th</sup> century from Spain (Michaud et al, 1988). Since then, traditional breeding has been applied on farm in almost all regions of France, producing landraces. Seed exchanges were frequent among different regions and with foreign countries (Julier, 1996). Hybridization between cultivated landraces of the subspecies *sativa* and wild populations of the subspecies *falcata*, naturally occurring in continental Europe, conferred frost resistance and variegated flower colour to cultivated populations. The first collections of landraces were set up during the first half of the 20<sup>th</sup> century by the plant breeding station of Versailles, a precursor of INRA, which delivered an extensive phenotypic description of them (Alabouvette and Méneret, 1935; Mayer et al, 1951). These studies and that of Demarly (1957) concluded that six main types of cultivated lucerne were present in France: ‘Provence’ in the south-east, ‘Poitou’, ‘Marais de Luçon’ and ‘Marais de Challans’ in the centre-west, ‘Flamande’ (or ‘Flemish’) in the north and ‘Ormelong’ in the south of the Paris Basin. These six types differentiated for traits like tap root, kidney-shaped seeds, pod coil numbers and frost resistance and for the frequency of plants with varie-



**Figure 4.** Geographical distribution of *L. perenne* natural populations maintained by the *Prairies* genebank. In the background, isothermality is the ratio ‘temperature diurnal range/temperature annual range’ in percentage. Values are computed from 1989–2010 climate norms. High isothermality values are typical of oceanic temperate climate whereas low values are typical of continental climate. Credit: Fabien Sampoux, project ColNat.Fourrage+ (AAC RPG 2018-2 CTPS GNIS)

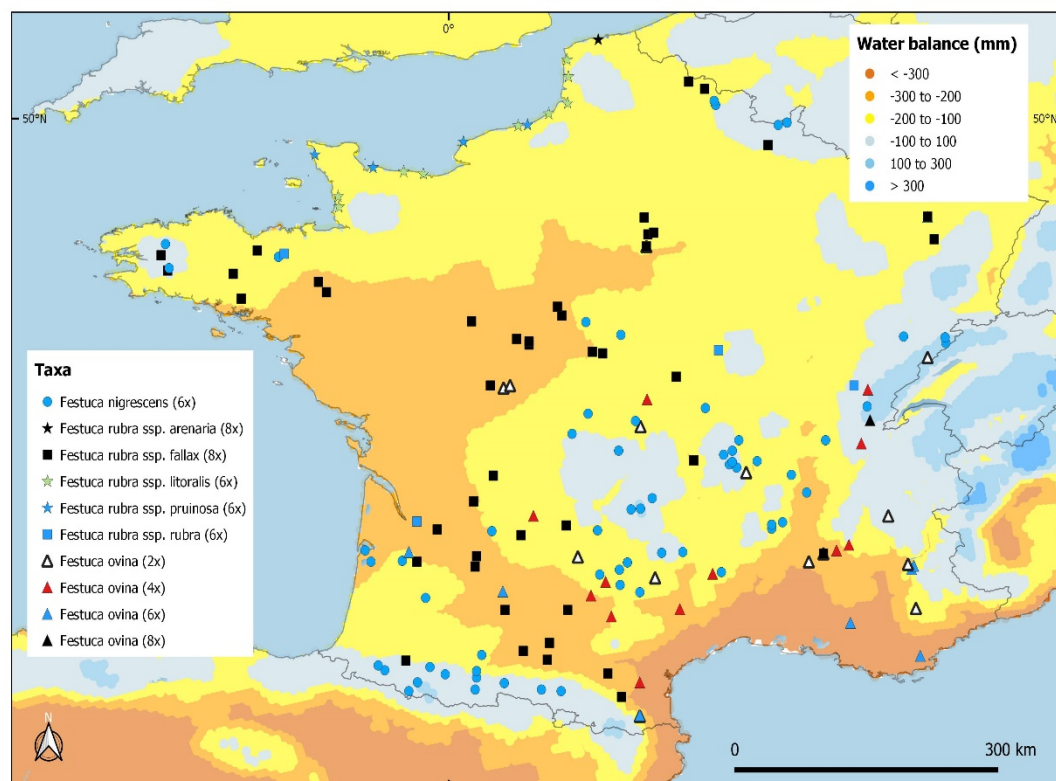
gated flowers (Julier, 1996). They were used to initiate breeding programmes and the very first registered cultivars (e.g. ‘Du Puits’) were produced after a brief selection in ‘Flamande’.

The six types of French landraces were transferred to the INRA forage plant breeding unit in Lusignan at its creation in 1960. In order to widen the genetic basis of its lucerne breeding programmes, the plant breeding unit of Lusignan received various cultivars, landraces and also wild populations from different countries in Europe, North Africa and America. A few of them were included in the genetic resources made publicly available, in agreement with provider requirements and plant breeding rights. As a noticeable event, a collection trip was undertaken by the INRA plant breeding unit of Lusignan in 1985 in Lorraine (east of France) after a witness mentioned the presence of some prostrate, yellow-flowered lucerne populations in this region. Two populations, named ‘Malzeville’ and ‘Marron’, were collected and multiplied, and a smooth selection was applied to remove cultivated off-types. These populations turned out to be typical of the Eurasian continental subspecies *falcata*. Furthermore, from 1985 to 1987, scientists of the INRA plant breeding unit of Montpellier organized collection trips in Spain and Portugal to collect wild populations of lucerne,

also named ‘Mielga’ populations (Prosperi et al, 1989). These populations, mainly located on roadsides and low-input grasslands, had a prostrate growth habit but their violet flower colour attested to their relatedness to the subspecies *sativa*. The phenotypic description of these wild populations showed various levels of introgression by cultivated materials (Prosperi et al, 2006). Some ‘Mielga’ genetic materials are currently available in the *Prairies* genebank (see below). Several studies, carried out by the INRAE plant breeding unit of Lusignan, provided extended analyses of the phenotypic and genetic diversity of lucerne genetic resources, including cultivars, landraces and natural populations (Julier et al, 1995; Crochemore et al, 1998; Herrmann et al, 2018; Pégard et al, 2023a).

### Genetic resources currently available

The numbers of landraces, natural populations and cultivars maintained by the *Prairies* genebank are reported in Table 1. The landrace accessions include the six original French landrace types (see above) as well as several landraces from northern Africa. The natural populations comprise five *M. sativa* subsp. *falcata* accessions including the ‘Malzeville’ and ‘Marron’ populations, one *M. sativa* subsp. *glomerata* accession as well as seven *M. sativa* subsp. *sativa* accessions including



**Figure 5.** Geographical distribution of *Festuca rubra* aggr. and *Festuca ovina* aggr. natural populations maintained by the *Prairies* genebank. In the background, the water balance is the cumulated rainfall minus the cumulated evapotranspiration of June, July and August. Values are computed from 1989–2010 climate norms. Credit: Fabien Sampoux, project ColNat-Fourrage+ (AAC RPG 2018-2 CTPS GNIS)

five 'Mielga' populations from Spain. Note that a large set of 'Mielga' accessions has recently been transferred from INRAE Montpellier to the *Prairies* genebank in Lusignan but these accessions are not currently ready for distribution.

There are 25 *M. sativa* subsp. *sativa* cultivars maintained by the genebank. They notably include several historical cultivars formerly grown in France, i.e. cultivars 'Coussouls' (1998), 'Janine' (1974), 'Luciole' (1965), 'Luisante' (1998), 'Lutèce' (1976), 'Luxor' (1977), 'Mireille' (1971) bred by INRA, cultivar 'Medalfa' (1988) bred by INRA and ACVF, and cultivars 'Du Puits' (1950), 'Gamma' (1952), 'Glacier' (1975), 'Elga' (1964), 'Emeraude' (1952), 'Etoile du Nord' (1965), 'Hybride de Crécy' (1974), 'Polder' (1972), 'Prima' (1963), 'Oméga' (1952), 'Orchésienne' (1952), 'Verneuil' (1968) bred by private breeders.

### Other genetic materials in collections

The seed-collecting campaign carried out in 2014 to collect natural populations of grass and legume species in southern France (see previous paragraphs) also enabled the collection of 11 populations of meadow fescue (*Festuca pratensis*), two populations of red clover (*Trifolium pratense* L.) and six populations of white clover (*Trifolium repens*). In 2017, another collecting trip

enabled the collection of 27 *Phalaris* natural populations (*Phalaris arundinacea* L. and *Phalaris aquatica* L.) in southern France and Corsica, which are currently in the process of seed regeneration.

Finally, the genebank maintains a small number of cultivars of red and white clovers, thimothy (*Phleum pratense* L.), vetch (*Vicia sativa* L.) and sainfoin (*Onobrychis viciifolia* Scop.), all from INRA breeding.

### Practical organization of the *Prairies* genebank

In 2008, the INRAE research unit UR P3F formally set up the *Prairies* genebank on its operational site in Lusignan in order to gather genetic resources of forage and turf species previously maintained in several INRAE sites across France. During the following years, substantial investments were achieved to equip the genebank with renewed facilities such as cold rooms for seed storage and a partitioned greenhouse to grow accessions in pollen isolation. Accessions were inventoried and a database was created to record all necessary information (passport data of accessions, quality and quantity of seed lots, characterization and evaluation data) and to trace the activity (seed quality control, seed regenerations, seed distributions, etc.).



For each accession, seeds harvested after a regeneration event are split into three seed lots: an active lot weighing from 200 to 800g which is stored in a cold room (+5°C and 30% hygrometry), a 20 to 30g safety lot stored at -20°C, and a 1 to 2g long-term conservation lot stored at -20°C in a different freezer than the safety lots. The genebank maintains active and safety seed lots only from the most recent regeneration event and long-term seed lots from all successive regeneration events and the initial seed batch introduced in the genebank. Germination tests are performed every three years on active lots, if they are not replaced by lots from a new regeneration event, as well as on safety seed lots if the germination percentage of active seed lots is below the established thresholds. Active and safety seed lots are regenerated when the germination percentage of active seed lots falls below 80%, i.e. every 10 to 15 years.

Species maintained in the genebank are allogamous and more or less self-incompatible. Because of this sexual reproduction biology, natural populations, landraces and cultivars (synthetic varieties) from these species are polygenotypic populations in principle at panmictic equilibrium. Therefore, the regeneration of accessions from these different kinds of genetic materials is performed by intercrossing a number of plants expected as sufficient to avoid genetic drift (i.e. 100 to 250 plants) in pollen isolation from any plant from the same species not belonging to the accession. Different means are implemented to ensure pollen isolation. In field conditions, accessions from grass species are grown in spaced spots within a triticale-rye crop which acts as a pollen barrier. Another way to achieve pollen isolation is the use of pollen-proof (for anemophilous grasses) or insect-proof (for entomophilous legumes) tents and a partitioned greenhouse with 12 confined compartments.

To date, 731 accessions made publicly available for sample delivery (Table 1) can be browsed online on the Siregal website of the Biology and Plant breeding department of INRAE (<https://urgi.versailles.inra.fr/siregal>), on the Florilege website (<https://florilege.arcad-project.org/fr/collections>) set up by the plant pillar (BRC4Plants) of the French National Research Infrastructure RARE (Bergheaud et al, 2025) and on the website of the European Search Catalogue for Plant Genetic Resources EURISCO (<https://eurisco.ecpgr.org>) with the holding institute field filled in as FRA001. These databases will be soon updated with the recently introduced accessions still missing in their records. All the species maintained in the *Prairies* genebank are listed in Annex I of the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA). Therefore, 531 of the publicly available accessions were notified in 2014 as a contribution of France to the Multilateral System of the ITPGRFA. An additional set of 321 accessions has been proposed in 2024 for further contribution to the Multilateral System (Table 1). Furthermore, passport data of 537 accessions from the natural diversity of grass species have been uploaded to the Global Biodiversity Information Facility (GBIF)

database (<https://www.gbif.org/publisher/0b5846f7-20b5-410a-93c5-5de83b522deb>).

The *Prairies* genebank was labelled as a Biological Resource Centre by the network IBiSA (French network of biology, health and agronomy infrastructures) in 2011 and approved as a genebank of the plant pillar of the French National Research Infrastructure RARE in 2021. It was furthermore officially recognized as a curator of plant genetic resources for food and agriculture by the French Ministry of Agriculture in 2020. The *Prairies* genebank manages the French network for conservation of forage and turf species genetic resources, which includes INRAE and private companies actively breeding these species in France. In 2023, the *Prairies* genebank as well as the biotechnology and chemistry facilities of UR P3F were acknowledged for ISO9001:2015 certification.

Over the five last years, the *Prairies* genebank has distributed around 1,300 seed samples to various recipients, which were, by decreasing order of magnitude, French and foreign academic research institutions, French and foreign plant breeding companies, farmers and hobby-growers.

### Uniqueness of the collections

The collections of natural populations maintained by the *Prairies* genebank originate from areas not or little represented in other genebanks. Notably, most of the grass and lucerne populations from northern Africa kept by the genebank are not maintained in their country of origin, and sometimes do not grow anymore in their site of origin, because of changes in climate and land use, excessive grazing or replacement by cultivars from modern breeding. Recent collections of natural populations undertaken by the INRAE research unit UR P3F in France and other European countries have confirmed that the *in situ* conservation of these populations is often threatened by the continuous regression of natural and permanent grasslands, climate change, and the corruption of their genetic integrity by over-seeding with unrelated cultivars. The *ex situ* collections of grass and legume natural populations maintained by the *Prairies* genebank thus make a unique, although non-exhaustive, contribution to the preservation of the genetic diversity of these species. Furthermore, the *Prairies* genebank is the only repository of INRAE cultivars removed from national lists. However, the polygenotypic nature and the allogamous sexual reproduction of forage and turf species make the seed regeneration of accessions costly, even though the seed market for these species, and consequently the means for genetic resources conservation, are relatively limited. International networks of genebanks, like the Forages Working Group of the European Cooperative Programme for Plant Genetic Resources (ECPGR), are thus essential to share conservation efforts in these species. In the coming years, the *Prairies* genebank will aim to achieve a better sampling of the French heritage for genetic resources of forage and turf species in its collec-

tions. Present collections of orchard grass and tall fescue natural populations only sample a limited part of the French territory, and it will be aimed to complete the collections through new sampling campaigns in regions not visited so far. It will also be considered to sample the French natural diversity of legume species, such as white and red clover. Another important goal will be to work towards a more representative collection of cultivars, bred by INRAE and also other breeders, that were grown in France since the 1950s for all forage and turf species that had significant use.

## Valorization of the collections

Initially set up to have genetic resources available to start breeding programmes, collections of natural populations may provide relevant materials to study the environmental distribution of inter- and intra-specific plant diversity. In this respect, 167 scientific papers from various international institutions have to date used the occurrence data of grass species forwarded by the *Prairies* genebank to the GBIF database. The main asset of these collections is their capacity to supplement occurrence data with phenotypic and genomic information already existing or newly generated. Earlier in this paper, we reported the use of phenotypic data to document adaptive differentiation in fine-leaved fescue taxa (Sampoux and Huyghe, 2009) and adaptive variability within perennial ryegrass (Balfourier and Charmet, 1991). More recently, the FACCE-JPI ERA-NET+ (<https://www.faccejpi.net/en/faccejpi/actions/core-theme-1/facce-era-net-plus-on-climate-smart-agriculture.htm>) project GrassLandscape gathered INRAE, IPK (Leibniz Institut für Pflanzengenetik und Kulturpflanzenforschung - Institute of Plant Genetics and Crop Plant Research, Germany), IBERS (Institute of Biological, Environmental and Rural Sciences, Wales, United Kingdom) and ILVO (Instituut voor Landbouw-, Visserij- en Voedingsonderzoek - Flanders Research Institute for Agriculture, Fisheries and Food, Belgium) to implement phylogeographic and landscape genomics analyses on a set of 470 accessions from the natural diversity of perennial ryegrass provided by 15 genebanks from European countries and the USDA. High throughput genotyping of these accessions allowed for the reconstruction of the expansion history of perennial ryegrass across Europe (Blanco-Pastor et al, 2019). Then, a multivariate landscape genomics analysis retrieved 633 potentially adaptive loci associated with either winter cold or summer drought and heat stresses and pointed phenotypic traits putatively conferring adaptation to these stresses (Blanco-Pastor et al, 2020). The adaptive relevance of these traits was further confirmed by a functional ecology approach (Keep et al, 2021). Results of the project GrassLandscape were then used to set up a European core collection of perennial ryegrass natural populations within the frame of an ECPGR Activity (Keep et al, 2020; Sampoux and Willner, 2022). Adaptive phenotypic and genomic variations revealed by the project GrassLandscape have furthermore been

used to assess the future of perennial ryegrass local populations exposed to climate change by implementing genomic offset analyses (Pégard et al, 2023b). For other forage species than perennial ryegrass, collections maintained in European genebanks, and beyond, could likely provide sufficiently wide sets of natural populations to set up environmental niche models of species (Sampoux and Badeau, 2009) and reveal adaptive phenotypic and genomic diversities within species (see for example Boller et al (2010) for genetic resources and diversity in forage species). This information could be used to implement predictive analyses, such as genomic offset analyses, to foresee spatial shifts of adaptive diversity required for adaptation to ongoing changes in climate and other environmental factors (Fitzpatrick et al, 2021; Gougherty et al, 2021; Hung et al, 2023; Aitken et al, 2024; Zou et al, 2024). On this basis, strategies for *in situ* conservation could be developed, taking into account ongoing environmental changes on a European scale. For most forage and turf species that can spontaneously persist and evolve in long-duration grasslands, *in situ* conservation planned in this way would be the cheapest and most sustainable means to maintain valuable functional and adaptive genetic diversity in the long term. Incorporating adaption to future environmental changes across territories in *in situ* conservation programmes would contribute to maintaining an economically viable forage production on involved grasslands that would thus help the sustainability of these programmes.

Results of the project GrassLandscape also showed that the diversity of perennial ryegrass forage cultivars currently grown worldwide represents only a small part of the natural diversity of the species (Blanco-Pastor et al, 2019). Moreover, the H2020 European project EUCLEG ([www.eucleg.eu](http://www.eucleg.eu)) led by UR P3F studied a worldwide set of lucerne landraces and cultivars, which revealed clear phenotypic and genomic differentiation related to geographic origin and evidence that the cultivar diversity grown in Europe represents a limited part of the whole genetic diversity of the species (Pégard et al, 2023a). These results suggest that natural diversity, landraces and old cultivars of forage and turf species may still provide potentially useful genetic variability to adapt grown cultivars to upcoming challenges. Such genetic resources may be useful sources to adapt species to new combinations of climate constraints (Blanco-Pastor et al, 2020) and to improve their tolerance or resistance to biotic stresses (Sampoux and Badeau, 2009; Annicchiarico et al, 2015). They could also be valuable sources to develop cultivars adapted to cultivation in species mixtures. During the last five decades, forage and turf species have intensively been bred for performances in pure stands and it may be assumed that phenotypes more adapted to interactions prevailing in species mixtures (Litrico and Violle, 2015; Sampoux et al, 2020) could be recovered from natural diversity or genetic material that experienced only limited breeding (e.g. old cultivars). Furthermore, with the transition to agroecological practices, some forage

species have emerged as service crops. They could be used as intermediary cover crops, e.g. Italian ryegrass, or as perennial living mulches, e.g. lucerne, red and white clovers, sainfoin (*Onobrychis viciifolia* Scop.) and birdsfoot trefoil (*Lotus corniculatus*). Cultivars bred for forage usage are probably not the best-adapted materials for these new uses (El-Ghazzal et al, 2024) and genetic resources may help to breed more suitable phenotypes.

However, introgressing allele diversity from raw genetic resources to elite cultivars may have become more and more challenging as continuously improved cultivars have been released. This may especially be the case for the allogamous and partially auto-incompatible forage and turf species, for which it can be assumed that the alleviation of the genetic load has been a substantial part of the genetic improvement (Kimbeng and Bingham, 1998; Annicchiarico et al, 2015). Genotyping based on genome sequencing should be expected to help the efficient introgression of desirable multi-genic features from genetic resources to elite germplasm within a reasonable timespan. While investigations such as the ones of the project GrassLandscape have proven that collections of natural populations can be used to reveal the signature of natural selection (loci whose allele frequency variation departs from neutral expectations), it could be expected that collections of cultivars punctuating the history of modern breeding may reveal the signature of artificial breeding (see for example Welcker et al (2022) for such approach in maize). Marker-assisted selection methods could then possibly be designed to optimize favourable allele frequencies at both loci involved in desirable natural adaptations and loci that determine agronomic performances in the cultivars of forage and turf species, essentially bred as synthetics. Efforts to broaden, phenotype and genotype the collections of the *Prairies* genebank are to be conceived in order to contribute in these directions.

### Author contributions

The manuscript was written by JPS with contributions from BJ and MG. All authors reviewed and commented the manuscript and approved the final version.

### Conflict of interest statement

The authors have no conflicts of interest to report.

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# Strengthening European research cooperation on plant genetic resources conservation and use

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**Abstract:** Plant genetic resources (PGR) are a vital research infrastructure and an important asset to increase the resiliency of agri-food systems, conserve agrobiodiversity and mitigate the effects of climate change. In the current scenarios of climate change and biodiversity loss, it becomes increasingly urgent to ensure the conservation of existing crop diversity and assure its availability for research and breeding to enable the development of new, adapted crops. Throughout Europe, more than 400 collections conserve PGR of over 6,500 genera, with more than 2 million accessions documented in the European Search Catalogue for Plant Genetic Resources (EURISCO). To make these resources available to breeders, more research investment in these collections is needed. Here, we analyze the participation of European genebanks in collaborative projects within the EU Horizon scheme as an indicator for the use of PGR collections in research. We highlight two Horizon projects, AGENT and G2P-SOL as well as the ECPGR initiative European Evaluation Network (EVA), which have brought together genebanks and other stakeholders to create tools and knowledge on PGR. Their experience could be translated into a dedicated, large European research infrastructure for PGR (GRACE-RI), suggested in the *Plant Genetic Resources Strategy for Europe* and currently in the concept phase by the Horizon Europe project PRO-GRACE. GRACE-RI will connect European research institutes involved in PGR conservation and research and will be key to ensuring access to well-documented and maintained PGR and methods for their characterization and utilization, preventing further loss of plant biodiversity which is increasingly threatening European agriculture and natural landscapes.

**Keywords:** plant genetic resources, genebank, research, breeding, documentation, infrastructure, biodiversity, conservation, sustainable use

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## Introduction

Plant genetic resources (PGR) are important assets to address the challenges associated with climate change, sustainability in agriculture and food and nutrition security, as they provide the genetic diversity necessary to develop adapted crop cultivars for agriculture in

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diverse and rapidly changing ecosystems (Hausmann et al, 2004; McCouch et al, 2013; Swarup et al, 2021). They are also an invaluable reservoir of genetic information that scientists can decipher thanks to modern technologies and the increasing understanding of the relationship between genomes and the metabolic functions of living organisms (Bilbrey et al, 2021; Derbyshire et al, 2022).

An important prerequisite to ensure PGR availability is their active conservation and documentation (Weise et al, 2020) with the involvement of all relevant stakeholders, including genebanks, *in situ* conservation sites, farmers, researchers and breeders (Engels and Ebert, 2024). PGR conservation falls under the responsibility of national programmes in each country, with international agreements such as the Convention on Biological Diversity (CBD) and its Nagoya Protocol (CBD, 2011), as well as the International Treaty for Plant Genetic Resources for Food and Agriculture (ITPGRFA, FAO (2009)) providing the legal frameworks for conservation and facilitated access to germplasm and its associated information and the shared benefits deriving from their use. Since the establishment of the first genebank collections in the 1950s, now more than 4.4 million accessions are documented in the worldwide data platform Genesys (<https://www.genesys-pgr.org/>).

These diverse national PGR collections are becoming more and more relevant for research and breeding programmes as they provide the wild relatives and locally adapted landraces necessary for breeding improved varieties. Therefore, ensuring the availability of these plant materials is of crucial importance (Gullotta et al, 2023). Adding genotypic and phenotypic information on accessions in the collections greatly facilitates their management and use, helping with identifying duplicates and selecting appropriate material for further studies and breeding activities (McCouch et al, 2013; Mascher et al, 2019). Accessing international funding through participation in collaborative projects and networks has been a way to improve knowledge about institutes' PGR collections and their management, build capacity and strengthen connections among stakeholders. At the European level, since 1980 the European Cooperative Programme for Plant Genetic Resources (ECPGR) has built a network for national PGR conservation programmes currently encompassing 36 countries, and has been instrumental in building capacity and developing tools for the use of the PGR community, including the European Search Catalogue for Plant Genetic Resources (EURISCO) (Kotni et al, 2023) and the European Genebank Integrated System (AEGIS). The latter aims at standardizing quality management across participating genebanks and establishing a European collection of unique PGR accessions, promptly available under the same terms and conditions. Still, overall funding is inadequate to effectively conserve, document and exploit the available PGR diversity, as outlined in the *Plant Genetic Resources Strategy for Europe* (ECPGR, 2021).

A worldwide survey conducted among stakeholders within ITPGRFA contracting parties identified a number of bottlenecks in the PGR use system, including insufficient support and implementation of national policy systems, lacking personal and institutional capacities, insufficient involvement of important stakeholders such as farmers and breeders as well as lack of awareness of the importance of PGR conservation in the general public (Kell et al, 2017). They also identified several important constraints for access to PGR and connected data, including insufficient characterization and evaluation across diverse gene pools, the fragmentation and inaccessibility of generated data and the difficulty to access material with specific traits. The survey further highlighted the value of collaboration, between public sector administrations and researchers as well as between the public and private sectors, in programmes towards sustainable use of PGR in research and breeding (Kell et al, 2017). Improving our knowledge of crop genetic diversity conserved across the world, in particular to promote coverage of genetic and phenotypic data on PGR as suggested by McCouch et al (2013) will enable focused research and breeding of well-adapted, nutritious and resilient crops.

With the world's biodiversity in decline (IPBES, 2019) and the loss of genetic diversity in our crops threatening food security (Pilling et al, 2020), more decisive actions and policies are thus needed to ensure the conservation of PGR for their use in research and breeding. In the recently published *Plant Genetic Resources Strategy for Europe* (ECPGR, 2021), issues and relevant gaps in conserving and facilitating PGR use across Europe were highlighted, and the authors called for a transformative change whereby Europe should reinforce its leading role in their conservation and sustainable use. The strategy's objectives by 2030 include the expansion, improvement and consolidation of *ex situ*, *in situ* and on-farm conservation activities, strengthened data management and germplasm information systems, increased access and sustainable use of PGR and monitoring of progress in PGR conservation and use (ECPGR, 2021). In order to ensure the availability of well-documented PGR for use by future generations, the strategy proposed a number of actions to strengthen national programmes, build capacity of conservation actors and promote international collaborations, involving all relevant stakeholders including the general public. This requires an appropriate policy and legal framework, combined with secure and appropriate financing to strengthen national and regional programmes as well as institutional and human capacity. A recommended key step to enable the transition to a fully functional European system effectively supporting high-quality research is the establishment of an efficient research infrastructure dedicated to PGR conservation, documentation, research and sustainable use.

In this paper, we provide an overview of European genebank collections documented in EURISCO and analyze the current engagement status of European

genebanks and other PGR-holding institutes in international projects funded by the EU as an indicator for the use of PGR collections in research. We provide examples of successful cooperation between research institutes, genebanks and other stakeholders in three European projects that highlight the usefulness of international collaboration in advancing research on PGR. Finally, we propose a coordinated European approach to PGR research involving a dedicated Research Infrastructure (GRACE-RI) that will enable effective conservation and sustainable use of PGR through the provision of a suite of relevant technical services to facilitate adaptation of European agriculture to the climate emergency and food security issues.

## Materials and methods

To conduct a summary analysis of the information stored in EURISCO to describe the current situation in terms of genebank documentation, data were extracted from EURISCO on 27/01/2025 and filtered for size of collections with regard to level of safety duplication, biological status and genera represented.

To map the involvement of European genebanks in such projects, we conducted a keyword search with relevant terms against the EU Horizon project databases (FP7, <https://data.europa.eu/data/datasets/cordisfp7/projects?locale=en>; H2020, <https://data.europa.eu/data/datasets/cordish2020projects?locale=en>; Horizon Europe, <https://data.europa.eu/data/datasets/cordis-eu-research-projects-under-horizon-europe-2021-2027?locale=en>; accessed on 10/09/2024). Out of 35,386 projects granted within the Horizon2020 framework, 424 projects contain the keywords ‘biodiversity’, ‘agriculture’, ‘breeding’, ‘crop wild relative’, ‘genetic resource’ or ‘plant’ in varying combinations in their titles and objective descriptions. This list was manually curated to eliminate projects that were not directly working on PGR or were involving only one institute, yielding 40 projects with multi-actor consortia working on PGR in the years 2014–2022 (Supplemental Table 1). A similar filtering approach for Horizon Europe projects yielded 40 PGR-related projects out of 13,215 total, which started in the years 2022–2024 and are ongoing (Supplemental Table 2). Within the Framework Programme FP7 funding scheme, which was active between 2008 and 2018, we identified 33 multi-actor projects related to the above keywords (Supplemental Table 3).

Projects were analyzed and grouped according to the crops and PGR studied and the overarching topics of their research questions. We then compared the involved project partners against the EURISCO institutes list (downloaded from EURISCO: <http://eurisco.ecpgr.org>, accessed on 23/04/2024) since these officially conserve germplasm that is part of the European countries’ national inventories. Some project partners

could represent multiple holding institutes (e.g. INRAE,<sup>1</sup> CSIC<sup>2</sup> or CREA<sup>3</sup>); in these cases, the projects were analyzed to match the correct institute. In this way, we identified 76 institutions from 25 countries listed in EURISCO that have participated in projects financed by the European Commission since 2008 (Supplemental Tables 1–4).

We contacted genebank curators and managers of the identified institutes with a short survey to collect additional information on their involvement in the listed projects, including whether or not they contributed PGR and how research materials produced in the projects were conserved and received responses from 35 institutes.

The qualitative analysis of Horizon2020 projects AGENT and G2PSol as well as the ECPGR European Evaluation Network (EVA) is based on data available to project partners among the authors.

## Results

### Overview of European genebank collections

Across Europe, more than 400 institutes in 43 countries conserve PGR in *ex situ* and field collections, with at present more than 2 million accessions recorded in EURISCO (Kotni *et al*, 2023). These holding institutes vary greatly in size, capacity and mandate within national conservation programmes, with documented collections of between one (recorded by 22 holding institutes) and more than 680,000 accessions (by the Nottingham Arabidopsis Stock Centre NASC, Figure 1A). Figure 1B shows the number of genebanks conserving the most represented genera (excepting *Arabidopsis*), with collections of more than 100 and 1,000 accessions emphasized, further highlighting the diversity in size of European genebanks. Thirty-four countries have safety-duplicated parts of their collections in genebanks located in other countries or in the Svalbard Global Seed Vault, although overall, only 5.4% of European *ex situ* accessions have been added to the Svalbard Seed Vault so far (Figure 1C, Asdal (2025)). The collections cover more than 6,500 different genera of crop species and their wild relatives (CWR) stored as seeds, *in vitro*, in cryo or field collections and provide long-term storage of the plant reproductive material. Reviewing the biological status of material in the collections (Alercia *et al*, 2015), wild or natural materials (code 1xx) represent around 13% of collections but have the greatest diversity with 6,185 genera (Figure 1D). Around 15% of the collections are traditional cultivars and landraces (code 300), with 599 genera represented. The largest part of the documented collections are

<sup>1</sup> INRAE, *Institut national de recherche pour l’agriculture, l’alimentation et l’environnement* (France’s National Research Institute for Agriculture, Food and Environment)

<sup>2</sup> CSIC, *Consejo Superior de Investigaciones Científicas* (Spanish National Research Council)

<sup>3</sup> CREA, *Consiglio per la ricerca in agricoltura e l’analisi dell’economia agraria* (Council for Agricultural Research and Economics, Italy)

breeding materials (code 4xx) and advanced/improved cultivars (code 500) with together ~44% of the documented accessions, covering 473 and 549 genera, respectively. Material of unknown status (999 or NA) makes up ~27% of accessions, representing more than 1,600 different genera, highlighting the need to improve knowledge of the European genebank collections and their documentation. A coordinated system for *in situ* conservation of PGRs and its documentation is still in its infancy, with a recent extension of EURISCO facilitating the documentation of *in situ* populations of CWR. Information on *in situ* conserved PGR is more difficult to review as data are slowly being inserted into the public domain.

### Survey of EU-funded research on plant genetic resources

In the European research strategy, funding has been made available through the 7<sup>th</sup> Framework Programme and Horizon schemes to support research on genetic resources and breeding, providing crucial innovation towards developing more sustainable agrosystems in the face of changing environments. Research and innovation actions create platforms for collaborative research consortia that connect institutions from diverse countries and with complementary expertise to produce valuable outputs for the European research agenda. Using a keyword search of all projects recorded in the EU Cordis database we identified 33, 40 and 40 multi-actor projects funded through the FP7, Horizon2020 and Horizon Europe frameworks, respectively (Supplemental Tables 1–3). We focused our survey on these funding mechanisms because they offer frameworks for international cooperation, cover all EU and associated countries and provide the backbone of European research funding. Additional analyses exploring other funding sources (national, bilateral, philanthropic, etc.) would complement our analysis. As per the project descriptions, these addressed various aspects of biodiversity research, agronomy, policy actions and development of research infrastructures. Many of these projects are transdisciplinary, working on the development of methods and practices on a variety of crops and agricultural systems, rather than focusing on specific PGR genera.

Overall, the number of projects on biodiversity, agriculture and PGR funded through EU research actions have increased over time reflecting the increasing importance of related topics in the European research agenda in Horizon Europe (Figure 2A), with an investment of more than 625 million euros. Considering the main topics and crops covered by these projects, there is a clear tendency towards cross-cutting projects covering multiple crops or agrobiodiversity in general, with a focus on breeding (Figure 2B). The main crops studied by these projects were cereals, legumes and Solanaceae, while other vegetables, perennials and non-food crops were represented in fewer projects (Figure 2C).

### Survey of research involvement of European genebanks

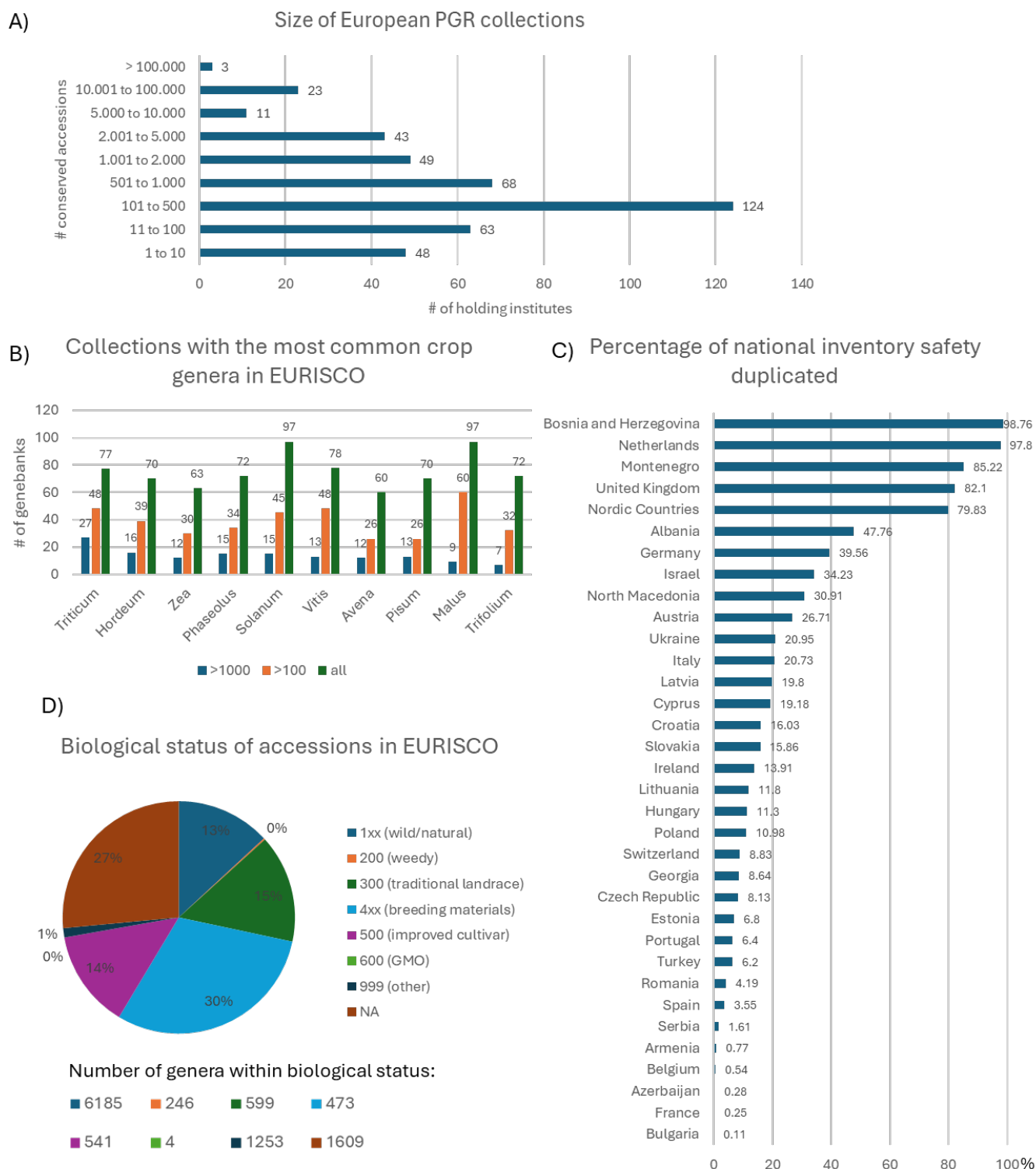
Within the above-mentioned projects we analyzed the involved project partners to identify those who are holding institutes of PGR included in EURISCO and thus potentially contributing to the projects by providing genebank materials. We identified 40, 28 and 25 projects in H2020, Horizon Europe and FP7, respectively, involving 76 organizations from 25 countries listed in EURISCO (Supplemental Tables 1–4). Some genebanks are embedded in larger organizations and universities (e.g. INRAE, WUR<sup>4</sup>, IPK<sup>5</sup>) and therefore their parent institutions may participate in projects in other capacities. Table 1 shows the participation of institutes listed in EURISCO in projects under the different funding schemes disaggregated by countries, highlighting that some countries and organizations are well represented in project consortia, as also reflected in the number of projects per institute (Supplemental Table 4). However, many countries and EURISCO institutes take part in only one project at a time, suggesting limitations exist in organizations to fully participate in and benefit from international research opportunities.

To better understand the role of EURISCO-listed institutes in European research, we contacted genebank managers of the identified organizations to confirm whether they provided PGR accessions to the Horizon or other European projects they participated in and received feedback from 35 institutes (Table 2). Of these, three did not contribute PGR to the projects they participated in, indicating that they played a different role in these projects. In total, respondents listed 29 projects funded by different mechanisms (e.g. Horizon, PRIMA or ERANET as well as the ECPGR EVA project) for which they had provided PGR accessions from their collections. Nineteen genebanks indicated that they had incorporated material developed in 15 Horizon projects back into their collections to make them available for further exploitation, mainly covering different cereals, legumes, Solanaceae and *Brassica* species. Based on the identified involvement of their institutes in European projects (Supplemental Table 4), these responses suggest that while some organizations are very active in Horizon projects, European genebanks and their collections are not systematically involved or effectively utilized in European PGR-related projects. In addition, European projects working on PGR appear to utilize plant germplasm that may not be documented within EURISCO and included in European national collections, showing an obvious gap in PGR documentation. Lastly, promising plant materials generated by European projects are not systematically introduced in genebank collections for further conservation and exploitation. It would be especially interesting to further research

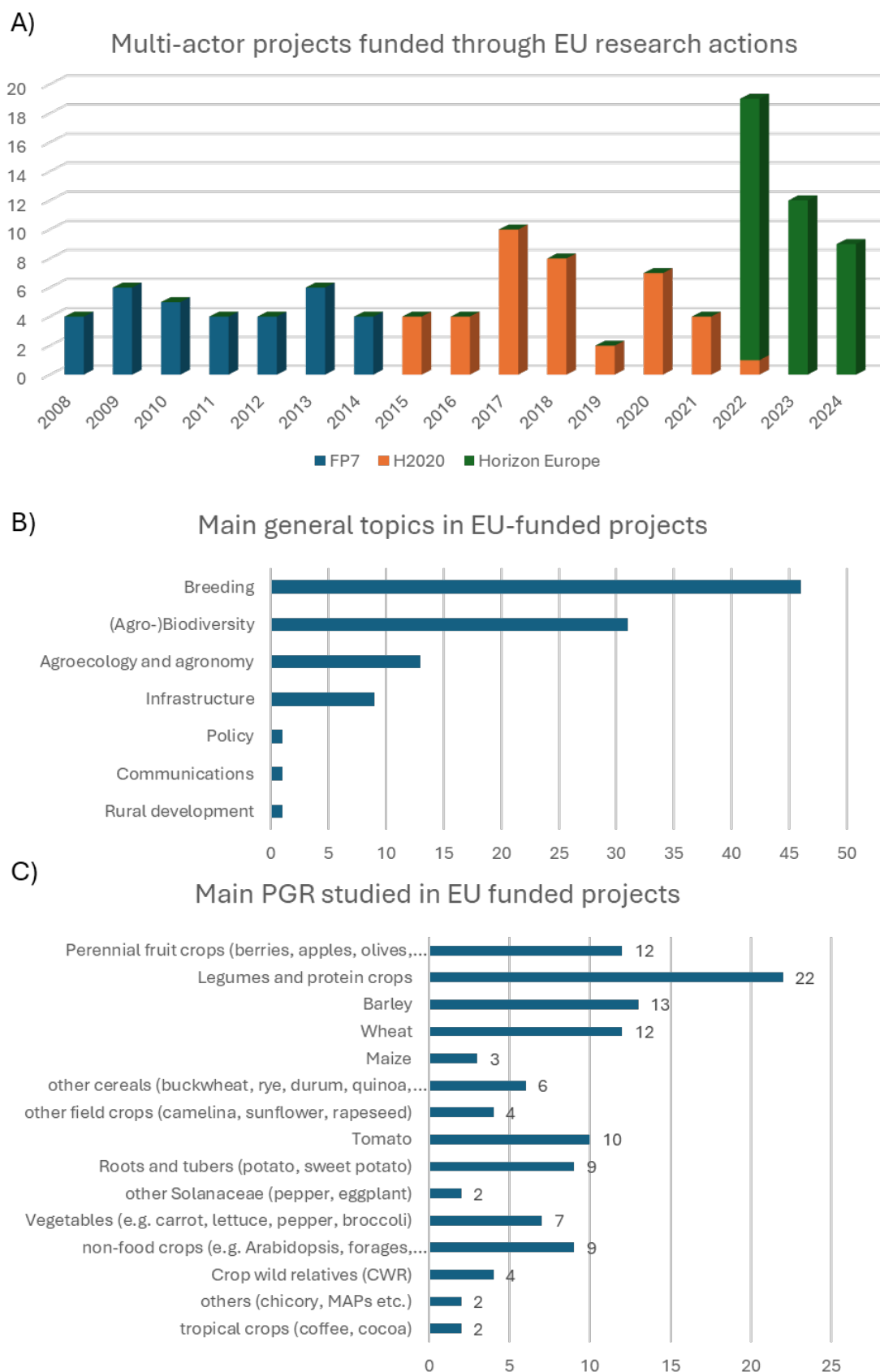
<sup>4</sup> WUR, Wageningen University & Research, The Netherlands

<sup>5</sup> IPK, Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung (Leibniz Institute of Plant Genetics and Crop Plant Research, Germany)





**Figure 1.** European plant genetic resources (PGR) collections documented in EURISCO (data from 27/01/2025). A) European holding institutes grouped by size of their collections documented in the EURISCO catalogue. In total, 432 institutes have deposited passport data for a total of 2,101,833 PGR conserved accessions in EURISCO as of that date. B) Distribution of main crop genera holdings among European holding institutes, with collections with more than 100 (>100) and 1,000 (>1,000) accessions indicated. The most represented genus *Arabidopsis* is mainly conserved at the Nottingham Stock Centre and not included in this analysis. C) Percentage of safety duplication of *ex situ* national inventories. D) Distribution of documented accessions according to biological status, with associated number of represented genera. Numbers for biological status 1xx and 4xx include several subclasses, which were combined for ease of viewing (Alercia *et al.*, 2015).



**Figure 2.** EU-funded multi-actor projects on plant genetic resources since 2008. A) Number of projects on biodiversity and plant genetic resources funded through European funding schemes 7<sup>th</sup> Framework Programme (FP7), Horizon 2020 (H2020) and Horizon Europe since 2008, listed by starting date. Data extracted on 09/10/2024, hence additional projects may still start in 2024. B) Main general topics and C) major crops studied in these projects.

**Table 1.** Participation of 76 EURISCO institutes from different countries in multi-actor projects on biodiversity and plant genetic resources funded by the European funding schemes 7<sup>th</sup> Framework Programme (FP7), Horizon 2020 (H2020) and Horizon Europe between 2008 and 2024. Details are included in [Supplemental Table 4](#).

Country	# Institutes	Participation in EU projects			Total
		FP7	H2020	Horizon Europe	
Austria	2	1	5	0	6
Belgium	4	5	7	11	23
Bosnia and Herzegovina	2	0	1	1	2
Bulgaria	1	0	1	1	2
Croatia	1	0	0	1	1
Czechia	2	0	3	2	5
France	9	15	25	15	55
Germany	4	7	10	7	24
Hungary	1	0	0	1	1
Israel	3	1	7	1	9
Italy	11	6	22	14	42
Latvia	1	1	1	0	2
Lithuania	1	0	1	1	2
Netherlands	1	10	16	8	34
Poland	4	2	6	0	8
Portugal	2	3	1	3	7
Romania	2	0	3	0	3
Russia	1	0	2	0	2
Serbia	2	1	4	5	10
Slovakia	1	0	2	0	2
Slovenia	2	0	3	4	7
Spain	9	5	22	12	39
Sweden	1	0	2	1	3
Switzerland	2	5	10	10	25
United Kingdom	7	14	12	12	38

the contributions of genebank collections in terms of the numbers of accessions provided and conserved for projects.

## Examples of successful cooperations of genebanks in international projects

### AGENT

In the H2020 project Activated Genebank Network (AGENT, <https://agent-project.eu/>) running from 2020–2025, partners created a network of genebanks and bioinformatics institutes with the aim to improve genebank operations and data management through the development of standardized approaches to data curation and management, data integration and analysis pipelines. The goal was to apply the concept of ‘genebank genomics’ (Mascher *et al*, 2019) to accession management, whereby genomics data would facilitate the identification of potential duplicate accessions and enable tracking the identity of accessions through regeneration cycles for quality management. In combination with relevant phenotypic data, genomics data would also allow prediction and association analyses to identify useful germplasm for further research and breeding.

During the project, 12 genebank partners (11 of which are in EURISCO) created precision collections (with an emphasis on unique material from the individual genebanks) and bridging collections (potential duplicates between different European genebanks) of the target crops wheat and barley. In total, 6,956 wheat and 5,315 barley accessions were genotyped and phenotyped for agronomic traits by the holding institutes. Additional trials for biotic and abiotic stress traits on subsets of the collections complemented the phenotyping and provided data for association analyses.

Furthermore, genebanks digitized historical data collected during regenerations of their collections, in some cases going back almost 80 years. These historical datasets have proven useful for predicting performance in view of various stresses (Gonzalez *et al*, 2021).



**Table 2.** EURISCO institute involvement and plant genetic resource (PGR) provision to European projects. Information represents survey responses received from Genebank managers. #N/A, not applicable.

WIEWS code	Institute, location	Involved in European projects	Provided PGR to projects	Conserved PGR derived from projects
BGR001	Institute for Plant Genetic Resources 'K.Malkov', Sadovo, Plovdiv district, Bulgaria	PRO-GRACE, AGENT, EVA	AGENT, EVA	AGENT
CHE001	Agroscope Changins, Nyon, Switzerland	AGENT, EVA	AGENT, EVA	#N/A
CHE063	ProSpecieRara, Basel, Switzerland	GenResBridge, BRESOV, Farmers Pride, Diversifood, PRO-GRACE	BRESOV	BRESOV
CZE122	Gene bank, Prague 6 - Ruzyne, Czech Republic	AGENT, ECOBREED, BRESOV, PRO-GRACE, EVA	AGENT, ECOBREED, BRESOV, EVA	AGENT, ECOBREED
DEU146, DEU159, DEU271	Genebank, Leibniz Institute of Plant Genetics and Crop Plant Research, Germany	EUCLEG, Farmer's Pride, GenRes Bridge, G2P-SOL, AGENT, INCREASE, PRO-GRACE, COUSIN, EVA, Legume Generation	EUCLEG, AGENT, INCREASE, EVA, Legume Generation	#N/A
ESP004	Centro Nacional de Recursos Fitogenéticos, Alcalá de Henares. Madrid, Spain	INCREASE, AGENT PRO GRACE, EVA	INCREASE, AGENT, EVA	AGENT
ESP009	Consejo Superior de Investigaciones Científicas. Misión Biológica de Galicia, Pontevedra, Spain	PRO-GRACE, EVA	EVA, MineLandDiv (SUSCROP-ERAnet 2022), Dromamed (PRIMA)	#N/A
ESP026	Generalidad Valenciana. Universidad Politécnica de Valencia. Escuela Técnica Superior de Ingenieros Agrónomos. Banco de Germoplasma, Valencia, Spain	TRADITOM, BRESOV, Farmers' Pride, G2P-SOL, HARNESSTOM, PRO-GRACE	TRADITOM, BRESOV, G2P-SOL, HARNESSTOM	#N/A
ESP032	Principado de Asturias. Servicio Regional de Investigación y Desarrollo Agroalimentario, Villaviciosa, Spain	BRESOV, INCREASE, LegumeGeneration	BRESOV, INCREASE, LegumeGeneration	BRESOV, INCREASE, LegumeGeneration
FRA010	Institut de Génétique Environnement et Protection des Plantes, Plant Biology and Breeding, INRAE Ploudaniel, France	G2P-SOL, BrasExplor, Nem-Emerge, Pro-Wild	G2P-SOL; Nem-Emerge; Pro-Wild	BrasExplor

*Continued on next page*

Table 2 continued

WIEWS code	Institute, location	Involved in European projects	Provided PGR to projects	Conserved PGR derived from projects
FRA065	Plant Biology and Breeding, INRAE Versailles, France	G2P-SOL, TRADITOM, GenRes Bridge, HARNESSTOM, AGENT, INVITE, GenBeCon, PROWILD, NEM-EMERGE, InnOBreed, PJI-FACCE GrassLandscape, PRIMA BrasExplor, PRIMA FREECLIMB, PRIMA DROMAMED, SusCrop ERANET MineLandDiv	All projects except GenRes Bridge, AGENT, INVITE	BrasExplor, InnOBreed; Harnesstom
FRA250	Institut Agro Rennes-Angers IRHS, Angers, France	OPTIMA, EVA	OPTIMA, EVA	#N/A
GBR004	Millennium Seed Bank - Royal Botanic Gardens Kew	PRO-GRACE, AGENT	#N/A	#N/A
GBR016	Genetic Resources Unit, Institute of Biological, Environmental & Rural Sciences, Aberystwyth University, United Kingdom	EUCLEG, CropDiva, Legume Generation, Legendary, EVA	EUCLEG, Legume Generation	#N/A
GBR140	Nottingham Arabidopsis Stock Centre, Loughborough Nottingham, United Kingdom	PRO-GRACE, PGR Secure	Any project that has requested them	<i>Arabidopsis</i> lines from various projects
GBR247	Germplasm Resources Unit, John Innes Centre, Norwich, United Kingdom	LegumeGeneration, ProWild, EVA	Legato, LegumeGeneration, ProWild, EVA	Gediflux, LEGATO
GBR251	The James Hutton Institute, Dundee, Scotland, United Kingdom	G2P-SOL, PRO-GRACE, EVA	G2P-SOL	G2P-SOL
HRV041	Faculty of Agriculture, University of Zagreb, Croatia	BELIS, GEroNIMO, GRASS Ceiling, PASTINNOVA, RESBIOS, TODO, PRO-GRACE, Strenght2Food, Farmers Pride, TREASURE, MendTheGap, EVA	EVA	#N/A
HUN003	Centre for Plant Diversity, Tápiószele, Hungary	PRO GRACE	#N/A	#N/A
ISR001	Department of Field and Vegetable Crops, Hebrew University of Jerusalem, Rehovot, Israel	G2P-SOL, EVA	G2P-SOL	G2P-SOL
ISR002	Israel Gene Bank for Agricultural Crops, Agricultural Research Organisation, Volcani Center, Bet Dagan, Israel	G2P-SOL	G2P-SOL	G2P-SOL

Continued on next page

Table 2 continued

WIEWS code	Institute, location	Involved in European projects	Provided PGR to projects	Conserved PGR derived from projects
ITA331	Facolta di Agraria, Università degli Studi di Catania, Italy	BRESOV, COUSIN	BRESOV, COUSIN	BRESOV
ITA363	Dipartimento di Chimica, Biologia e Biotecnologie, Università degli Studi Perugia, Italy	Farmers Pride, PRO-WILD	#N/A	#N/A
ITA382	CREA-Centro di Ricerca Genomica e Bioinformatica - Sede di Fiorenzuola d'Arda, Italy	NEURICE, BEST-CROP, GP2-SOL, PRO-WILD, PRO-GRACE, EVA	GP2-SOL, PRO-WILD, EVA	NEURICE, BEST-CROP, GP2-SOL, PRO-WILD
ITA383	CREA-Centro di Ricerca Cerealicoltura e Colture Industriali - Sede di Vercelli, Italy	AGENT, PRO-GRACE, EVA	AGENT, EVA	AGENT
ITA391	CREA-Centro di Ricerca Orticoltura e Florovivaismo - Sede di Pontecagnano, Italy	G2P-SOL, PRO-GRACE, EVA	G2P-SOL, EVA	G2P-SOL
ITA394	CREA-Centro di Ricerca Zootecnia e Acquacoltura - Sede di Lodi, Italy	BELIS, DIVINFOOD, INTERCROPVALUES, LIVESEEDING, INVITE, LIVESEED, REMIX, EVA	EVA	#N/A
ITA436	Istituto di Bioscienze e Biorisorse, Consiglio Nazionale delle Ricerche, Bari, Italy	PRO-GRACE, EVA	EVA	#N/A
LVA024	Institute of Agricultural Resources and Economics, Riga, Latvia	LIVESEED, EVA	EVA	#N/A
NLD037	Centre for Genetic Resources, the Netherlands, Wageningen, Netherlands	PRO-GRACE, G2P-SOL, AGENT, PrepActGR, Framers Pride, GenRes Bridge, EVA	AGENT, G2P-SOL, EVA	#N/A
POL003	Plant Breeding and Acclimatization Institute, Blonie, Poland	AGENT, INCREASE, RustWatch, G2P-SOL, ECOBREED, DETECTIV	INCREASE, AGENT, G2P-SOL	AGENT, INCREASE, G2P-SOL
PRT001	Banco Português de Germoplasma Vegetal, Braga, Portugal	Farmer 's Pride; LIVESEED, PRO-GRACE, EVA	LIVESEED, EVA	LIVESEED
SRB062	Institut for forage crops Krusevac, Krusevac, Serbia	EUCLEG, BELIS, EVA	EUCLEG, BELIS	#N/A
SVK001	National Agricultural and Food Centre (NPPC), Research Institute of Plant Production (RIPP), Piestany, Slovakia	RustWatch, Ecobreed, AGENT, EVA	Ecobreed, AGENT, EVA	Ecobreed
SVN019	Crops and Seed Production Department, Agricultural Institute of Slovenia, Ljubljana, Slovenia	Ecobreed, MedVitis, BrasExplor, Diversilience, INCREASE, Liveseeding, Root2Res, EVA	Ecobreed, MedVitis, BrasExplor, Diversilience	#N/A



To manage the data, the bioinformatics partners involved in the project (IPK, INRAE, WUR, RBGK<sup>6</sup>) created a range of tools, starting with standard data collection templates, an online data curation tool, a web application, REST interfaces using the Breeding API specification (Selby *et al.*, 2019) and various data analysis pipelines, which allow the users (primarily genebank data managers) to effectively manage and exploit the data on their collections (Beier *et al.*, 2022; Berkner *et al.*, 2022, 2024; El-Hanafi *et al.*, 2023). Several in-project training events and datathons were an opportunity for capacity building and four rounds of genebank peer reviews following the ECPGR AQUAS protocol have provided useful feedback on genebank operations and identified gaps and areas for improvements (<https://www.ecpgr.org/aegis/aquas/peer-visits>).

### European Evaluation Network (EVA)

Some of the tools developed by AGENT build on project outputs of the ECPGR initiative European Evaluation Network for PGR (EVA, <https://www.ecpgr.org/eva>). This network brings together genebanks, public research institutes, private sector breeders and farmers' organizations in public–private partnerships to jointly evaluate genebank accessions for relevant traits in multilocation trials across Europe. Activities are typically provided in-kind and are distributed among partners according to their expertise and capacity. These activities include provision of accessions, regeneration of material (where necessary as single-seed descent lines or crosses), evaluation in the field, lab or greenhouse, genotyping, data curation and data analysis. ECPGR funds, so far provided by the German Ministry for Food and Agriculture, have been essential to kick-start activities that would be difficult to carry out as in-kind inputs, especially seed multiplication or genotyping. Participation of breeding companies was incentivized by the agreement to enforce a 3-year embargo on the publication of evaluation data outside the consortium. The crop-specific networks have been operating since 2019 and currently have more than 120 partners including around 50 private breeding companies working on 15 crops. So far, the networks have generated phenotypic data on more than 5,000 accessions in almost 400 trials, where they have so far collected more than half a million datapoints on more than 200 different traits (Table 3).

The use of standard phenotyping protocols, along with standardized metadata and phenotypic data collection templates, was imperative for the integration of data collected by different partners in more than 100 locations. The EURISCO-EVA Information System, a project-internal platform, developed using the same framework as the public EURISCO catalogue, enabled effective data management and analysis (Kumar *et al.*, 2024) and provided the template for the AGENT

database structure. Within their collaborative networks, EVA partners have developed a new tool for the genotyping of lettuce (Tripodi *et al.*, 2023) and have investigated the genetic diversity of European carrot and maize accessions (Goritschnig *et al.*, 2023; Balconi *et al.*, 2024), generating valuable knowledge for the genebanks and research and breeding communities. The plant material exchange within the networks is governed by the rules of the ITPGRFA Multilateral System (FAO, 2009), using their Standard Material Transfer Agreement (SMTA) also for crops not included in Annex 1, thus facilitating access to PGRFA for further research and development activities by all partners.

### G2P-SOL

The Horizon 2020 project G2P-SOL ([www.g2p-sol.eu](http://www.g2p-sol.eu)) was active from 2016 to 2021 and involved 19 partners (including 13 genebanks of which nine are listed in EURISCO) as well as 12 external collaborating institutions from three continents. G2P-SOL focused on four major Solanaceae crops (tomato, potato, eggplant, pepper), for which the partner genebanks held around 65,000 accessions, of which around 14,000 CWR (Table 4).

The project consisted in five phases: 1) inventory of the partner genebanks, in which the images and passport data from the different genebank information systems were collected, manually curated and published, 2) genotyping of a subset of approx. 40,000 accessions with 2,000–10,000 high-quality SNPs and analyses of population structure, duplicates and taxonomic classification (Toppino *et al.*, 2021), 3) establishment of core collections of around 350–400 accessions (both cultivated and wild) for each crop, representing the worldwide genetic and phenotypic diversity of each genepool (e.g. Nankar *et al.* (2020)), 4) phenotyping of the core collections in multiple locations for resistance to biotic and abiotic stresses, agronomic, and (limited to the three fruit crops) metabolic traits (Gramazio *et al.*, 2020), and 5) pre-breeding, in which markers for described and novel traits from CWRs were established and the traits pre-bred into the genetic background of each cultivated species (Stefa' *et al.*, 2020). G2P-SOL has been classified as a success story by the European Commission, and its core collections are available under SMTA and with phytosanitary certificates from partner genebanks acting as distribution points.

## Discussion

### Benefits of international cooperation in PGR research

In the three projects highlighted above, partners described the possibility of engaging in active networks and exchanging pre-competitive ideas and knowledge as primary benefits of participation. Novel traits for a more sustainable agriculture have been identified and some partners have already applied approaches and

<sup>6</sup> RBGK, Royal Botanic Gardens, Kew, United Kingdom

**Table 3.** Summary of partnership and outputs of five crop-specific ECPGR European Evaluation Network EVA (data as of 30 June 2024).

	All Networks	EVA Wheat Barley	EVA Carrot	EVA Lettuce	EVA Pepper	EVA Maize
Crops	8	3	1	2	1	1
Accessions	5,092	3,608	67	367	183	867
Partner institutes	89	47	14	12	15	18
Countries of operation	33	25	8	8	13	9
Experiment locations	119	58	14	6	11	30
Traits	285	46	138	24	26	51
Trials with available data (2020–2024)	384	265	27	14	15	63
Phenotypic datapoints available	539,678	318,891	95,695	10,717	24,016	90,359

**Table 4.** Summary of Solanaceae accessions included in the G2P-SOL collection by participating genebanks. AVRDC, the World Vegetable Center, Taiwan (TWN); IPK, Leibniz Institute of Plant Genetics and Crop Plant Research, Germany (DEU); HUJI, The Hebrew University of Jerusalem, Israel (ISR); INRAE, Institut national de recherche pour l’agriculture, l’alimentation et l’environnement, France (FRA); CIP, International Potato Center, Peru (PER); WUR, Wageningen University & Research, the Netherlands (NLD); UPV, Universitat politècnica de Valencia, Spain (ESP); JHI, James Hutton Institute, United Kingdom (GBR).

Partner Country	AVRDC TWN	IPK DEU	HUJI ISR	INRAE FRA	CIP PER	WUR NLD	UPV ESP	JHI GBR	All others	Total
Tomato	8,260	3,840	8,100	1,600	0	1,332	2,220	0	390	25,352
Of which wild	812	26	100	200	0	108	220	0	50	1,466
Potato	0	6,020	0	1,500	6,000	1,446	0	2,300	390	17,266
Of which wild	0	2,845	0	500	1,800	1,243	0	1,400	300	7,788
Pepper	8,235	1,530	0	1,460	0	1,010	1,400	0	850	13,635
Of which wild	464	63	0	46	0	783	80	0	56	1,436
Eggplant	3,713	110	0	2,015	0	510	260	0	280	6,608
Of which wild	1,499	0	0	1,120	0	373	70	0	40	3,062
All four crops	20,208	11,500	8,100	6,575	6,000	4,298	3,880	2,300	1,910	62,861
Of which wild	2,775	2,934	100	1,866	1,800	2,507	370	1,400	446	13,752

tools developed by these projects to other species and in other projects. Early career researchers in the AGENT and G2P-SOL projects highlighted the opportunity to learn diverse aspects ranging from fieldwork to data curation and analysis, which was facilitated by mentors from different project partners. In general, the standardization approaches followed by both AGENT and EVA are prime examples of how access to data can be facilitated according to FAIR (Findable, Accessible, Interoperable, Reusable) principles (Wilkinson et al, 2016), providing pipelines for data curation and management that will allow their reuse in future investigations. However, while these projects have shown a possible new way of working for genebank data management and collaboration, its long-term adoption and implementation involving the majority of European genebanks and covering more crops would require significant investment and political commitment to provide the necessary framework. At present, the ECPGR Secretariat is best positioned to disseminate standards and methodologies for genebank operations and crop evaluations throughout their European networks.

An assessment of the long-term impact of EU-funded research projects overall is difficult to produce but should consider the availability of generated materials,

tools and project data for further research and the level at which project outputs have been exploited after the end of the projects. A well-known issue with project data is that they are often stored in fragmented form, and websites or databases are only maintained for a limited period after projects end. All these considerations call for the need to operate within a more coherent framework that facilitates and promotes genetic resources conservation, documentation and sustainable use, as the *Plant Genetic Resources Strategy for Europe* (ECPGR, 2021) is advocating and a suitable EU Research Infrastructure might implement.

Our preliminary survey of genebanks involved in EU-funded Horizon projects showed that some genebanks are very active in participating in European research, contributing both materials and relevant expertise in conservation, cultivation, breeding and documentation. On the other hand, a significant number of genebanks with relatively large collections do not participate in many European projects, limiting the use of these collections in international projects and the benefits to organizations from knowledge exchange. In addition, much of the funded research has been focusing on a few staple crops, e.g. cereals, legumes, Solanaceae and Brassicaceae, with minor crops only recently

receiving more attention (Figure 2, Supplemental Tables 1–3). The involvement of genebanks in crop improvement, especially in research and pre-breeding projects, should also ensure that the final products (in terms of germplasm materials) of these projects are either fully incorporated into their collections or otherwise made available for further distribution and exploitation (Hanson *et al*, 2024). It should be noted that inclusion in a genebank collection implies conservation in perpetuity. For some material, such as breeding or research material, this might not always be the proper approach and novel solutions to making project materials available for future use will be needed. Follow-up studies are also needed on the effective use of PGR in breeding and to assess the impact of publicly funded research on PGR. The survey analyzed in this paper highlights the importance of PGR use in a collaborative research framework at both the regional and international levels. This is believed to be the most promising in terms of exploitation of the immense public good that is maintained by genebanks, often with insufficient public funds but with a significant potential economic value (Gollin, 2020). Considering the growing threat of genetic erosion resulting from the changing climate and changing habitats it will also be important to mainstream *in situ* conservation activities coupled with the assurance that populations of CWR and wild food plants are made available for use in breeding and research (Khoury *et al*, 2022).

The experiences from the collaborative projects described in this paper, as well as reports from other successful projects at the European level, highlight the benefits to be gained from international cooperation of genetic resources institutes to increase our knowledge on PGR and make them accessible for further research and breeding. Collaborative activities in PGR research and breeding, through the implementation of public–private partnerships and multi-actor projects are imperative to realizing the potential of European PGR in strengthening the bioeconomy along the whole agri-food value chain (Vangheluwe *et al*, 2023). Tools and partnership models have been developed, but they may remain ineffective without a permanent coordinated approach and support for the long-term exploitation of project outputs. Similarly, a variety of technical challenges in information management as well as political and institutional considerations for access to PGR will need to be addressed in novel cooperative approaches to enhance innovation in PGR research (Halewood *et al*, 2018). The *Plant Genetic Resources Strategy for Europe* (ECPGR, 2021) identified the need for long-term European cooperation, which could be realized through the establishment of a European Research Infrastructure fully dedicated to PGR, currently in the concept development phase through the project PRO-GRACE (<https://www.grace-ri.eu/pro-grace>).

## A European Research Infrastructure to facilitate consolidated research on PGR

The Horizon Europe project PRO-GRACE involves genebanks, research institutes, and non-governmental and stakeholder organizations in developing a framework for collaboration at the European level to assure availability and access to PGR for breeding and research. Within this project, standards have been proposed to provide guidance on PGR documentation, enabling effective linkage between conserved accessions, associated Multicrop Passport Descriptors (Alercia *et al*, 2015), and associated phenotypic and genotypic data. Surveys have established a baseline of full implementation of quality management systems (QMS) in genebanks and informed deliverables developing recommendations for implementation of QMS in genebanks and the establishment of a Genebank Certification System, ensuring reliable conservation of and access to PGR conserved in genebanks (Van Hintum and Wijker, 2024). Gap analyses and surveys have identified a clear lack of consolidated PGR research infrastructures within Europe and provided evidence for a need to establish a Europe-wide distributed research infrastructure that would effectively ensure high-quality conservation of PGR, as well as develop state-of-the-art methods, tools and services for their use in research, breeding and cultivation. This approach represents a great opportunity to further develop EURISCO into a FAIR-compliant European PGR information system that is more closely linked to regional and global platforms and integrates previously untapped data from additional sources and further data domains. The concept for a future European Research Infrastructure on PGR (GRACE-RI) covers important aspects which support the implementation of the *Plant Genetic Resources Strategy for Europe* and align with needs of stakeholders from conservation and use: 1) enhance all PGR collections across Europe to accepted standards through implementation of quality management and upgrade of genebank procedures, 2) improve and consolidate PGR documentation through advances in data integration and management, 3) develop multi-omics and phytosanitary technologies for PGR characterization, enhancing their availability for breeding, 4) assure access to all PGR across Europe through supporting genebanks in meeting legal and phytosanitary requirements, 5) mainstream *in situ* conservation of PGR, particularly of CWR, to facilitate their availability for research and breeding, and 6) develop scientific services destined to the scientific and breeding communities, as well as programmes for capacity building, training and education to achieve the above-mentioned objectives. Participating countries and institutes will thus create a relevant and well-defined service for the user community, with a significant reduction of redundancies and increased efficiency through the pooling of capacities and expertise. In contrast, continued inaction in this field would mean surrendering to the loss of agricultural and general plant biodiversity which is already threatening our agriculture and ecosystems.

## Conclusion and outlook

Proper management of PGR is becoming increasingly important due to the growing demand for access to PGR for breeding of new varieties and thanks to better breeding techniques using genebank accessions as raw materials. At the same time, the need to conserve is expanding due to climate change and increasing genetic erosion. Against this backdrop, international research projects have provided genetic resource centres with the possibility to participate in collaborations, improving their capacity, gaining knowledge about their collections and thus adding value to their holdings. The creation of a dedicated Research Infrastructure for PGR, as proposed by PRO-GRACE, will allow the European conservation, research and breeding communities working on PGR to better organize and monitor their activities, reduce redundancies, improve processes and conservation, develop novel methods, with the aim to ensure continued access to and utilization of high-quality PGR materials and related information. This will offer current and future researchers access to PGR and methods for their study and valorization, which are necessary for addressing the main challenges of the present time and ensuring a sustainable and biodiverse agriculture in Europe in the future. Providing a stable political and financial framework for international research collaboration on PGR will enable Europe to firmly establish a primary role in developing science-based solutions to the challenges of the twenty-first century.

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## Supplemental data

**Supplemental Table 1.** EU-funded multi-actor projects on PGR within the H2020 funding scheme (active 2015–2027).

**Supplemental Table 2:** EU-funded multi-actor projects on PGR within the Horizon Europe funding scheme, which have started by mid-2024.

**Supplemental Table 3:** EU-funded multi-actor projects on PGR within the FP7 funding scheme (2008–2018).

**Supplemental Table 4:** European genebanks participating in Horizon multi-actor projects funded through the European funding schemes 7th Framework Programme (FP7), Horizon 2020 (H2020) and Horizon Europe since 2008.

## Conflict of interest

The authors declare no conflict of interest.

## Author contributions

SG, SW, NS, GG and LM developed the concept of the paper, SG wrote the first draft and analyzed the data. All authors commented on the paper and contributed to the writing.

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# The French INRAE Biological Resource Center for pome fruits and roses: Plant and DNA collections of traditional and research genetic resources

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**Abstract:** The Biological Resource Center (BRC) 'Pome fruits and roses' is hosted by INRAE near Angers, France. It includes collections of apple, pear, quince and rose. The apple, pear and quince collections are preserved in orchards on site and as DNA samples. Research accessions of the *Rosa* genus are preserved both in the field and as DNA samples, whereas traditional accessions are primarily maintained as DNA samples. Traditional roses accessions are preserved in the field by different private and public rose gardens in France.

The main BRC's mission is to preserve these biological resources while improving and optimizing its collections. It also aims to gather and enrich data associated with the conserved genetic accessions, in particular by recording and centralizing phenotypic and genetic characterization data. This ensures the provision of suitable biological material and data for research and selection purposes. Additionally, these data support genetic resources management, especially in the framework of the apple and pear French networks coordinated by BRC. Thanks to these data, pedigrees could be unravelled and association studies implemented.

**Keywords:** genetic resources, Maloideae, *Rosa*, diversity, genebank, DNA

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## Introduction

The French National Research Institute for Agriculture, Food and the Environment (INRAE) has historically hosted collections of traditional pome fruit genetic resources in Angers, France. Cultivars of apple (*Malus* Mill. spp.), pear (*Pyrus* L. spp.) and quince (*Cydonia*

Tourn. ex Mill. spp.) were mainly introduced in the collections in the 1950s and 1960s from French nurseries, horticultural societies and botanical gardens. Botanical species were also introduced at this time from various European botanical gardens. Most of the accessions of cider apple, perry pear, apple and pear rootstocks came from field surveys carried out in the same years. In addition, the collections have been continuously enriched for breeding and for distinctness,

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uniformity and stability (DUS) testing activities, as well as for the conservation of heritage genetic resources. In particular, a duplication of old French varieties – identified as at risk of disappearing following a national genetic resources survey – was carried out in the 2000s.

The pome fruit collection of research genetic resources began to be built up in the 1980s with the creation of haploids, dihaploids and aneuploids. This collection was then considerably enriched since the 2000s thanks to the genetic mapping progenies that have served as references in a series of European projects.

The rose (*Rosa* L. spp.) collection was launched in 2003 when the *Rosa* genus was chosen as a model ornamental plant due to its economic importance and biological characteristics among ornamental woody plants. Since then, research has focused on understanding the key processes involved in quality development in ornamental plants such as plant architecture, flowering and disease resistance. This required the introduction and creation of original research genetic material, mainly progenies for genetic mapping. Due to their high importance in research, these mapping progenies have been preserved for further studies. These genetic resources are now being used to support national and international scientific collaborations. In addition, a DNA bank of wild individuals of *Rosa gallica* L. and traditional varieties conserved in rose gardens was established to study the evolution of the genetic variability in the *Rosa* genus.

The INRAE ‘Pome fruits and roses’ Biological Resources Centre (*RosePom* BRC) that gathers these collections is now hosted by the Research Institute on Horticulture and Seeds and the Horticultural Experimental Unit.

### Collection description

For clarity and simplicity, accessions conserved in *RosePom* are classified into two categories: traditional genetic resources and breeding/research genetic resources. These terms are defined in accordance with the classification proposed by the Multi-Crop Passport Descriptors (MCPD V.2.1) (Alercia et al, 2015) for the biological status of accessions. Traditional genetic resources include: (1) wild relative species (code 100), i.e. presumed wild specimens collected in a wild environment; (2) traditional cultivars (code 300), comprising heritage cultivars (French or foreign old cultivars of heritage interest), other old cultivars and botanical accessions of species (i.e. presumed wild specimens grown in botanical gardens, sometimes originating from seeds collected in other botanical gardens where some natural hybridization may have occurred between different conserved accessions, including old varieties); and (3) advanced or improved cultivars (code 500), which are cultivars obtained after 1945. Note that botanical accessions of species may or may not be declared of heritage interest, depending on the criteria used to define ‘heritage interest’. These criteria are currently under discussion in France. Breeding/research genetic resources

(code 400) include, among others, unnamed breeders’ material (codes 410 to 420), cytogenetic stocks (code 422), and mapping populations (code 423). Note that we prefer to use the term ‘mapping progenies’ rather than ‘mapping populations’, since a progeny is the result of a cross between two parents, whereas a population may consist of several unrelated individuals.

In summary, *RosePom*’s collections include traditional resources of agricultural, industrial, economic, scientific, social and cultural interest as well as research resources, much of which consist of material for genetic and genomic studies.

More specifically, *RosePom* conserves approximately 8,800 reproductive clonal accessions (accessions conserved as plants in the field with the capacity to participate in the reproduction process), mainly from three genera, *Malus*, *Pyrus* and *Rosa* (Figure 1), each represented by multiple species. This includes around 3,500 traditional accessions (cultivars, botanical accessions and wild relatives) and 5,300 breeding/research accessions (breeding unnamed progenitors, research accessions composed of cytogenetic stocks and mapping progenies). *RosePom* conserves also several thousand additional non-reproductive accessions, in the form of leaves or DNA only, particularly for the *Rosa* genus.

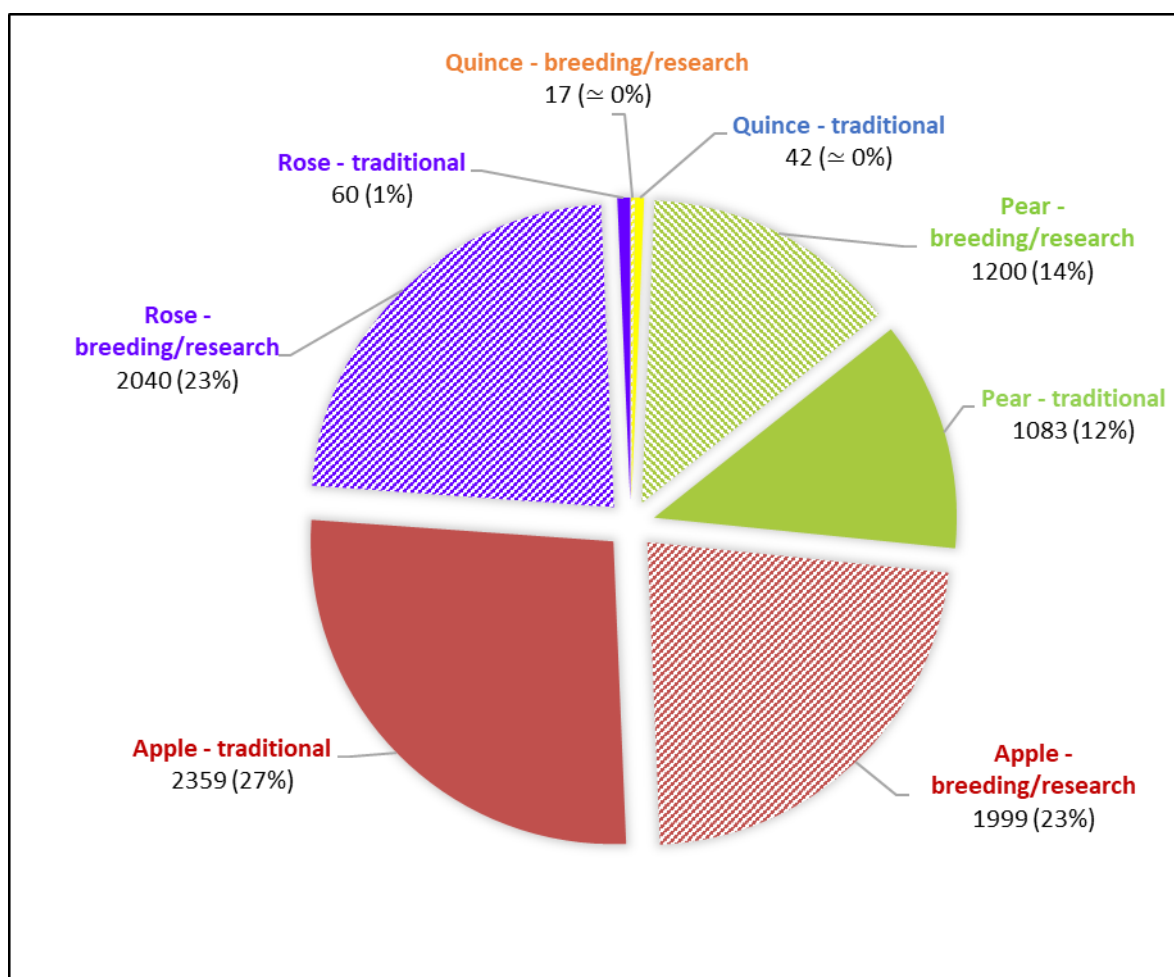
### Pome fruits

The pome fruits collection contains 4,300 apple, 2,300 pear and 60 quince accessions. These reproductive accessions have been introduced from different geographical origins and according to their use in fruit production, as rootstocks, for ornamental use or for research purposes. They therefore represent a large genetic diversity.

Traditional conserved genetic resources include: (1) wild accessions of relative species of *Malus*, *Pyrus*, *Cydonia* from all over the world including 47 taxa of western and eastern *Pyrus* species, (2) botanical accessions of *Malus*, *Pyrus*, *Cydonia*, (3) old or new cultivars of rootstocks of *Malus*, *Pyrus*, *Cydonia*, (4) old or new cultivars of dessert or cider apple (*Malus domestica* Borkh.), dessert European pear (*Pyrus communis* L.), perry pear (*Pyrus nivalis* Jacq.), nashi (*Pyrus pyrifolia* (Burm.f.) Nakai) and quince (*Cydonia oblonga* Mill.), and (5) old or new cultivars of ornamental apple and pear (various species and many interspecific cultivars). Among these, nearly 1,500 accessions are old cultivars from France (840 apple accessions, 640 pear accessions), old cultivars from other countries in Europe and some well-known old cultivars from America, Asia and Africa. In addition, a UPOV international reference collection of nearly 1,000 accessions of recent apple and pear cultivars complements the *RosePom* resources.

Resources originating from breeding and pre-breeding programmes include over 1,000 accessions of material collecting traits of agronomic interest, in particular, (1) for apple, resistance to scab (*Venturia inaequalis* (Cooke) Winter), fire blight (*Erwinia amylovora* (Bur-





**Figure 1.** Reproductive collections: species groups and types. Repartition of the number of accessions of rose, apple, pear and quince according to the type of resources (traditional or breeding/research).

rill) Winslow *et al.*) and powdery mildew (*Podosphaera leucotricha* (Ellis & Everhart) Salmon), fruit texture, (2) for pear, resistance to scab, fire blight and pear psylla (*Cacopsylla pyricola* (Förster), and (3) for rootstocks, dwarfing, resistance to fire blight and abiotic stresses. It also includes 16 intergeneric hybrids between *Pyrus*, *Malus* and *Cydonia*.

Resources originating from research programmes include original material, in particular 46 accessions of haploids and double haploids and 1,400 accessions of mapping progenies segregating for disease and pest resistance. For apple, the two most important mapping progenies conserved by *RosePom* were created by crossing genotypes exhibiting different quantitative trait loci architecture (identified thanks to previous not conserved mapping progenies) for apple scab resistance: (1) 'TN10-8' hybrid crossed by the cultivar 'Fiesta' (260 accessions), and (2) 'Discovery' crossed by 'TN10-8' hybrid (149 accessions). For pear, *RosePom* conserves in particular a mapping progeny segregating for resistance to pear psylla and obtained by crossing the interspecific *Pyrus bretschneideri* Rehd.  $\times$  *Pyrus communis* L. hybrid PEAR3, resistant to pear psylla, with the susceptible European pear cultivar 'Moonglow' (234 accessions).

From the point of view of a classification based on the type of use, among the accessions of cultivars and unnamed breeding material, there are 320 rootstock accessions (of which nearly 200 of *Malus*, 80 of *Pyrus* and 45 of *Cydonia*), 4,800 accessions of dessert or cider apple, dessert European pear, perry pear, nashi and dessert quince, and 120 accessions of ornamental apple and pear.

## Roses

The rose collection conserves traditional biological resources (mostly in a non-reproductive form) and research genetic resources (in reproductive and non-reproductive forms).

Traditional resources include wild roses, botanical roses and cultivated varieties. Three thousand seven hundred (3,700) wild specimens are mainly of the species *R. gallica*, collected from more than 80 locations spread over 54 municipalities in France, with in general, 8 individuals per plot, depending on the size of the plot. Three sites, near Toulouse, France, were sampled more densely. Around 1,500 *Rosa multiflora* Thunb. accessions were collected in semi-natural/sown (MCPD code 130) sites in France. Botanical roses and cultivars

were sampled from 2004 to 2018 in French rose gardens and the Sangerhausen Rosarium (Germany). Sampled cultivars were mostly bred during the 19th and 20th centuries. The high genetic diversity of these accessions enables research teams to assess the impact of evolutionary history, natural selection and human activity on the *Rosa* genus.

Research resources are made up of mapping progenies or accessions selected for their particular characteristics. More specifically, these genetic resources include three diploid pseudo-F1 progenies linked by the use of the same male parent, an accession, abbreviated as Rw, which is an interspecific hybrid of *Rosa wichurana* Crép., conserved in the *Jardin de Bagatelle* in Paris, France. The three female parents are: *Rosa chinensis* Jacq. ‘Old Blush’, introduced into the United Kingdom by James Colvill around 1795 as ‘Parsons’ Pink China’, the ‘Fairy’, Polyantha variety bred in the United Kingdom in 1932 by Ann and John Bentall, and ‘H190’, a dihaploid obtained by haploidization of tetraploid *Rosa* ‘MEIrilocra’, commercially known as ‘Zambra’ bred in 1980 by Marie-Louise Meilland (Meynet et al, 1994). The size of the three progenies is roughly 1,050, 100 and 590 offspring. These segregating progenies, whose parents have a contrasting phenotype for different morphological traits (recurrent blooming ability, flower duplication, shrub habit) or disease resistance, can be used to answer questions about the genetic determinism of traits of interest.

## Conservation

### Conservation types

The main infrastructures (Figure 2) used by *RosePom* for the conservation of accessions are fields (15.5ha, including 1.5ha protected by Alt’Carpo nets, nets designed to prevent the entry of codling moth), insect-proof tunnels (480m<sup>2</sup>), freezers at -20°C and three cryopreservation tanks (total volume of 1,400L). *RosePom* also has access to glasshouses, a container yard, *in vitro* culture laboratories, cold chambers and -80°C freezers.

Reproductive resources are conserved as plants in the field or in pots in insect-proof tunnels, as shoots in *in vitro* culture and as budwood in liquid nitrogen (cryopreservation). Non-reproductive material such as leaves or petals are generally freeze-dried and stored at room temperature or in a cold chamber; DNA extracts are stored in freezers at -20°C.

Depending on various criteria, including in particular the value of interest, the rarity of the biological material and the type of biological material to be conserved, the conservation method or combination of conservation methods is defined according to four categories: (1) high level of conservation (at least two specimens) using a combination of conservation systems with special care for the specimen, (2) high level of conservation (two specimens) with one type of conservation system and special care for the specimen, (3) low level of

conservation (one specimen) with special care for the specimen (i.e. propagation to be planned if the specimen is in danger of being lost), (4) low level of conservation (one specimen) without propagation even if the specimen is in danger of being lost.

### Pome fruits

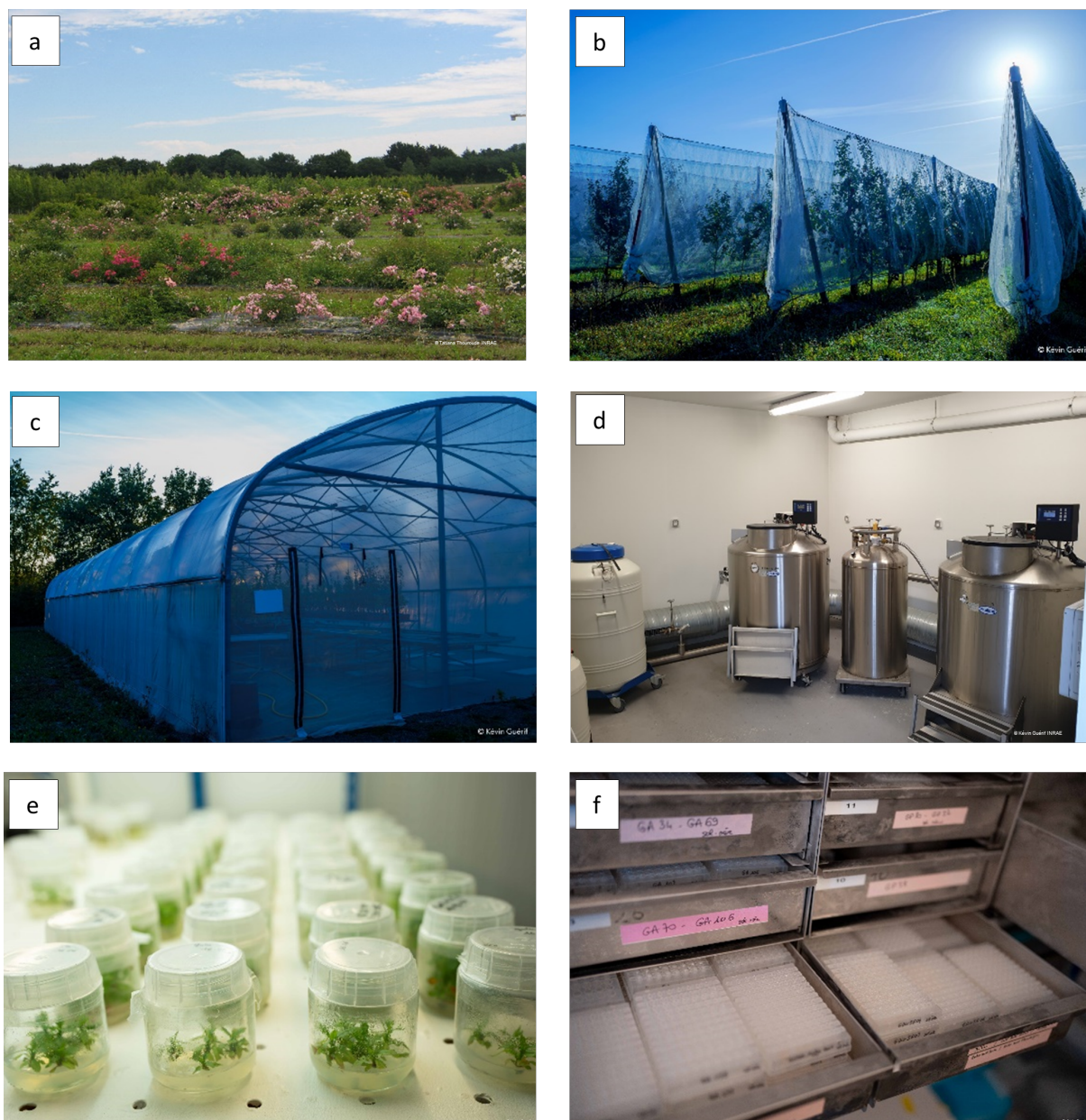
For pome fruits, the highest level of conservation is currently used for old French varieties, which are part of the French national heritage, with: (1) a specimen in an orchard protected by Alt’Carpo nets, used to protect trees from the insects that transmit fire blight (one of the main causes of apple and pear trees loss), (2) another specimen in an insect-proof tunnel, which provides protection from damage by pests and diseases transmitted by insects, such as pear decline (*Candidatus Phytoplasma pyri* Seemüller & Schneider) transmitted by pear psylla, and (3) ideally a specimen in cryopreservation. Cryopreservation is in progress for apples, though it remains a lengthy process requiring special care during the dehydration and freezing steps, which depend on the variety. For pears, the success rate is very genotype-dependant and is therefore not currently used. High-level conservation is also applied to specific apple and pear research resources, such as haploids and doubled haploids. These accessions, which tend to grow poorly in orchards, are maintained in *in vitro* culture chambers as well as in the field. Conversely, the lowest level of conservation is currently used for some progeny collections where the loss of some accessions is less detrimental to the overall collection.

In the orchard, trees are planted in the field with a distance of 4m between rows. Space between trees within rows ranges from 1.2m for those used only for conservation, to 2.5m between plants in the row for trees used both for conservation and other purposes (observation of the fruit and architecture of the trees, use as female parent in cross-breeding for research or pre-breeding, production of budwood).

Depending on the intended use beyond conservation, trees are managed with minimal pruning and without fruit thinning or according to industrial fruit production methods or pruned specifically to provide budwood.

Orchards are irrigated, except when planted in naturally wet soils, and trellised, except for trees pruned for budwood production. Trees are fertilized with organic fertilizers, except when the aim is precise fruit characterization – in this case, mineral fertilizers are applied to the soil. In all cases, mineral foliar fertilizers are applied. Trees are subject to preventive or curative phytosanitary treatment for diseases such as scab, powdery mildew and pests, in particular rosy aphid (*Dysaphis plantaginea* (Passerini)) and pear psylla, in accordance with sustainable agriculture standards.

Potted plants are grown in 3L pots for apples and 7.5L pots for pears and maintained in screened tunnels. Plants are spaced 25cm apart and heavily pruned to keep them small (about 1m high). Automatic watering in the tunnels is scheduled twice a day at fixed times, with frequency, amount and method varying according



**Figure 2.** Infrastructures used for the conservation of the accessions: a, field; b, orchards under Alt'Carpo nets; c, insect-proof tunnels; d, cryotanks; e, *in vitro* chamber for the conservation of living plant material; f, freezers at  $-20^{\circ}\text{C}$  for the conservation of non-reproductive material.

to the season and weather conditions. Phytosanitary treatments are applied when pests or diseases threaten the plant's survival.

In addition to conserving accessions in our facilities, the preservation of old French cultivars, is managed at the national level through a national network for pome fruits genetic resources. The aim is twofold: first, to ensure that each heritage cultivar is conserved in at least two locations by different institutions; and, second, to ensure that some heritage cultivars are not conserved in too many copies, in order to optimize human and material resources.

### Roses

For roses, shrubs are planted on agricultural tarpaulins in the field with 3.5m between rows and 1 or 1.5m

between plants in the row. The large space between rows was chosen to accommodate farm equipment used for pome fruits. Annual pruning is carried out when winter temperatures become stable, i.e. between late November and early January, followed by a copper treatment to protect the plant from fungal diseases.

Soil analysis is carried out to adjust the availability of nutrients to the needs of the plants. Fertilizers are applied to support the flowering period. A powdery mildew treatment in early spring and one or two aphicides per season are applied to these resources. Maintaining the edges of the agricultural tarpaulins and the base of the roses represents many hours of work. These plots are not irrigated.



Traditional resources are conserved at *RosePom* only in a non-reproductive form, such as leaves or DNA. The wild accessions are conserved as reproductive forms in wild habitats, which in France are under the aegis of national botanical conservatories. Traditional varieties are being preserved in rose gardens, which are owned by the public sector or by private enthusiasts.

## Health monitoring

For pome fruits, plant health monitoring is carried out throughout the year, but more intensively between April and October. It mainly involves visual inspection, but may also include analyses to confirm a diagnosis. The pathogens observed are mainly regulated organisms, including fire blight, European canker (*Neonectria ditissima* (Tulasne & C. Tulasne) Samuels & Rossman) and pear decline. For roses, field plants are regularly inspected for the absence of visible symptoms.

## Identity validation after propagation and authentication

For pome fruits, after each cycle of propagation of the accessions, each plant planted in the field or in pots for conservation is tested with a set of five microsatellite (SSR) markers to check that the molecular identity of the plant obtained after propagation corresponds to the original plant. In the case of mutants, a visual comparison of the tree and fruit of the original plant and the new plant is necessary. This process generally takes one year (if the SSR control is sufficient) to three or four years (if it is necessary to check the fruit). The original trees are kept until all these checks are made.

Collaborative projects at European and international levels have enabled the comparison of molecular profile varieties from different collections in Europe and the United States, leading to the assignment of a unique code to each variety. This work helped identify duplicates of a variety represented by different accessions under different names and to determine the most probable varietal name of an accession with the same profile. This does not replace the historical authentication of varieties (i.e. checking the accuracy of the given variety name) but improves the knowledge of the identity of the accessions (Durel et al, 2023).

For roses, new plants are tested after propagation, where necessary with SSR markers, to validate their conformity to the mother plant.

Authentication of old cultivars is based on historical data and the expertise of rose garden managers, but it is rarely fully certain.

## Accession description

Molecular and phenotypic characterization covers both traditional and breeding/research resources and is essential to ensure their trueness-to-type. To date, molecular characterization has mainly been carried out using SSR markers revealed by capillary electrophoresis or sequencing. Additionally, single nucleotide polymor-

phism (SNP) markers are now being used by research projects and revealed by high-throughput technologies. So far, 68% of pome fruit accessions and 41% of rose accessions have been genotyped.

*RosePom* carries out a part of the characterizations and also centralizes data generated by research teams using the *RosePom*'s genetic resources or supplying *RosePom* with new resources and associated data.

For pome fruits, *RosePom* uses a minimum list of descriptors for the management of pome fruit collections, including phenotypic characteristics (such as flowering time and harvest time) and simple morphological characteristics (such as fruit colour, shape and size, and tree habit). Characterization is based on the European Cooperative Programme for Plant Genetic Resources (ECPGR) reference documents (Lateur et al, 2022a,b) and the UPOV Guidelines for the distinctness, uniformity and stability of apple (UPOV, 2023) and pear (UPOV, 2000). Phenotypic characterization is carried out for certain characteristics (disease resistance, flowering, architecture, fruit quality, etc.) in the field, in the greenhouse or the laboratory.

For roses, the first characteristics used to help verify the identity of an accession are the colour of the flower, the number of petals and the plant's habit. Many other traits of morphological, disease susceptibility or biochemical nature, for example, can be phenotyped through research projects. The data obtained is then centralized by the *RosePom* information system.

## Distribution

This material is regularly used in research and breeding programmes and is also distributed to third-party organizations and the international scientific community. Around 1,000 to 1,500 accessions are distributed per year mainly through collaborative projects. Depending on the availability of material, *RosePom* will distribute, in accordance with national and international regulations in force: (1) plant material in the form of plants or samples (grafts, cutting, pollen, leaves, petals, fruits, etc.), (2) extracted plant material such as DNA, and (3) data associated with this plant material, such as phenotypic or molecular data. The distribution service is available to public or private research bodies, institutes or breeders mainly in the frame of cooperative projects. It is also available to public or private actors in biodiversity conservation (conservatories managed by amateur associations or local authorities, nurseries specialized in the sale of old cultivars).

*RosePom* offers the opportunity to obtain resources on request by emailing [crb-rosepom-contact@inrae.fr](mailto:crb-rosepom-contact@inrae.fr). Requests must be made in advance, considering legal and administrative constraints and the nature of the material requested. Distributions are made after the signature of a contract between INRAE and the recipient, with different requirements for the use of the resource, depending on the nature of the resource and the use



planned by the recipient. *RosePom* does not guarantee the availability or quantity of requested material.

### Quality management systems

*RosePom*'s quality management system has been ISO 9001:2015 certified since February 2023. The certification covers the organization of activities related to the acquisition, conservation, characterization and distribution of *RosePom*'s genetic resources and associated data. The aim is to effectively meet the needs of its users. It ensures the traceability of living plant material, non-reproductive plant material and associated data.

The orchard is managed in accordance with the ISO 14001 environmental management system and the French 'Vergers Ecoresponsables' label.

### Use of the resources

#### In breeding

*RosePom* collections have long been used as a source of germplasm for pre-breeding and breeding pome fruit programmes. These programmes have resulted in the release of 36 new plant breeders' rights (PBR) varieties. Pre-breeding programmes are currently being carried out in partnership with CEP-INNOVATION and NOVADI breeding companies.

*RosePom*'s wild species and old apple and pear cultivars have long been tested for resistance to diseases and pests, particularly scab, fire blight, powdery mildew and pear psylla, and the more interesting accessions have been used to introduce resistance genes and QTLs coming from different genetic backgrounds by crossing with modern cultivars. The improved genetic stocks obtained have been introduced into *RosePom*'s breeding material collections for use in the development of new varieties of dessert apple, cider apple and pear. Collections from research programmes, particularly mapping progenies established to study pest and disease resistance and fruit quality, have also been used to select improved material for dessert apple and pear, with the knowledge gained from these mapping progenies allowing the use of marker-assisted selection to pyramiding resistances to diseases and pests.

Western and eastern *Pyrus* species from *RosePom* have been used in rootstock breeding to produce rootstocks more tolerant to global warming (six taxa including: *P. cordata* Desv., *P. amygdaliformis* Vill., *P. amygdaliformis* var. *persica* (Pers.) Bornm., *P. elaeagnifolia* Pall., *P. syriaca* Boiss.), biotic stress (*P. calleryana* Decne. and *P. betulifolia* Bunge) or to induce dwarfing of scions (*P. nivalis* Jacq.). Perry pear (*P. nivalis* Jacq.) cultivars conserved by *RosePom* have been used in the breeding of dwarfing rootstocks, as cytochemical analyses have shown that this compartment is divided into two sections: one of triploid genotypes, with a high vigour segregation in their progenies, and one of diploid genotypes, which are very homogeneous in terms of vigour. This triploid compartment has been used to create dwarfing rootstocks.

The cultivar collections of apple and pear varieties (dessert apple, cider apple, pear, rootstocks and ornamental apple and pear) have been used as reference material for DUS testing for official variety registration and PBR at the national and European levels. Thanks to these large collections, INRAE is an entrusted examination office to carry out the DUS technical examination on behalf of the Community Plant Variety Office (CPVO).

#### In research

Identity, diversity, relatedness and traits of interest are elucidated by characterizing the resources of *RosePom*.

#### Pome fruits

**Characterizing genetic diversity.** More than 20,000 apple cultivars are documented worldwide. An in-depth analysis of the genetic diversity, structure and relatedness of old and recent apple cultivars conserved in *RosePom* and in institutional and associated germplasm collections at the French and European levels has been carried out by comparing more than 3,000 accessions from more than 20 European countries, USA and Canada, using SSR genotypic profiles (Leforestier *et al*, 2015; Suprun *et al*, 2015; Lassois *et al*, 2016; Urrestarazu *et al*, 2016; Durel *et al*, 2023). The highlighted main characteristics are a weak geographic structure and an important gene flow on a European scale, resulting from the numerous exchanges of graftwood over the centuries. A similar approach is also currently developed for European pear (Denancé *et al*, 2019; Bassil *et al*, 2023; Durel *et al*, 2023).

An apple core collection (287 accessions) has been developed from *RosePom* (Lassois *et al*, 2016) and further phenotyped for various traits (Lopez *et al*, 2015) including scab and fire blight resistance. Further, large multi-generation pedigrees were reconstructed from very old founders to elite cultivars thanks to a large SNP dataset, enlightening the history of apple empirical selection and the unexpected major role of a very limited number of founders such as the French cultivar 'Reinette Franche' and 'Margil' from the Renaissance period (Muranty *et al*, 2020; Howard *et al*, 2021). The amazing empirical selection of a high frequency of triploid cultivars (~20% of old apple cultivars are triploids) was also deciphered thanks to SNP-based pedigree analyses (Howard *et al*, 2023). Thanks to this work, INRAE's elite breeding population is now connected back to old founders with an accurate description of the transmission of genomic fragments across generations, giving a complete picture of haplotype sharing among all individuals.

#### Genetic mapping of disease/pest resistance factors.

Identifying new resistance genes/QTLs in pome fruit is an important goal to help breeding, especially for those pathosystems not yet sufficiently explored. Thanks to the conserved mapping progenies, numerous QTLs for pear resistance against scab, fire blight and psylla were identified (Montanari *et al*, 2015, 2016b; Perchepped *et al*, 2015, 2016). In apple, a major resistance gene

against rosy apple aphids was fine-mapped thanks to plant accessions conserved in *RosePom* (Dall'Agata et al, 2018) with further development of tightly linked markers useful for breeding. Significant QTL (epistatic) interactions have been observed in both apple and pear progenies, which may correspond to the combined effect of favourable alleles for two or three genes along a metabolic pathway to promote strong resistance (van de Weg et al (2018) for apple/fire blight, Perchepped et al (2016) for pear/psylla). In traditional accessions, attempted genome-wide association studies (GWAS) for both apple scab and fire blight have so far been rather unsuccessful, although scab tests have been conducted with various strains in the greenhouse to account for *V. inaequalis* genetic diversity. For the *V. inaequalis*-apple interaction, numerous low-frequency gene-for-gene relationships in the core collection studied may have hampered QTL detection and may require a redesign of this collection. In contrast, GWAS performed for flowering and harvest dates or for eco-physiological traits successfully identified genomic regions controlling these traits (Urrestarazu et al, 2017; Coupel-Ledru et al, 2022).

Three scab resistance QTLs identified in the 'TN10-8' x 'Fiesta' progeny were subjected to in-depth analysis with the ultimate goal of cloning them and analyzing the metabolic pathway they control, in particular by looking for accurate co-localizations of QTLs controlling either disease resistance or metabolic compounds (Lapous et al, 2023). One (qT1) exhibits a specific interaction with *V. inaequalis* strains and has been shown to finely co-localize with the major scab resistance gene Rvi6/Vf. For the other two (qF11 and qF17), haplotype-sharing analysis allowed us to trace them in various genetic backgrounds along the reconstructed pedigree. In particular, qF17, initially mapped at almost the same position in two unrelated backgrounds, actually corresponded to the same SNP-based haploblock inherited from an as-yet-unknown common (and certainly ancient) ancestor (Durel, 2019).

The conserved progenies also allowed a very original work to be carried out, analyzing the combination of genetic (intrinsic) and plant resistance inducer (PRI)-induced resistances to diversify/complement apple disease control methods. Putative synergistic or antagonistic effects between the two types of resistance were investigated using a genetic mapping approach. The F1 population 'TN10-8' x 'Fiesta' was genotyped and phenotyped for scab and fire blight resistance without and with PRI pretreatment (with acibenzolar-S-methyl – ASM –, a functional analogue of salicylic acid). QTLs (including qT1, qF11, and qF17) were detected in both situations, but with a rather limited impact of ASM on QTLs effect, indicating a mainly additive effect of both resistance mechanisms (Bénéjam et al, 2021, 2024).

In addition, other traits of interest were phenotyped on apple and pear mapping progenies, namely fruit texture/colour and sublethal genes (Ben-Sadok et al, 2015; Montanari et al, 2016a; Moriya et al, 2017).

**Apple genome sequencing.** One of the double haploid accessions conserved by *RosePom*, the double haploid 'Golden Delicious' line (GDDH13), also coded X9273, obtained at INRA in 1963, has been used, thanks to the homozygosity of this line, to produce a high-quality de novo assembly of the apple genome (Daccord et al, 2017).

## Roses

**Characterizing genetic diversity.** In the cultivated compartment, with more than 30,000 varieties, rose breeding activity has been very intensive, especially since the 19th century when old varieties of various origins and wild accessions were hybridized. The biological resources of 1,228 garden roses (botanical roses and old varieties) stored in *RosePom* (leaves and DNA) were genetically characterized using 32 microsatellite primer pairs (SSR). The genetic structure was revealed. The study of its evolution during the 19th century showed that the genetic background of European rose hybrids displayed a shift from an old European genetic background to an Asian one (Liorzou et al, 2016). This result is consistent with the introgression of old Chinese garden roses into European hybrids, especially for their continuous flowering. Indeed, the genotyping of the *copia* allele of the *RoKSN* gene, a homologue of *TFL1* responsible for continuous flowering, on 270 accessions showed that this allele was selected in Europe during the 19th century (Soufflet-Freslon et al, 2021). All this work was carried out using an interdisciplinary approach, collaborating with historians to answer the same questions through both genetic resources and archival research, while considering the historical construction of rose diversity.

The 20th century was marked by the intensification of specific uses, such as cut and pot roses and the globalization of rose production and markets. These specificities were studied in a larger interdisciplinary approach with geographers, sociologists, economists and historians. Accessions were collected from rose gardens and private companies to constitute a large sample representative of time periods, usages and kinds of breeders. The first results on 1,796 accessions genotyped with 23 microsatellite primer pairs showed a decrease in genetic diversity during the 20th century, in line with the dominance of Tea hybrids. Cut roses appear to be a subset of garden roses.

In the wild compartment, 219 *Rosa gallica* populations represented by 901 plants collected in France by *RosePom* or by associated research teams and partners throughout Europe were genotyped by SSR sequencing of amplified fragments from 29 SSR primer pairs together with 717 cultivated accessions in order to investigate the diversity structure and origin of French populations. Diversity in France is much more structured than elsewhere in Europe, which can reveal multiple origins (Pawula, 2023).

Furthermore, the identification of clones and sports (vegetative mutants), thanks to genotyping and phenotyping characterizations, is in progress.

**Genetic mapping of traits of interest.** With research genetic resources, genetic maps have been developed using SSR or SNP markers, allowing to anchor the genome sequence of Old Blush (Hibrand-Saint-Oyant *et al.*, 2018) and to identify regions with important genes controlling traits of interest, such as floral characteristics, fragrance, disease resistance (Lopez-Arias *et al.*, 2020) and prickly development (Zhou *et al.*, 2020). For example, a study on the fragrance components (more than 30 components) was carried out on two pseudo-F1 populations conserved in *RosePom*'s fields. Three genes were identified for the production of: geraniol, *RhNUDX1-1* located on linkage group 2 (Magnard *et al.*, 2015); 2-phenylethanol, *RhPAAS* located on linkage group 6 (Roccia *et al.*, 2019); and farnesol, *RhNUDX1-2* located on linkage group 7 (Sun *et al.*, 2020).

All of these research projects have greatly enriched *RosePom*'s collections, either in terms of biological material or associated data. These resources and associated data are valuable for expertise, e.g. in DUS testing for pome fruits, or for supporting urban management decisions through knowledge of wild *R. gallica* populations.

## Outreach

From an outreach point of view, *RosePom* is regularly presented to various audiences, including the general public, schoolchildren, students, professionals and scientists from other disciplines, such as historians, who see *RosePom* as a living archive. This is achieved through visits, stands at trade fairs and conferences.

## Participation in networks and working groups

*RosePom* is one of the INRAE Biological Resource Centers that have joined forces with other French public institutions (CIRAD – French Agricultural Research Centre for International Development, IRD – French National Research Institute for Sustainable Development, and Institut Agro – French Institute for Higher Education & Research in Food, Agriculture & the Environment) in the BRC4Plants pillar (Bergheaud *et al.*, 2025) of the National Agronomic Resources Research Infrastructure (AgroBRC-RARE). *RosePom*, as a member of the BRC4Plants pillar, is linked to the national structure coordinating plant genetic resources (Duval *et al.*, 2023). Since 2023, the pome fruit collection has been one of the collections for which INRAE is recognized as an official manager of Plant Genetic Resources for Food and Agriculture by the French Ministry of Agriculture. This collection is also labelled as a 'National Collection' by the Conservatoire des Collections Végétales Spécialisées (Conservatory of Specialized Plant Collections).

At the national level, *RosePom* leads a national cooperative network on pome fruit genetic resources involving public and private partners, in accordance with the strategy drawn up as part of the National Charter for the Conservation of Genetic Resources. It is also involved as an expert in the national cooperation network on roses, whose members are French rose gardens.

At the international level, *RosePom* is involved in the European Cooperative Programme For Plant Genetic Resources (ECPGR) *Malus/Pyrus* Working Group, which helps to define common standards and protocols for these species. It is also involved in the ECPGR Cryopreservation Working Group.

## Conclusion

The *RosePom* BRC plays an important role in the conservation of genetic resources of major fruit and ornamental plants, especially pome fruits. It has acquired considerable expertise in understanding the diversity of these genetic resources, both wild and cultivated. The conservation and knowledge of these genetic resources have proved very useful for three main activities: breeding, research and DUS testing. Interest in these resources is growing as crops are subject to many changes, in particular due to new regulations on the use of phytosanitary products, the emergence of pathogens and pests, and climate change. However, the conservation of these resources is costly in terms of human and material resources and faces multiple constraints. In order to maintain the collections in good condition despite these challenges, the management of all stages of the process must be carefully organized. The quality management system helps to achieve this objective. As highlighted by Engels *et al.* (2024), collaborative networks are of primary importance for an efficient genetic resource conservation system. The organization in a network with different parties at the national and European levels makes it possible to share experiences, reflections and work to reduce the technical and financial constraints and optimize the process of conservation and valorization of these resources. Nevertheless, in order to enhance this activity of common interest for society, we emphasize the need to continuously raise awareness among the general public, researchers and policymakers of the importance of these genetic resources in view of their potential use in the future.

## Author contributions

Writing – original: draft AP and LF, with contributions from all co-authors. Rose collections were described especially by AP, FF, TT, AC. Pome fruits collections were described especially by LF, CED, MHS, KG, ALH. Photos were provided by KG and TT. Writing editing: all authors, especially AP, LF, HM, FL. Review editing: AP and LF.



## Conflict of interest statement

The authors have no conflicts of interest to report.

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# Community seedbanks in Europe: their role between *ex situ* and on-farm conservation

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**Abstract:** This article enlightens the role of community seedbanks (CSBs) in Europe within the plant genetic resources for food and agriculture community and their role in the conservation and sustainable use of agrobiodiversity in complementarity with the *ex situ* management system. For more than 40 years, CSBs around the world have emerged as part of the so-called informal seed system to counteract the loss of locally adapted varieties through the development of collective seed systems. Most of the studies and articles refer to experiences from the Global South, whereas a comprehensive analysis of the impact and role of CSBs on seed systems in industrialized countries is still missing. This paper provides three case studies describing the community seed banking experience of different European organizations: Pro Specie Rara (Switzerland), Arche Noah (Austria) and Rete Semi Rurali (Italy). These organizations exemplify the diversity among European CSBs and their efforts to engage with genebanks and the formal seed system.

**Keywords:** community seedbanks, agrobiodiversity, participatory plant breeding, on-farm conservation, seed networks

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## Introduction

This article describes the role of community seedbanks (CSBs) in Europe in the plant genetic resources for food and agriculture (PGRFA) community and their contribution to the conservation and sustainable use of agrobiodiversity in complementarity with the *ex situ* management system. Recently, two EU Horizon projects (DYNAVERSITY: [www.dynaversity.eu](http://www.dynaversity.eu) and Farmers' Pride: <https://more.bham.ac.uk/farmerspride/>) have worked on this complementarity promoting the emergence of a European network of actors involved in PGRFA conservation and use. Both projects have involved CSBs in their activities and developed manuals and guidelines for the management of CSBs in connection with public genebanks. But what are the main differences between CSBs and genebanks?

If the complementarity between *ex situ* and on-farm conservation of PGRFA has been accepted by the scientific community in the last 20 years, less attention has been given to investigating the role of CSBs in

relation to these two systems. Only a few scholars have studied such collective endeavours (Vernooij *et al.*, 2015) while national seed policies hardly include CSBs among the relevant institutions for conservation and sustainable use of PGRFA. A quantitative study on the impact of CSBs and civil society organizations (CSOs) in the European conservation system of PGRFA is still lacking. Many questions about their role can be addressed. How can they act as an intermediate between genebanks and farmers/gardeners? How can they increase the awareness of citizens on biodiversity conservation? How can they work with public research centres to support participatory plant breeding programmes? How can they diversify European seed systems and, subsequently, food systems?

It becomes important to better understand the functions as well as the practical and collective actions of CSBs, based on concrete experiences. This article aims to fill this gap, presenting three case studies from Italy, Switzerland and Austria, based on the

personal experiences of the authors, who work with the three organizations, and on the results of surveys and workshops organized within the framework of four EU-funded research projects – DIVERSIFOOD (<https://diversifood.eu/>), DYNAVERSITY, Farmers' Pride and PRO-GRACE (<https://www.grace-ri.eu/pro-grace>).

Genebanks and CSBs should not be considered as competitors but as complementary actors, each having specific objectives, targets and rules as summarized in Table 1 (Bartha et al, 2021). Perhaps the most relevant difference is that genebanks are committed to the long-term conservation of PGRFA and to granting facilitated access to a wide range of users, while CSBs aim at short-term conservation and easy availability of seed for the aims of the community managing the CSB. This complementarity means that together, they can contribute to creating inclusive and integrated conservation strategies at national and regional levels.

## Community seedbanks in Europe

For more than 40 years, CSBs around the world have emerged as part of the so-called informal seed system to counteract the loss of locally adapted varieties through the development of collective seed systems (Vernooij et al, 2015). Most of the studies and articles on the subject refer to experiences from the Global South. However, a comprehensive analysis of the impact and role of CSBs on seed systems in industrialized countries is still missing.

In 2017, within the framework of the European project DIVERSIFOOD, a group of seed networks including Arche Noah, Rete Semi Rurali (RSR), ProSpecieRara (PSR), Réseau Semences Paysannes (RSP) and Red de Semillas (RdS) organized a regional survey on CSBs in Europe, to start understanding their distinctive features. The results of the survey, briefly presented in this paper, showed the great diversity of initiatives in terms of age, size and internal structures. Differences emerged also in their approaches to PGRFA management, with some leaning towards a more 'dynamic management' approach. Since 2005, the number of CSBs in Europe seems to be rapidly growing, at least in some regions (Koller and Bocci, 2018).

The first CSBs in Europe were established in Northern and Central European countries (Austria, Denmark, Germany, the Netherlands, Sweden, Switzerland, United Kingdom), mainly by seed saver organizations and managed by gardeners. The US-based Seed Savers Exchange inspired the birth of many of these organizations. The UK Garden Organic's Heritage Seed Library, known at the time as Henry Doubleday Research Association, was founded in 1975. ProSpecieRara (Switzerland) was founded in 1982 and Arche Noah (Austria) in 1990. Since then, other organizations have sprung up in European countries, with an accelerated increase occurring since the mid-2000s, especially in France and Spain. The DIVERSIFOOD survey was able to map only a few experiences from south-eastern Europe.

The survey revealed that there is not one type of CSB that fits all. This diversity is related to the fact that CSBs in Europe have followed two different pathways. In the older CBSs in Europe, mainly from central and northern Europe, private gardeners had a key role, following the example of the seed savers in Australia and the USA. On the contrary, in southern Europe small farmers played a major role, adapting the experiences of social movements in the Global South, where CSBs emerged to provide seeds to farmers in marginal areas or after conflict. However, most experiences converge around the key ideas of diversity, conservation, exchange, community and sovereignty. Regardless of the crop, most of the accessions they conserve are landraces, farmers' varieties, old commercial varieties (open-pollinated varieties) or breeding populations.

What is important to note is that many initiatives have moved from just conservation to more dynamic approaches, where participatory and decentralized plant breeding plays an increasing role. This change was achieved through dedicated training activities for all the members of the CSBs, which raised awareness of breeding for diversity and local adaptation. Many CSBs have also been able to create strong links with citizens, often using public campaigns focused on the importance of plant and seed diversity, and protecting local varieties. Through these activities, they have promoted more diversified, sustainable and resilient food systems that are better suited to face climate challenges.

The main obstacles raised by the participants to the survey have been the lack of financial resources and an enabling legal environment. In fact, seed and food policies have often promoted uniform and formal seed systems, through regulations on seed marketing. For example, the interpretation of seed exchanged by farmers varies across countries in Europe: some countries allow it, while others consider it as commercialization following the rules of seed marketing. Only recently more diversity entered this picture with the concept of Conservation Varieties (EU Commission directives 62/2008 (EU, 2008), 145/2009 (EU, 2009) and 60/2010 (EU, 2010)), even if its impact is still questioned (Didonna et al, 2024). Networking and cooperation as well as mutual support and social learning have been indicated as strategies to overcome these barriers.

Within this large and diversified movement of seed conservation, sharing and breeding, the idea of creating a European umbrella organization emerged as members of different CSBs started connecting through European meetings. In 2005, European seed networks organized the first European meeting – 'Let's Liberate Diversity' – in Poitiers, France. After that meeting, RSP, RSR and RdS started the process of formalizing a regional-wide organization which would group the different associations involved in seed saving, on-farm conservation and agrobiodiversity management. After seven years of negotiations and meetings, the European Coordination Let's Liberate Diversity (ECLLD) was formally registered in 2012 as a non-profit organization in Belgium, and as of



**Table 1.** The main conceptual framework of community seedbanks (CSBs) and genebanks (Bartha *et al*, 2021).

	Genebank	Community seedbank
<b>Organizational structure</b>	Public Institution	From single-person initiatives to community-based organizations (association, foundation, network without legal status, etc.).
<b>Actors</b>	Centralized structure Employees (scientists, practitioners), occasionally farmers and breeders (if project available)	Partly decentralized (network structure) Network member volunteers (gardeners, farmers, horticulturists, etc.), employees (scientists, practitioners).
<b>Funding structure</b>	State, public–private partnerships (PPP), projects	Private (members, sponsors, foundations, etc.), public (European, state, region, municipality), commercial activities, non-profit organizations (NPO)
<b>Communication strategy; Know-how transfer</b>	Towards scientific and breeder community. Case by case policymakers too. Specific and science-focused communication. Specific communication to farmers.	Broad public (sponsors, donors), practitioners (farmers, gardeners, horticulturists), governmental decision-makers and politicians. Integrative and comprehensive communication.
<b>Quality management for plant genetic resources</b>	Aiming at a common and internationally agreed certification system based on protocols and standardized procedures. Monitoring only internal genebank activities.	Aiming at quality systems that are best adapted to the needs and actual situation (financial and structural) of the CSB. Monitoring is based on the control of the whole network.
<b>Choice of plant material</b>	Based on national breeding programmes, genebank managers' interest, national agrobiodiversity strategy (if existent). Only recently international coordination and sharing of responsibilities (e.g. AEGIS, A European Genebank Integrated System).	Based on CSB strategy developed by network members based on public or founder's interests as well as financial and network capacity. Often local, national or regional focus.
<b>Breeding</b>	Providing PGR for breeders for targeted breeding activities mostly for resistance. Aiming at specific and homogenous varieties. Varieties adapted to industrial agriculture.	Evolutionary breeding mostly aims for tolerance. Varieties with a less homogenous calibration spectrum. Aiming at varieties that keep their adaptation capacity to different agricultural systems.
<b>Governance</b>	Public mission based on national and international law/agreements/protocols  Hierarchic, top-down  Bound to governmental obligations	Based on common agreed values, shared visions and missions and agreed statutes and bylaws. Social aspects are key. From hierarchic to democratic structures, bottom-up Civil society organizations, representing the interests of the community
<b>Access to materials</b>	Mainly through the easy standard material transfer agreement (SMTA) and the rules of the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA)	It could vary from one CSB to another. There are CSBs fully compliant with ITPGRA, others that have specific mutually agreed terms
<b>Type of materials</b>	Mainly old varieties and landraces in the public domain. Some conserve also breeding lines or commercial varieties with dedicated access rules	From old varieties and landraces in the public domain to new heterogeneous materials bred through participatory breeding programmes

2024, it brings together 22 organizations from 21 countries, encompassing over 170 national organizations. ECLLD is dedicated to promoting the dynamic management of cultivated biodiversity and farmer-led seed systems across Europe and aims to bring diversity back into our food systems. By connecting CSBs, researchers, civil society groups, seed savers, and farmers, ECLLD operates through three core focus areas: policy, community seedbanks, and communities. As a platform for policy engagement, ECLLD supports capacity-building initiatives and fosters exchanges among its members

to enhance their advocacy efforts toward policies and regulations that promote and sustain agrobiodiversity. In the area of CSBs, ECLLD empowers local groups by facilitating knowledge sharing, supporting exchanges on practices, and the integration of participatory plant breeding (see as reference the three Technical Manuals on CSBs, Galluzzi *et al* (2021c,b,a) <https://liberatediversity.org/knowledge/readings/>). Finally, through its work on communities, ECLLD fosters peer-to-peer learning, enabling stakeholders to share practices, exchange experiences, innovate, build connections and collectively

drive action on cultivated diversity. Events like the Let's Liberate Diversity and Let's Cultivate Diversity forums, are central to these community-building efforts (for further details visit [www.liberatediversity.org](http://www.liberatediversity.org)).

### Three case studies

Our case studies describe the CSB experience of three different European organizations which are ECLLD members: Pro Specie Rara (PSR, Switzerland), Arche Noah (Austria) and Rete Semi Rurali (RSR, Italy). They were chosen since they exemplify the diversity among European CSBs and are well connected to their respective national systems for PGRFA conservation. Moreover, the three organizations have all made efforts, although in different ways, to engage with the so-called formal seed systems, with activities such as seed marketing, registration of local varieties/populations, characterization of the accessions conserved and traceability of the work of the CSBs through dedicated databases.

For each organization, we will describe the history, turning points, PGRFA managing system, the networks they are involved in and their perspectives. A brief summary of the main characteristics of the three organizations is presented in Table 2. They have different members (single persons in the case of PSR and Arche Noah, and other organizations for RSR), activities (PSR is working also on animal breeds), facilities and access rules. PSR and RSR have integrated the easy standard material transfer agreement (SMTA) of the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) for providing accessions for research and breeding, meanwhile Arche Noah has its own access rules with an obligatory compliance check: if companies or organizations work with GMOs or patents, they are excluded from seed access.

### Rete Semi Rurali

#### History

Rete Semi Rurali (RSR) is the Italian seed network, an umbrella non-profit association grouping organizations involved in the sustainable use of agrobiodiversity, within an agroecological framework. RSR was set up in 2007 by seven founders; in 2024 it consisted of 36 profit and non-profit members. RSR's mission is to diversify our food systems, starting from seeds and varieties. Its projects are directed primarily towards increasing diversity in agricultural systems, starting with the management of diversified seed systems in organic farming. RSR's strategy aims to recognize the role of farmers and other actors in breeding and seed production and enable a legal framework for the dynamic management of agrobiodiversity (Bocci and Galluzzi, 2015). RSR activities cover four different work areas:

1. Action research: this area supports the diversification of agricultural systems, by promoting a different model of agricultural research which brings research back to farmers' fields (decentralization)

and involves different food system actors (participation).

2. Community seedbanking: this area supports diversified seed systems by promoting CSB development at a local level (see Figure 1).
3. Communities: working on seeds means working with the communities that grow them, process and consume their products. RSR works to build and support vibrant communities in which food system actors interact with each other, being aware of their complexity and diversity.
4. Policies: to promote changes in agricultural systems, it is necessary to build a political, legal, economic and social environment that makes these possible. RSR is active in fostering this enabling environment at local, regional, national and European levels.

In 2013, after the ECLLD meeting Let's Cultivate Diversity in Tuscany, RSR started to create its own CSB dedicated to different cereal species. Subsequently, the CSB expanded its structures and functions, engaging new members, opening hubs in new regions, establishing field trials for different crops, and engaging the communities in the evaluation of varieties and sensory analysis of the products (Petitti et al, 2022). Great emphasis was placed on participatory approaches to bottom-up seed system innovation, focused on the development and dissemination of dynamic crop populations and their management within organic farming systems (De Santis et al, 2022). The concept of Community Biodiversity Management (DeBoeuf et al, 2013) was adopted, believing in its great potential for change and adaptation, and for making agricultural systems the places where site-specific innovation takes place.

Each year, RSR's CSB organizes two seed distribution campaigns, one for winter and one for spring crops, through which a catalogue of landraces and populations is released and from which farmers or gardeners can ask for small seed samples. RSR has developed a dedicated material transfer agreement to trace the exchange of the materials and keep track of the flows from the CSB. The samples distributed are small, but generally larger than the ones of formal genebanks. For more uniform varieties, RSR provides around 200 or 300gr and for populations up to 2kg, to avoid reducing the diversity by sampling a small amount of seeds.

In 2019, RSR inaugurated its new headquarters, called the House of Agrobiodiversity, a multifunctional space, which includes the first agrobiodiversity library in Italy, a fully operational seedbank and a training centre. In 2022 RSR launched DIVERSITAS – the Digital Ecosystem of RSR. It collects and manages all the accessions in the CSB and the data from the experimental fields. DIVERSITAS is designed to track the flow of seeds in and out of the CSB.

#### Turning points

From 2010–2019, thanks to EU projects SOLIBAM ([www.solibam.eu](http://www.solibam.eu)) and DIVERSIFOOD, RSR moved from

**Table 2.** Main features of Rete Semi Rurali, ProSpecieRara and Arche Noah

	<b>Rete Semi Rurali</b>	<b>ProSpecieRara</b>	<b>Arche Noah</b>
<b>Date of founding</b>	2007	1982	1990
<b>Website</b>	<a href="https://rsr.bio">https://rsr.bio</a>	<a href="http://www.prospecierara.ch">www.prospecierara.ch</a>	<a href="http://www.arche-noah.at">www.arche-noah.at</a>
<b>Legal structure</b>	Non-profit organization; Umbrella org.	Foundation	Association
<b>Board, staff</b>	5 board members, 2 employees, 15 consultants	7 board members, 35 employees	9 board members, 57 employees (approx. 39 full-time equivalents)
<b>Members</b>	35 entities (profit and non-profit)	13,000 donors and 4,400 active seed savers and rare-breed holders. Farmers, breeders, gardeners, researchers, etc.	10,000 members + 7,000 extra donors
<b>Mission</b>	Diversification of farming and seed systems	Maintain and promote the genetic and cultural diversity of plants and animals.	Conservation and development of crop diversity in regional and Europe-wide networks and advocacy for an enabling policy framework
<b>Main collections</b>	3,487 accessions. Crops: soft and durum wheat, barley, rye, oat, other cereals, rice, maize, tomato, sunflower, soybean, chickpea, bean and lupine.	5,600 cultivated plants and 32 rare breeds	5,500 seed accessions and 550 fruit cultivars
<b>Main activities</b>	Central seed storage (climatic chamber and freezer). Database: DIVERSITAS.	Central seed storage (climatic chamber and freezer), tuber storage facility (climatic chamber), nursery and greenhouse and tunnel. Webpage for seed and breeds exchange. Database for dynamic on-farm management	Central seed storage (climatic chamber and freezer), one visitor's garden and one multiplication garden incl. tunnels. Online shop and shop in the visitor's garden. Internal and external database.
<b>Main network activities</b>	Seed saving, knowledge exchange, courses, markets, collection holders, data collection, breeding	Seed saving, knowledge exchange, courses, markets, collection holders, data collection,	Seed saving and regular multiplication incl. data collection, educational programme with approx. 50 courses per year, political campaigning, participatory vegetable breeding networks, Arche Noah Diversity Farms
<b>Main projects (2024 status)</b>	Implementing the ITPGRFA in Italy, ECPGR EVA Network, 5 Horizon Europe projects	72 different projects; Label for PSR products; Horizon 2020 projects; Projects within the frame of the national action plan for PGRFA	No patent on seeds-campaign, Participatory vegetable breeding, Fruit Monitoring Austria, Supporting community biodiversity management in South-Eastern Europe via Small-Scale Grants. Online seed savers index

the mere preservation of local varieties and landraces to actively breeding for diversity, developing evolutionary populations of soft and durum wheat (*Triticum aestivum* L. and *T. turgidum* subsp. *durum* (Desf.) Husn.), and barley (*Hordeum vulgare* L.). This work on participatory and decentralized plant breeding, and in particular on evolutionary populations, was done in collaboration with Dr Salvatore Ceccarelli, a breeder who worked at the International Center for Agricultural Research in the Dry Areas (ICARDA), one of the SOLIBAM partners,

and then directly with RSR. ICARDA's evolutionary populations of soft and durum wheat and barley were evaluated and tested in different and contrasting farming environments (Bocci *et al.*, 2020; Ceccarelli and Grando, 2020). This move from agrobiodiversity conservation to breeding for diversity was the first important turning point in the history of RSR. Over time, the work on wheat and barley was expanded to other crops: rice (*Oryza sativa* L.), tomato (*Solanum lycopersicum* L.), oat (*Avena sativa* L.), lupin (*Lupinus*





**Figure 1.** Community seedbank at Rete Semi Rurali

*albus* L.), sunflower (*Helianthus annuus* L.) and recently other legumes, following the increasing interest among farmers in crop populations for organic farming systems.

In the beginning, the populations remained within RSR's network and were exchanged during the seed campaigns, since marketing this kind of seed was not legally possible. In 2014, a second relevant turning point occurred: thanks to the lobbying done by SOLIBAM partners, mainly the Organic Research Centre (UK), Fibl (Switzerland), Itab (France) and RSR, the European Commission opened the space for marketing the seeds of these populations by an experimental derogation (EU Commission implementing decision 150/2014 (EU, 2014)). Using this derogation, RSR supported farmers to engage in the process of seed production, multiplication and marketing of the populations they were growing and adapting, by registering as small seed companies. In 2017, the first soft wheat population was officially certified by public authorities and two farmers (one in Tuscany and one in Sicily) started marketing its seeds. At the same time, RSR developed its label for the seed packages using and adapting the open-source pledge promoted by the Open Source Seed Initiative in the US (<https://osseeds.org>).

The last relevant turning point was the approval of the new EU regulation 848/2018 (EU, 2018) on organic production and labelling of organic products that entered into application in January 2022. This

regulation created a new varietal category, the Organic Heterogeneous Material (OHM), which took up the concept of populations contained in the Decision of 2014. Since then, RSR has worked to support the implementation of OHM in Italy, notifying one rice, one sunflower and one soft wheat OHM.

### **Collaboration with institutions**

Since its foundation, RSR has collaborated with the Ministry of Agriculture, being one of three partners of the national programme for the implementation of the ITPGRFA. This programme involves 29 research facilities of the Council for Agricultural Research and Agricultural Economics Analysis (CREA), the Institute of Plant Genetics of the National Research Council (CNR) in Bari and RSR. Thanks to the programme, RSR supports its CSB as well as the Italian delegation within the ITPGRFA framework on negotiations related to the sustainable use of agricultural biodiversity and Farmers' Rights.

Thanks to the above national programme and its involvement in Horizon projects, RSR has developed dedicated agreements with a range of European organizations, including one with the CNR genebank in Bari for the multiplication and regeneration of some of the accessions conserved there. Other agreements on participatory and decentralized plant breeding have been signed with the Universities of Florence, Bari, Turin, Milano Bicocca, Viterbo and the Sant'Anna



School of Advanced Studies in Pisa. The aim of these agreements is to place RSR as an intermediary organization between the public research system and farmers.

### Networks

RSR is a member of several Italian alliances and networks including Azione TerrAE, the Coalition for Agroecological Transition (<https://azioneterrae.com>). This coalition is made up of 7 international cooperation associations (ACRA, CISV, COSPE, DEAFAL, LVIA, Mani Tese, Terra Nuova) and two Italian and European civil society networks (RSR and Agroecology Europe), engaged in experimentation, promotion, training and dissemination of different aspects of agroecology, involving both research and farmers' organizations. Azione TerrAE plays a crucial role in the promotion of agroecology in Italy and West Africa, while the role of RSR is to strengthen the link between good farming practices and seed systems, putting the development of diversified seed systems at the core of activities of the Coalition. At the national level, RSR has also been involved in the campaign *Cambiamo Agricoltura*, which unites over 70 organizations actively engaged in the negotiations of the Common Agricultural Policy (CAP).

At the European level, RSR is a full member of the European Consortium for Organic Plant Breeding (ECO-PB, <https://www.eco-pb.org/>), which aims at facilitating knowledge exchange and supporting breeding programmes for organic farming. As mentioned, RSR is one of the founding associations of the European Coordination Let's Liberate Diversity.

At the international level, RSR is a member of the Global Coalition of Open Source Seed Initiatives (GOSSI, <https://www.opensourceseeds.org/en/goSSI>), an international coalition of organizations, individuals (farmers, seed keepers, plant breeders, activists) working to ensure that seeds can be freely used and shared in perpetuity.

RSR contributes actively to the debate and negotiations on the European regulatory framework and is involved within the ITPGRFA in the development of policies on the sustainable use of PGRFA and Farmers' Rights.

### Perspectives

RSR has become a complex, inclusive and dynamic network dealing with local field experimentations as well as international processes. It aims to maintain a fruitful dialogue among practitioners, researchers and policymakers. Its work demonstrates that we must enlarge the vision of agrobiodiversity, focusing not only on mere conservation but on innovation and breeding for diversity, i.e. delivering new varieties that are sufficiently diverse (rather than narrowly responding to the standard criteria of distinctness, uniformity and stability (DUS) of modern varieties) to be able to adapt to climate change and low-input farming systems. The belief in the importance of diversifying seed, farming and food systems is the reason why RSR has recently

moved towards projects and research activities that involve not only seed diversification and breeding but also intercropping, rotations and soil microbiome. These will be the challenges for RSR in the coming years. Regarding the CSB and its database DIVERSITAS, the next steps will be the possibility of implementing the ITPGRFA easy SMTA directly from its website for the accessions distributed from the CSB and the digital object identifier (DOI) for some of the conserved accessions.

## ProSpecieRara

### History

PSR was founded in 1982 in St. Gallen (Switzerland) and its first activities were related to safeguarding rare breeds. In 1985, collecting activities for fruits, field crops and vegetables started. In 1988, the first employee was hired with a fixed salary. Around the same time, the network of seed savers was established and the seedbank (called 'seed library') was founded. Very successful TV broadcasts and some national exhibitions about rare breeds and fruit varieties organized by PSR and its partners and volunteers helped to raise awareness among the broader public. In the 1990s, the first private foundations started funding the projects of PSR and private donors supported the organization as well as many volunteers who helped to propagate seeds as seed savers. In addition, many breeders joined the different breeding associations created by PSR to coordinate the conservation of the different endangered breeds. After 15 years of existence, PSR encountered about 2,000 donors, 250 seed savers and over 2,000 breeders organized in 15 different breeding associations.

### Turning points

The first important turning point for PSR's activities was the ratification of the Convention of Biological Diversity by Switzerland in 1995, followed by the development of the National Plan of Action for the Conservation of Plant Genetic Resources for Food and Agriculture (NAP-PGRFA) in 1998. The Department of Agriculture decided to create the Swiss Commission for the Conservation of Cultivated Plants (SKEK/CPC) in which PSR became a leading member of the governing body to implement the NAP-PGRFA. This mandate was and still is accompanied by some funds (3.2 million CHF per year). At the time, the Commission developed a conservation strategy with conservation standards for various crops and a national database that relates to the European Search Catalogue for Plant Genetic Resources (EURISCO). PSR's own database has an interface with the national database to transfer and exchange passport and characterization data. Today about 15% of the turnover of PSR is covered by these public funds.

A second important turning point was the collaboration with COOP, the biggest supermarket chain in Switzerland. Together with this impactful partner, PSR participated in one of the biggest national exhibitions called EXPO02, which attracted millions of people over

six months. The interest of the visitors in the topic was so big that COOP decided to fix the collaboration through a contract. This collaboration has lasted until today.

Accordingly, PSR's focus shifted more and more from pure conservation towards the sustainable use and development of PGRFA. PSR developed a label for the promotion of traditional and endangered varieties to keep or reintroduce them in the value chain. Aside from the COOP outlet, farmers and horticulturists who are part of the PSR conservation network use this label for their own marketing activities, helping them showcase the added value of their products to consumers. In 2023, PSR's database revealed that about 600 people use the PSR label and surveys reported that around 30% of the Swiss population know this label.

Another important turning point in the Swiss PGRFA conservation activities occurred when the Government implemented article 147a in the agriculture law (SR 910.1 Art. 147a LwG):

*"The Confederation may promote the conservation and sustainable use of genetic resources. It may manage genebanks and conservation collections or have them managed and support measures such as in situ conservation, in particular with financial contributions."*

Under this provision, PSR and other stakeholders in this field could apply for funding for activities going beyond pure conservation such as on-farm development and improvement of PGRFA.

Thanks to the development of a suitable legal framework (e.g. the national long-term strategy for PGRFA and its accompanying measures) as well as through adequate funding opportunities, PSR was able to grow further, engaging new stakeholders for on-farm management of PGRFA. Today governmental financial support constitutes only about 20% of their total turnover but is still crucial because it guarantees the financing of conservation activities that depend on a long-term financing source (e.g. on-farm fruit and berry collections).

### Development and monitoring

ProSpecieRara went on to develop its network of seed savers (for vegetable species) and collection holders (for fruits and berries). Today, 400 seed savers maintain 1,208 vegetable varieties, 270 people care for 1,012 ornamentals, 1,000 people host 2,436 fruit and 422 berries varieties all over the country. The PSR staff is responsible for managing the network and monitoring conservation activities. For seeds, this work is facilitated by the central seed library, located in Wildeggen (AG) and consisting of a climatized room with 2,000 accessions stored as seeds. This repository functions as a backup of the *in situ* collection, with seed savers regularly sending back a reference seed lot of the variety/accession they maintain and regenerate. The reference lots are sown in PSR's different demonstration gardens, where quality and varietal identity are verified. All the exchanges between the seed savers and PSR are registered in a specific database for on-farm conservation and can be traced back. Every year, the 1,653 label holders

(i.e. registered and validated seed savers) receive a request to fill in a checklist to describe the status of the genetic resources they are maintaining. This checklist is provided by PSR on its portal (<https://www.prospecierara.ch/it.html>). Based on the results of this monitoring activity, PSR delivers a report about the status of each of the 5,600 accessions maintained by the people of the network. This report also allows the seed library manager to establish how many seed lots are in the genebank and their storage time. The manager will also know how many seed savers are maintaining the accession, how many are marketing seeds, fresh and processed products and how many of them are offering seeds on the variety finder portal of PSR. By compiling all this information, that is facilitated by the database, the manager can judge the conservation status of an accession and decide if an accession is endangered and must be propagated quickly or not. In addition, different training courses are organized for beginners and advanced seed savers to improve their knowledge and skills.

### Sustainable use

Over time, PSR's activities shifted more and more from pure conservation to sustainable use of PGRFA and the development and improvement of varieties, e.g. for niche markets and with niche varieties. The shift of PSR's activities towards sustainable use was backed by a national law (SR 916.151.1 Art. 2.4 - 2.7, 27, 29) for the marketing of seeds brought into force in 2010: besides registered and certified varieties, a new category called 'niche varieties' was created to include varieties that don't fulfil the DUS criteria. The government considered that certain varieties could be very interesting for niche markets or small-scale farmers or private gardeners (see Figure 2).

Allowing to register these varieties and being able to place them on the market would enrich the diversity of vegetables and field crops in the fields and on consumers' tables. This positive legal environment led to breeding activities to improve landraces, minor or 'opportunity' crops, or develop populations, with the support of government and private (e.g. COOP) funds.

Some of the breeding activities included participatory methods and, in some cases, citizen participation when a broader range of information about plant growth and development in different agricultural and horticultural contexts had to be collected and compiled. While often breeding activities take the form of mass selection to advance a population variety, in some cases, cross-breeding is the only way to make a variety fit for on-farm utilization (see Table 3).

### Further development as a CSB

In the future, open-pollinated crops will become even more important for alternative, independent, locally adapted and innovative agricultural systems, a grass-roots alternative to hybrids that are more and more taking over intensive and industrial agricultural production of food (IPES-Food, 2016). On the other hand, citizens



**Figure 2.** Carrot variety ‘Gniff’ from Ticino from Pro Specie Rara collections being commercialized

**Table 3.** Cross-breeding activities initiated by ProSpecieRara (PSR) for new niche varieties

Species	Varieties	Origin	Breeding
<i>Daucus carota</i>	‘Gniffola’	Landrace ‘Gniff’ x ‘Purple Haze’	Sativa Rheinau, 2012–ongoing
<i>Solanum lycopersicum</i>	‘Cuore di Bue’	‘Cuore di Bue’ x resistant varieties against <i>Cladosporium</i> sp.	Sativa Rheinau, 2012–ongoing
<i>Brassica rapa</i> subsp. <i>rapa</i>	‘Albedo Viola’ (rejected name) ‘Guringa’	Several old varieties of PSR, the Swiss Genebank and from commerce landrace ‘Bosco Gurin’ x several varieties of the same type	Sativa Rheinau, 2016–2024
<i>Allium cepa</i>	‘Piri’	Old variety ‘Birnenförmige’ x (‘Yankee F1’ x (‘Rijnsburger’ x ‘Yankee F1’))	Sativa Rheinau, 2012–2022
<i>Tragopogon porrifolius</i>	Salsify populations	11 salsify lines from PSR, the Swiss Genebank and from commerce	PSR & Sativa Rheinau together with PSR network (participatory breeding), 2024–ongoing
<i>Cucurbita pepo</i> var. <i>cylindrica</i>	Striped zucchini	Old variety ‘De Gênes Striée Vert-Jaune’ x other striped zucchini	Sativa Rheinau, 2024–ongoing

are increasingly interested in agroecological food production systems that foster biodiversity, protect the environment and provide healthy food for them. This is one of the reasons why urban farming and gardening movements are popping up all over the country in and around cities. Well-educated young people are applying new and better-adapted farming systems to local conditions. Several community-supported agriculture systems around cities like Geneva, Basel or Zurich have contacted PSR to get access to bigger amounts of seeds to be able to start their trials on a bigger scale and select those varieties that fit best to their local and specific needs and establish production plots for the marketing of produce. For PSR

this development causes different challenges: first, quantities of seeds requested are greater than those normally distributed for purely experimental purposes; second, these new actors require training in order to acquire the ability to produce and regenerate their own seed each year. Finally, these users demand more detailed information about the PGRFA stored in PSR’s seed library. To face these challenges, PSR is considering a series of new developments. To produce and distribute greater quantities of seed to growers, it could become a small seed company or start collaborating with local seed companies. In general, PSR would like to act as a knowledge hub or be part of a knowledge platform that provides



information to growers and helps them to make decisions tailored to the specific environmental and/or economic and social conditions they operate in – a kind of one-stop shop for farmers.

### Networks

Since the early 2000s, PSR has entered several partnerships with research institutions, whether within the framework of the NAP-PGRFA for the inventory, description and conservation of PGRFA, or within the framework of production and distribution with COOP. An important factor in the success of many of these collaborations was the clear definition of partners' roles. The typical collaboration between PSR and research institutions covers three steps along the continuum from conservation to the sustainable use of PGRFA:

1. Conservation: *ex situ*, i.e. *in vitro* and genebank conservation by research institutions (e.g. Agroscope) linked with the *in situ* and on-farm conservation by PSR and its partners. The research institutions use their scientific expertise to carry out *ex situ* conservation measures. On the other hand, PSR has an interest in testing PGRFA in on-farm conditions to possibly promote them among farmers, while network partners can use their PGRFA collections as sources of material for the multiplication and commercialization of seeds.
2. Evaluation: genetic and morphological characterization by research institutions (e.g. Agroscope) linked with the quality assessment, value description (e.g. cultural history, or market value), and determination of the utilization potential by PSR. While research institutions provide the expertise and technical infrastructure for characterization, PSR and its network can evaluate the PGRFA in terms of their socio-economic impact.
3. Environmental adaptation for agricultural use: selection of PGRFA, breeding activities and marketing activities by PSR and partners linked with agronomic evaluation and case studies by research institutions with a strong link to farmers (e.g. FiBL). PSR initiates breeding activities to recover the varieties and bring them to the market. The 'ProSpecieRara' label provides a tool to enhance the product's credibility with consumers. On the other hand, it is important for farmers to know the expected quality and quantity of the PGRFA available before accepting it for large-scale cultivation on their farms. Research organizations advise farmers about the agronomic and quality features of the PGRFA available through PSR enabling them to make informed choices depending on their context and needs.

### Perspectives

PSR is dedicated to further developing the plant and animal genetic resources it maintains in its broad network. As this example shows, there are strong incentives for collaboration between CSBs, research

institutions and genebanks. There is a great need for research on the characterization and evaluation of PGRFA, and the role of research institutions and genebanks is undeniable. At the same time, PSR experience shows that successful promotion of PGRFA is only possible with many dedicated actors, which come together in the CSB network. This collaboration will not only enable better integration of PGRFA into society through innovative agroecological farming systems but will also return a wealth of real-world data and experiences about PGRFA to the research institutions.

## Arche Noah

### History

The development of Arche Noah can be divided into four phases:

1. Pioneer phase. Emerging from predecessor organizations, Arche Noah was founded in 1990 by farmers and gardeners who had formed a network among crop collectors and seed savers. Early on, the organization issued a *Seeds Handbook* (not published, for members only) to stimulate the exchange of seed and other plant reproductive materials and began compiling a collection of rare and endangered varieties. The 1990s were a time of rapid growth of the collection, stimulated by collecting missions (Austria, Croatia, Romania) but also by research into commercial open-pollinated and heirloom varieties. Eventually, in 1994, the organization opened a garden for crop multiplication that was also open to the public for educational purposes. Seed production was organically certified which gave it a unique position among seed savers organizations as well as in the agricultural scene. Arche Noah was a pioneer in the then-emerging Austrian organic farming scene.
2. Differentiation phase. Around 2000, the organization had become well-known and grew to 6,000 members. The annual plant market attracted many visitors and became a meeting point for the organic gardening community. Arche Noah took up the direct marketing of transplants. To manage risks from commercial activities and to clearly separate commercial from non-profit activities, a company with limited liability was founded. Along with these developments, the number of employees at the organization rose and departments developed, specializing in plant collections (seed archive, fruit collection), gardening, seed savers activities, sales and event management. An early act of policy advocacy was to secure exemptions for unregistered PGRFA under the Austrian National Seed Legislation. This was achieved through persistence, as well as a rare window of opportunity for alternative farming approaches at that time. Since then, the legal exemptions have allowed for marketing small quantities of seeds, making Austria a unique environment for heir-



loom varieties in the EU. Beyond that, Arche Noah advocated for lighter registration procedures for Conservation Varieties. The demand for seeds was growing and it became necessary to open the registration process for plant materials not conforming to industrial standards and feasible for small seed companies. In the educational sector, a series of books was launched, setting off with a handbook about seed gardening (Heisteringer, 2013). In 2008, the education programme on organic gardening and crop diversity was expanded, comprising nowadays up to 80 courses per year, training people in seed-saving techniques, crop diversity and gardening skills.

3. Integration phase. In the late 2000s, a bundle of new strategic targets pushed the enlargement of the organization. With the slogan ‘Eating up what we want to save’, Arche Noah built a wider network with home gardeners and farmers, incentivizing access and marketing of rare varieties in the form of seeds, plantlets and products through different channels (plant markets, Arche Noah shop, farmers markets). The focus shifted from ‘home gardeners’ to the urban consumer as a new target group. This was accompanied by systematic on-farm research – also within EU projects – to evaluate varieties and their potential uses. The cooperation with the organic seed company Reinsaat led to an ever-increasing demand from consumers and retailers. Since then, Arche Noah has been registering several heirloom varieties in the EU catalogue per year and offering seeds via the Arche Noah webshop.
4. Association phase. As of 2010, the political and international cooperation expanded: Arche Noah hosted several international policy workshops and advocated for seed law issues, in some cases directly in Brussels. In 2015, the cooperation with the campaign ‘No patents on seeds’ started. At the same time, Arche Noah started to coordinate the Austrian participatory tomato breeding network. The financing structure was expanded thanks to Austrian and European research funds and new fundraising models, first targeted sponsorships for on-farm breeding and multi-location fruit conservation. Since then, the number of employees and supporters of Arche Noah has remained stable, while noticing a shift from association members to sole financial donors.

### **Multiplying accessions and making accessions available**

In comparison to public genebanks, the association focuses more on cooperation with farmers and gardeners in terms of conserving, describing, spreading and further developing PGRFA. The activities of Arche Noah centre on the seed archive and the gardens which are responsible for maintenance, variety descriptions and seed quality testing. Documentation of each variety or

accession is supported by a database of text descriptions and photos for each variety or accession, complemented by data about the origin, plant health status, quantity and germination performance of harvested or stored seed lots. Recently, the database was enforced with crop-specific descriptors, mainly based on UPOV and IPGRI standards, but supplemented by more user-related descriptors such as taste, utilization class, yield or lodging.

Distribution of plant material (mainly seeds) is done via various production lines (Table 4). Seed surplus of non-registered varieties from the multiplication garden are offered as ‘Treasures from the Seed Archive’ in strictly limited quantities in accordance with the Austrian Seed Regulation revised in 2006 (RIS, 2025). Two additional production lines pursue the aim to provide seeds of registered varieties and varieties of non-regulated species on a regular basis to a wider audience, having the Arche Noah webshop and local sale points as main channels, but also serving major retailers such as a supermarket chain. These marketing activities are carried out by the associated company *Vielfalt Erleben*, which is fully owned by the non-profit Arche Noah. Most of the offered varieties are registered in the EU variety catalogue as “varieties with no intrinsic value for commercial crop production but developed for growing under particular conditions” and are multiplied by contracted farmers. The main target group for Arche Noah seeds are amateur gardeners in Austria and neighbouring countries (see Figure 3). Accessibility of rare fruit varieties is limited to a set that is managed by certified organic nurseries cooperating with Arche Noah. Due to plant health restrictions and the complexity of handling planting material (seasonality, storage, shipping), the assortment of available varieties is less dynamic compared to seed crops.

### **Conserving and managing PGRFA**

In Austria, there are approximately 150 active seed savers in the network of Arche Noah, contributing through growing, multiplying and collecting varieties. Some of them manage their private collection of varieties (with or without links to the Arche Noah seed archive), but many engage in coordinated conservation activities of the organization. As ‘guardians’, they test varieties of the seed archive in their location and collect data. Depending on season, location and personal preferences, they contribute new or confirm previous observations, and thus, add to the wealth of knowledge and perspectives regarding crop diversity. These seed-saver programmes cover both seeds and tubers as well as fruit crops. Seed guardianship can be permanent or temporary (alternating varieties annually). Fruit guardians are designated permanently with a minimum number of ten trees per location. To facilitate the exchange of plant material and contacts among seed savers and the broader public, Arche Noah digitalized the former *Seeds Handbook* to an online private database where seed and fruit tree savers can indicate their varieties via text and photos.

Table 4. Seed marketing lines of Arche Noah (2024 status)

	Line proven for home garden	Rare Vegetables	Treasures from the Seed Archive
Distribution start year	2009	2014	1998
Main target group	Beginner and advanced home gardeners	Advanced home gardeners	Advanced and professional home gardeners
Criteria for taking into the collection	Robust varieties of well-known vegetable crops, with reliable yield and good taste	Lesser-known vegetable crops, underutilized	History, traits, utilization and/or special usage properties
Flexibility	Consistent collection, 1–3 varieties change per year	Consistent collection, 1–3 varieties change per year	Annually or biennially changing collection
Variety registration	Registration usually as "varieties bred for cultivation under special conditions"	Usually, no registration necessary if species are not listed	No registration necessary subject to restrictions and quantity limit set out in the Austrian Seed Regulation 2006
Number of varieties	53	18	approx. 100
Varieties also sold to retailers	31	12	0 limited quantities due to legal restrictions



Figure 3. Sale of Arche Noah seeds at the annual seed fair in Wien (Austria).

Farmers multiply seeds in larger quantities for Arche Noah's seed sale. Some of them run farms specialized in vegetable seed production, and others are biodiverse farms selling vegetables or other produce. Together with Arche Noah, they carry out maintenance breeding of seed archive materials by positive or negative mass selection and selection of elite plants. The aims are to maintain the phenotype and to maintain or improve plant health and sensory quality. In addition to this partnership for seed multiplication, Arche Noah licences a label to organic farms which produce and sell rare varieties. In 2024, 32 farms registered for this label, the so-called Arche Noah Diversity Farms.

Some of the partner farmers of Arche Noah not only multiply, produce and sell rare varieties, but actively engage in further developing varieties by cross-breeding and selection. In 2010, the working group *Bauernparadeiser*, a participatory breeding group on tomatoes, was founded on the initiative of farmers due to a lack of organically bred tomato varieties to suit the needs of direct marketing. Conventional tomato breeding mainly breeds hybrids which neither can be reproduced on the farm nor adapted to local conditions, and sensory quality is often neglected. Currently, the group consists of 12 organic Austrian farmers, three research institutions and Arche Noah. The goal is the development of tasty and reproducible (open-pollinating) tomato varieties, featuring resistance to plant pathogens, by means of crossing heirloom and modern varieties. The group works, first, on varieties for greenhouse cultivation, resistant to the fungal pathogen *Cladosporium fulvum*, to Tobacco Mosaic Viruses and common root diseases and, second, on varieties for outdoor production, resistant to the fungal pathogens *Phytophthora infestans*, Early Blight (*Alternaria* spp.) and Septoria leaf spot (*Septoria lycopersici*), and less susceptible to fruit cracking. Since 2020, Arche Noah has coordinated participatory screening and breeding activities within other vegetable crops, such as sugar pea and winter radish.

### Networks

Apart from previously mentioned partners in the multiplication and marketing of plant material, Arche Noah is a partner in various research projects, being well-connected with national universities and research stations. The latter are permanent partners in screening and breeding activities. There is loose contact with several other vegetable breeders, mainly in Austria and Germany. Further, Arche Noah is an active member of the Balkan Seed Network Association. The organization was founded in 2021 by 16 organizations and institutions active throughout Southeastern Europe. The purpose of the network is to increase the conservation and sustainable use of PGRFA in agriculture. Activities aim at stimulating resilient food systems and establishing a paradigm of collaboration within the wider Balkan region, which has historically been shaped by conflict. The network consists of seed savers, breeders, scientists, farmers, gardeners, associations, organizations, research institutes

and educational institutions. In addition, being an active member of the Balkan Seed Network Association, Arche Noah has been supporting seed savers organizations in Southeastern Europe by awarding small-scale grants, provided by foundations.

### Perspectives

Arche Noah calls itself 'The Association for Preserving and Developing the Diversity of Cultivated Plants' and cooperation with diverse network partners is extremely relevant. Arche Noah considers both preserving and developing as equally important activities. Regarding conservation, Arche Noah makes increased use of long-term conservation at sub-zero temperatures of accessions, to enhance the capacities to study and distribute the most valuable accessions for use. The participatory breeding activities account for the necessity to let the accessions adapt and improve according to the needs of home gardeners and farmers. Arche Noah does not intend to be a sole breeding organization by any means, but rather a motivator and catalyst for organic breeding in Austria. Since breeding and cultivating diversity also requires suitable frameworks, Arche Noah wants to create the appropriate awareness and the political foundations so that the development and marketing of diverse seeds does not only happen in niches but can also be economically successful on a broader scale. Under the prevailing market economy conditions, diversity is a massive business disadvantage. It would therefore be naive to expect private and for-profit companies to do this work. However, since the preservation and further development of diverse seeds represent an indispensable basis for humanity's livelihood, it cannot be left solely to the dynamics of the market. Arche Noah therefore claims that conserving and breeding for diversity should become part of public services and corresponding programmes should largely be publicly financed.

### Conclusions

The three cases presented here show that CSBs in Europe can maintain and manage thousands of varieties, landraces and populations within broad networks of different actors such as private gardeners, farmers and horticulturists. They operate in diverse, decentralized agricultural and climatic environments. Their activities can be framed as Community Biodiversity Management, and they focus not only on mere conservation but also on the dynamic management of PGRFA. This allows evolutionary and adaptive processes to happen. All the described CSBs are well connected to the local/regional communities they are operating in. Because they provide facilitated access to PGRFA, mostly open-pollinated varieties free of intellectual property rights, they are an excellent partner for agricultural movements that advocate for the enhancement of diversity in farming systems. They can be an excellent partner for researchers too, because they can act as bridges between scientific and tacit knowledge and help scale up interesting and crucial ideas for the future of our seed and food system.



Moreover, CSBs can play an important and complementary role with regard to the *ex situ* system. In fact, they can be considered as an intermediary between farmers and the genebanks, receiving small samples from the banks and multiplying them before distributing them to farmers. Propagation activities of the CSBs, in collaboration with their networks, offer the opportunity to provide a larger quantity of seeds to the final users. Very often the small quantities of seed coming from genebanks are considered as an obstacle by farmers, who do not necessarily know that providing larger quantities is not the mandate of these institutions. The multiplication and regeneration done by CSBs can provide useful information about the agronomic value of PGRFA, which leads to a better understanding and knowledge of the variety or landrace itself. These processes, done by the CSB members, operate in very diverse agricultural and horticultural systems, under different climatic conditions and within different social and economic contexts. This opens opportunities to collect site-specific information on how PGRFA perform and could help in coping with climate change and other challenges in the future, providing knowledge that is also useful for future breeding for diversity efforts.

As described by the three case studies, the turning points for CSBs are at least partially related to a change in the legal system. These changes allowed RSR to market the seeds of populations, PSR to market the seeds of niche varieties and Arche Noah the ones of conservation varieties. This means that the operativity of such organizations is concretely impacted by the legal and political environment in which they are embedded, and which has historically been conceived to promote DUS varieties while leaving diversity outside of the picture.

As of the time of writing this article, the EU is reforming the rules on seed marketing, with a proposal released by the European Commission in July 2023, voted by the Parliament in April 2024 and revised by the Council during 2025, before the final approval by the Trilogue involving all three bodies. The proposal contains a series of derogations to the conventional system to allow more diversity and actors in the seed sector. For the first time, the concept of “dynamic management of diversity” by farmers is mentioned in a legal text and participatory plant breeding is defined as an activity which develops locally adapted varieties. Moreover, an article is dedicated to the exchange between farmers, with the aim of creating a harmonized rule across Europe with less space for different national interpretations. All these points need to be maintained in the final regulation, if an enabling environment is to be created in Europe. Only in this way the development of CBSs, a relatively new and highly relevant actor in the PGRFA community, can be supported and the space for more diversity be created in our seed and food systems.

## Author contributions

Conceptualization, RB, MGS, BB, HM, MA; methodology, RB, MGS, BB, HM, MA; validation, RB, MGS, BB, HM, MA; formal analysis, RB, MGS, BB, HM, MA; investigation, RB, MGS, BB, HM, MA; resources, RB, MGS, BB, HM, MA; data curation, RB, MGS, BB, HM, MA; writing-original draft preparation, RB, MGS, BB, HM, MA; writing-review and editing, RB, MGS; visualisation, RB, MGS; supervision, RB, MGS; project administration, RB, MGS; funding acquisition, RB, MGS, BB, HM, MA.

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## Conflict of interest statement

The authors declare no conflict of interest.

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# History and future of industrial crop accessions preserved by CREA-CI in Bologna and Rovigo, Italy

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**Abstract:** The conservation and exploitation of industrial crops at the Cereal and Industrial Crop Centre of the Council for Research in Agriculture and Economics (CREA-CI, the Bologna and Rovigo Research Centres) date back to the beginning of the 20th century and has led to the development of a germplasm bank containing 2,237 accessions. This collection reflects the multidisciplinary approach to the study of these crops and consists of wild relatives, traditional ecotypes and landraces collected in Italy and Europe, breeding lines and populations, as well as ancient and modern varieties. The main crops of this collection are sugar beet (*Beta vulgaris* L., 381 accessions), flax (*Linum usitatissimum* L., 283 accessions), hemp (*Cannabis sativa* L., 90 accessions), potato (*Solanum tuberosum* L., 45 accessions), sunflower (*Helianthus annuus* L., 95 accessions), several species of the Brassicales order (75 accessions), castor bean (*Ricinus communis* L., 18 accessions) and grain legumes (1,250 accessions).

This germplasm is maintained according to international standards; most of the accessions are stored in triple-layer vacuum bags and generally kept in two separate locations, at -20° to -25°C and/or in a cold chamber under low temperature (5°C) and low humidity, while the potato collection is maintained *in vitro*. Each of these crops has been studied using different approaches, including genetic and genomic studies as well as chemical analyses.

This article describes the genesis and the evolution of the collection preserved at CREA-CI and how these plant genetic resources are fundamental to facing climate change, and ensuring global food security and environmental sustainability.

**Keywords:** agrobiodiversity, plant germplasm, *ex situ* conservation, industrial crops, genebank

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## Introduction

Global climate change's effects on agricultural production are becoming increasingly evident (Kumar *et al*, 2022).

In addition to increasing drought and heat stress, factors such as overurbanization, deforestation, habitat destruction and soil depletion are responsible for

the occurrence of pest and disease populations, creating unfavourable future conditions for agricultural production.

In the context of a continuously growing global population, biodiversity conservation becomes a *conditio sine qua non* for guaranteeing food security and universal access to food – in other words, safeguarding our future (McCouch *et al*, 2013; FAO, 2024).

Unfortunately, in the past, breeding efforts have focused only on improving a few species and crop traits,

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leading to genetic bottlenecks and a sharp reduction in crop biodiversity (Reynolds and Atkin, 2021).

The changing environmental scenario poses an urgent need to modify the strategy for developing new plant varieties resistant to climate variations (Pixley and Cairns, 2023). Plant Genetic Resources (PGR), i.e. the cultivated germplasm, particularly landraces, wild relatives and exotic germplasm, are essential sources of genetic variability, valuable crop traits and foreign alleles that can help mitigate abiotic and biotic stresses and a reduction in agricultural production caused by climate change (Maxted *et al.*, 1997; Mercer and Perales, 2010; Lopes *et al.*, 2015). The need for *ex situ* conservation is, therefore, undeniable, as it ensures that PGR can be utilized in future breeding and genetic improvement programmes.

The first to draw attention to the importance of PGR for food security and to the danger of genetic erosion (loss of genetic diversity) was the Russian scientist Nikolai Vavilov. Vavilov himself described the importance of the Mediterranean region, including Italy, as a centre of origin of cultivated plants and biodiversity (Vavilov, 1926, 1992). A significant part of the Italian crop genetic diversity, accumulated over the centuries, has been lost due to genetic erosion, caused since the 1960s–1970s by the rapid spread of a few species and new and modern varieties. Paradoxically, it is not incorrect to say that agriculture itself has been the main cause of the decline in crop genetic diversity (Antonelli *et al.*, 2020). This process accelerated enormously with the abandonment of mountainous and marginal areas.

CREA, the Italian Research Council in Agriculture and Economics (i.e. the leading Italian research organization dedicated to the agri-food supply chains, supervised by the Italian Ministry of Agriculture, Food Sovereignty, and Forestry, MASAF), with its 12 different research centres, maintains a huge germplasm collection of 40,186 accessions, including cereals, vegetables, fruits, forages, industrial crops, forests and woody crops and medicinal plants. CREA characterizes and preserves these PGR with various conservation strategies: seed banking, tissue culture and arboreta (Vaccino *et al.*, 2024).

Within CREA, the Research Centre for Cereals and Industrial Crops (CREA-CI) is the one with the largest collection, with 16,469 accessions, and the number is constantly growing. Among CREA-CI Research Centres, the Institutes of Bologna and Rovigo have historically dealt with the characterization and breeding of major and minor industrial crops since their foundation.

The Bologna and Rovigo Research Centres are closely linked with two renowned Italian agronomists, Francesco Todaro (1864–1950) and Ottavio Munerati (1875–1949), respectively, who, at the beginning of the last century, were key figures in the advancement of Italian agriculture.

Francesco Todaro, professor of Agronomy and Crop Cultivation at the Alma Mater in Bologna, in 1920

was the founder and first Director of the Istituto per l'Allevamento Vegetale dei Cereali of Bologna (Institute of Plant Breeding for Cereal Cultivation) (Regio Decreto, 1920); in this Research Institute, now CREA-CI Bologna, he continued the characterization and breeding of cereals that had first begun at the Royal Station of Agriculture in Modena and after at the University of Bologna ([https://archiviostorico.unibo.it/System/27/508/todaro\\_francesco.pdf](https://archiviostorico.unibo.it/System/27/508/todaro_francesco.pdf); Felice (2011)). Not only cereals, but also forage crops such as alfalfa (*Medicago sativa* L.) and traditional fibre crops such as hemp have been the focus of this Institute from the outset. Over the decades, the number of agricultural species conserved grew in parallel with the number of researchers working in Bologna. Interest in grain legumes can be traced back to the 1960s, followed by other species, mainly sunflower, potato, flax, Brassicaceae, castor bean, etc.

The Rovigo Beet Institute was founded in 1914 by Professor Ottavio Munerati, who had already recognized in 1908 that the substantial contribution of sugar beets to technical, economic and social progress in agriculture required a significant genetic improvement programme, including this species in crop rotations (Munerati, 1933). The current headquarters of the Institute was built in 1951 with financing from the Marshall Plan. These funds were granted by American geneticists in recognition of Professor Munerati's long-standing partnership with American research institutions (Coons *et al.*, 1955).

The germplasm collection maintained in Bologna and Rovigo (Table 1) fully reflects the multidisciplinary work of agronomists, breeders, geneticists, chemists, biochemists and phytopathologists who have contributed over more than 100 years of the centres' history, and it now consists of 2,237 accessions. These are wild relatives, traditional ecotypes and local varieties collected in Italy and Europe, breeding lines and populations, as well as ancient or modern varieties, many of which developed in our research centres.

**Table 1.** Crop accessions maintained at CREA-CI Centres of Bologna and Rovigo.

Crop	No. of accessions
Beta spp.	381
Brassicales order	75
Castor bean	18
Flax	283
Grain legumes	1,250
Hemp	90
Potato	45
Sunflower	95
<b>Total number</b>	<b>2,237</b>

The vast majority of conserved accessions can be consulted within the European Search Catalogue for Plant Genetic Resources (EURISCO) (<http://eurisco.ecpgr.org/>; Weise *et al.* (2017); Kotni *et al.* (2023)), but missing from the database are all breeding materials, pop-

ulations, segregating progenies, lines under selection, and all accessions under phytopathological observation because, as far as possible, we try to conserve healthy material from the phytosanitary point of view. Considerable effort goes into the conservation of the collection. Since each crop species has unique characteristics and requirements, a specialized expert is assigned to oversee each crop.

Conservation, except for potato and some chemotypes of hemp, is mostly carried out in the form of seeds placed under vacuum-sealed, trilaminar aluminium bags and stored in low-temperature environments: (1) in cold chambers for the largest quantities of seed to be preserved (from 4° to 9°C depending on the species and facilities available, with low humidity; in our cold rooms, over 500g per accession are stored for about five years); (2) in freezers (from -20° to -25°C) for long-term preservation, up to ten years depending on the species and with a weight per sample of about 100 to 300g per accession. Seed viability tests are carried out at the moment of storage and on a regular basis to monitor losses in viability during storage. In our experience, each species has different times of seed viability decline, depending on multiple factors. Consequently, seed regeneration activities are planned according to the different needs of the various PGR.

### Beta collection

The seed collection consists of 381 accessions of the genus *Beta*, stored under low temperature (5°C) and low relative humidity (<10%) conditions.

One hundred ten (110) of these accessions are pollinators, while 271 are 'hybrids,' including some crosses of particular interest for genetic and genomic studies between the two interfertile subspecies of the genus *Beta*, *vulgaris* x *maritima*. The collection maintains many diploid accessions, 361, compared to only 20 tetraploids. Some of the accessions are suited for autumn sowing, and several show resistance to *Rhizomania*, *Cercospora* leaf spot, nematodes, and *Rhizoctonia* spp. (Figure 1). Pollinators, male sterile lines and O-type lines are well represented and have been the core of the breeding activity of the last years.

The Rovigo CREA-CI collection's main goal is to preserve the lines resulting from many years of genetic improvement work to develop *Beta* cultivars appropriate for autumn sowing.

Autumn varieties must have certain specialized traits, such as tolerance to low temperatures and the capacity to survive the potentially harsh climate over the winter. Reduced sensitivity to vernalization is another important trait that minimizes bolting when the crop resumes vegetative growth in spring.

Bolting, the first visible sign of reproductive transition in sugar beet, causes the mobilization of reserve sucrose from the roots, resulting in a loss of sugar content; additionally, the roots of bolted plants are more fibrous and of poor technological quality (Biancardi, 1999).

Therefore, selection must be accurate, using more effective methods, including inbreeding. However, bolting resistance should not be pushed to excessively high levels to avoid depressing sucrose production and causing problems in seed multiplication. Among the accessions, 8 tetraploid pollinators and 11 hybrids show identified traits of bolting resistance, with varying levels of productivity.

Another very important trait for sugar beet, well represented in the CREA-CI collection, is resistance to *Rhizomania*. This disease is caused by the Beet Necrotic Yellow Vein Virus (BNYVV) transmitted and inoculated into the roots by the fungus *Polymyxa betae*.

Selection has achieved significant milestones in the last 40 years, allowing high protection of crops with the use of resistant varieties. The first source of resistance to *Rhizomania* was found at the end of the 1960s in one accession of Italian origin, which also showed good resistance to *Cercospora beticola*. Starting from 1977, using germplasm preserved in the Rovigo *Beta* collection, mass selections were carried out on various monogerm male-sterile (CMS) lines and their maintainers (O-Type), and in 1988 a pollinator (RO401) was released and subsequently exploited to create several commercial varieties.

A recent study of *B. vulgaris* subsp. *maritima* populations, which Ottavio Munerati began collecting in the 1920s, discovered a significant association between hybrids *vulgaris* x *maritima* and resistance to *Rhizomania* (Biancardi et al, 2012).

The *B. vulgaris* collection preserved at CREA-CI, considering accessions with *Rhizomania* resistance, consists of 44 tetraploid pollinators and 191 hybrids.

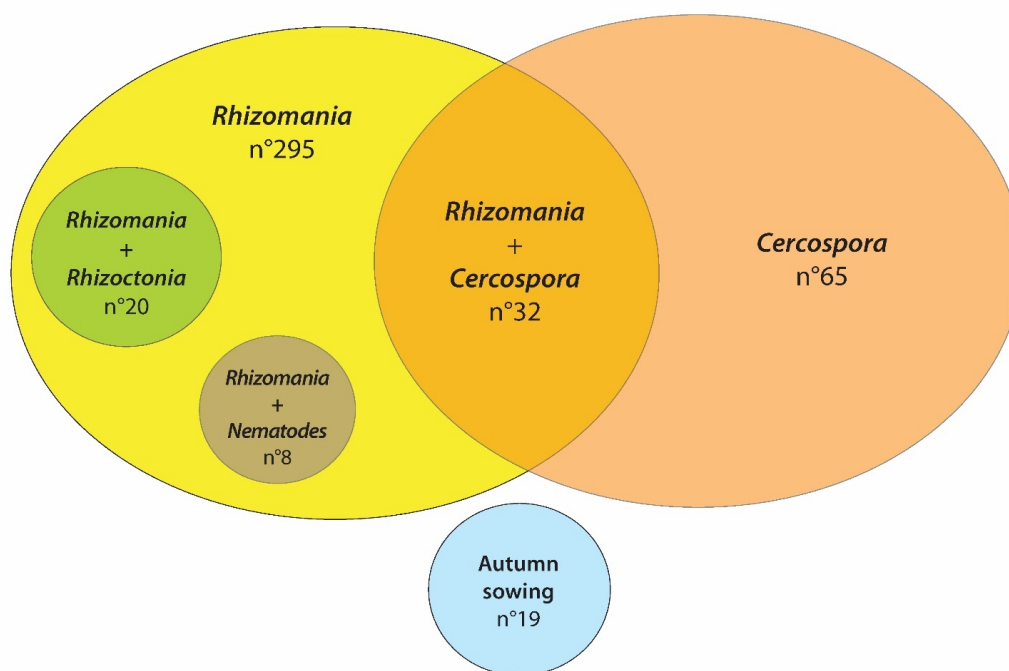
The disease caused by the fungus *C. beticola* is certainly today the main factor of productive and qualitative losses for beet cultivation in Italy and worldwide. This fungus causes characteristic necrotic spots on the foliage, leading to rapid desiccation.

The precise start date and basis of the selection for *Cercospora* Leaf Spot disease at the Rovigo Research Institute are unknown. Although Professor Munerati left behind some important publications, much of the knowledge from his notes and field annotations was lost during World War II; however, it is certain that by 1925 he had available disease-resistant lines whose seeds he made available for experimental trials in the USA (Biancardi et al, 2012).

The origin of this resistance is probably to be found in a progeny derived from crosses with the wild beet *B. vulgaris* subsp. *maritima*, which grew and still grows spontaneously along the Po di Levante embankment.

Subsequently, he started a breeding programme to eliminate some negative characters of *B. vulgaris* subsp. *maritima*, such as shallow root and tendency to annuity. Given the state of knowledge at the time, it was a challenging work, but Munerati managed to generate cultivable lines, albeit late-season, with increased sugar content and resistance to *Cercospora*, drought, and curly top disease.





**Figure 1.** Resistance traits distribution in the *Beta* CREA-CI collection of Rovigo (*Beta vulgaris* subsp. *vulgaris* x *maritima*)

His results were so interesting that with the seeds sent to the USA, it was possible to improve yields in California, where curly top disease was rampant, as well as in Colorado and Michigan. Notably, in 1946, the professor downplayed these results attributed to him, saying it was only a “modest contribution,” while after 90 years we now know that this was the only existing contribution to *Cercospora* resistance. Only in 2000, Biancardi, a former director of the Rovigo Centre, and other researchers showed that resistance to *Cercospora* is polygenic, relying on at least 4-5 gene pairs with effects that vary according to the level of infection (Koch and Jung, 2000; Skaracis and Biancardi, 2000).

The CREA-CI collection also includes, in addition to 17 tetraploid pollinators and 16 resistant hybrids, a certain number of accessions (20 pollinators and 12 hybrids) that combine *Rhizomania* resistance with *Cercospora* tolerance; these accessions are of particular interest in the perspective of ‘pyramiding’, i.e. stacking agronomically important genes in a single beet crop.

To conclude this brief overview of the *Beta* germplasm preserved at CREA-CI in Rovigo, it should also be noted the presence of other accessions that combine multiple resistance traits, particularly *Rhizomania* resistance with nematode resistance (8 pollinators) and *Rhizoctonia* resistance (2 pollinators and 18 hybrids).

In recent years, efforts have been focused on pollinator seed reproduction with resistance to *C. beticola* and on collecting wild material from the Po Delta area (Figure 2).

Breeding activities are also underway, with a particular focus on developing hybrids resistant to water stress and heat waves that have characterized recent years.

## Brassicales collection

For almost 30 years, CREA-CI in Bologna has been conducting applied research on plants of the Brassicaceae family which belongs to the Brassicales order, characterized by the presence in the plant tissues of the glucosinolate-myrosinase system, an effective defense strategy against many pathogens and insect pests (Liu et al, 2021).

Glucosinolates are specific secondary metabolites which, after a pathogen attack, are hydrolyzed by the endogenous myrosinase enzyme and release breakdown products, among which are the isothiocyanates (ITCs) with biocidal effects (Lazzeri et al, 2004).

There are over 140 glucosinolates identified (Blažević et al, 2020), with different profiles distinguishing genera and species of Brassicaceae (Agerbirk et al, 2021), a rich source of biodiversity, distributed worldwide with about 372 genera and 4,000 species.

The biofumigation technique, an environmentally friendly alternative to chemical fumigants, was developed by examining different Brassicaceae species for their ITCs biocidal properties as green manures in field applications (Lazzeri et al, 2003; D’Avino et al, 2004), displaying other environmental benefits, such as soil fertilization and biostimulant properties (Lazzeri et al, 2013). Furthermore, Brassicaceae seeds are characterized by an oil content ranging from 10% to 45% of their dry mass and by a variable fatty acid composition providing tribological features for lipochemistry formulations (Moser and Vaughn, 2012).

A seedbank was established over 20 years ago to conserve the germplasm of cultivated and wild



**Figure 2.** a, Collecting *Beta vulgaris* subsp. *maritima* on the Po River delta; b, A *B. vulgaris* subsp. *maritima* plant.

species, mainly of non-food Brassicaceae, provided by germplasm banks or by seed companies in order to identify new plants with high-value green chemicals to be studied by agronomists and chemists. The collection currently includes 84 accessions.

The species of the collection were characterized by evaluating: (1) their adaptability to cultivation in central-north Italy, where our experimental fields are located, selecting for high biomass yield and hardiness; (2) their seed glucosinolate profile and content according to the EU official ISO 9167-1 method, as described in the EU Commission Regulation No 1864/90 (EC, 1990), and based on the HPLC (High-Performance Liquid Chromatography) analysis; (3) their seed oil content and fatty acid composition, determined according to Conte et al (1989); Angelini et al (2015). Each accession was duplicated at least once every five years to regenerate the seeds.

To date, the Brassicales collection includes 73 species of Brassicaceae, 1 species of Cleomaceae, and 3 species of Resedaceae (see Supplemental Table 1 for a list of species). Within Brassicaceae, 53 are wild species; the remaining are mainly of *Brassica* genus, currently cultivated and selected mostly for their high biomass yield, hardiness and specific glucosinolate content in seeds or epigeal tissues, to be used as biofumigant green manures (*Brassica juncea* (L.) Czern.) or as biofumigant meals and pellets with fertilizing and amendment properties (*Brassica carinata* A. Braun and *Brassica nigra* (L.) W.D.J. Koch) (Lazzeri et al, 2013).

The CREA-CI collection contains varieties registered in the Italian Variety Catalogue such as *Brassica carinata* 'ISCI 7', *Brassica juncea* 'ISCI 99' and 'ISCI 20', *Brassica juncea* 'ISCI100red' and *Eruca sativa* (L.) 'Cav. Nemat',

included as components in patented biofumigant pellets, liquid foliar and root treatments (Figure 3).

An interspecific variation of the glucosinolate profiles among the species of our collection has been found, allowing us to characterize and identify most of them (Agerbirk et al, 2021).

The seed fatty acid analysis showed that in more than half of the species, primarily in the cultivated ones, a monounsaturated fatty acid is predominant, above all the erucic acid (C22:1), while in most of the wild species a polyunsaturated fatty acid, the alpha-linolenic acid (C18:3) is the most abundant (see Supplemental Table 1) (Lazzeri et al, 2013).

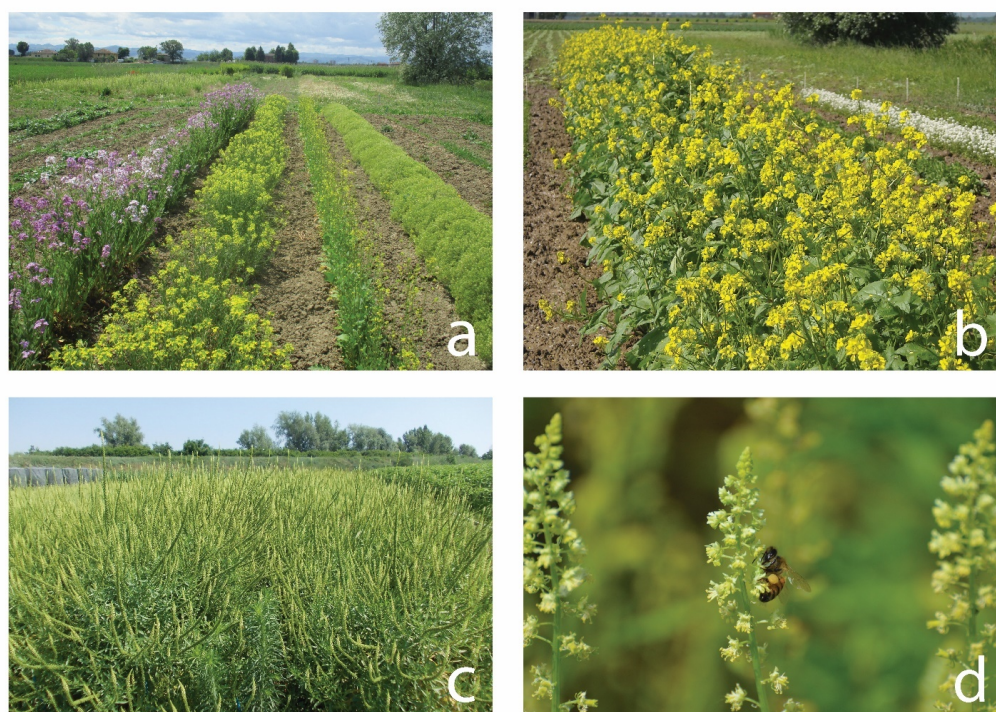
Many species of our collection are potential multifunctional plants, exhibiting different functions often exploited in the past. Some like *Barbarea verna* (Mill.) Asch., *Diploaxis eruroides* (L.) DC., *Raphanus raphanistrum* L., and *Rapistrum rugosum* (L.) All. have traditionally been locally consumed as edible plants. Others, such as *Barbarea vulgaris* W.T. Aiton, *Brassica montana* Pourr., *Diploaxis muralis* (L.) DC., *Diploaxis tenuifolia* (L.) DC., *Hesperis matronalis* L., and *Sinapis arvensis* L. are not only edible but have also been used as officinal plants (Figure 4a and b). Beyond their officinal values, *Isatis tinctoria* L. and *Reseda luteola* L. have been considered dyeing plants since mediaeval times (Figure 4c).

Considering that many Brassicaceae are melliferous (Filipiak, 2024), during their field cultivation and characterization, we observed that some of them were selectively attractive to pollinators. Our further interest was to obtain a preliminary visual estimate of the attractiveness of these species to honeybees and wild pollinators to identify the most visited.





**Figure 3.** a, Inflorescences of *Brassica juncea* 'ISCI 100red'; b, *Brassica juncea* 'ISCI 99' at full flowering; c, *Brassica juncea* 'ISCI 20'; d, Flowers of *Eruca sativa* 'Nemat'.



**Figure 4.** a, Wild species of the collection (Brassicaceae), at different flowering times during their field reproduction; b, *Sinapis arvensis* L. (Brassicaceae) at full flowering, a wild and indigenous species traditionally referred to as edible and officinal, melliferous and very attractive to pollinators; c, *Reseda luteola* L. (Resedaceae), at full flowering, a dyeing and officinal indigenous plant, a good melliferous species, attractive mainly to honeybees; d, Inflorescences of *Reseda lutea* L. (Resedaceae) at full flowering visited by a honeybee.

We then distinguished them for their different flowering times to hypothesize a long-term supply of food resources.

We focused on *Reseda lutea* L., a rustic wild species from the Resedaceae family, well adapted to extreme climatic conditions (Figure 4d), and attractive to different pollinators. Both these properties are fundamental for including a species in agroecological practices.

The collected data about the diversity of glucosinolate and fatty acid content and profile indicate the great potential of Brassicales germplasm to be used in more sustainable practices in agricultural systems offering a variety of environmental benefits, ranging from crop protection through biofumigation to increased soil fertility and agroecosystem resilience.

### Flax collection

Since 1988 (EEC Directive 1272/88, EC (1988)), flax/linseed (*Linum usitatissimum* L.) cultivation was favoured as an alternative crop introduced to face the EU deficit of oil for non-food uses (Zanetti et al, 2013).

Since 1989, field research at the former Experimental Institute for Industrial Crops in Bologna, now CREA-CI, has evaluated agronomic practices for the reintroduction of this crop (Cremaschi et al, 1995), while a flax and linseed germplasm collection has been established under the FAO-funded 'Risorse Genetiche Vegetali [plant genetic resources]' RGV Programme (Vaccino et al, 2024).

The current germplasm collection comprises 283 accessions with worldwide origins (Figure 5).

Given that flax/linseed breeding had been suspended in Italy for decades, the evaluation of the available genetic materials was a key priority in identifying cultivars adapted to the Italian climate.

Traditionally grown in autumn in Southern Italy until the middle of the last century, linseed has regained popularity also as a functional food due to its oil

and oil-derived products, opening up new commercial prospects. We therefore steered the research from flax genotypes towards linseed accessions and their seed oil content and fatty acid characterization.

The seed stock is regenerated at least once every five years when morphological observations, using appropriate descriptors according to Community Plant Variety Office technical protocol (CPVO, 2014), and phenological characterizations are regularly scored in order to assess the accession adaptability to the growing environment.

The germplasm was enriched by selected crosses between linseed varieties and ecotypes best performing in our area located in central-north Italy.

Systematic observations between 1989 and 2022 resulted in a dataset encompassing up to 252 varieties, possibly the most significant source of knowledge on the adaptation of this crop in Italy. This dataset was thoroughly examined, and summary data on oil content and fatty acid composition are shown in Figure 6 (Fila et al, 2024).

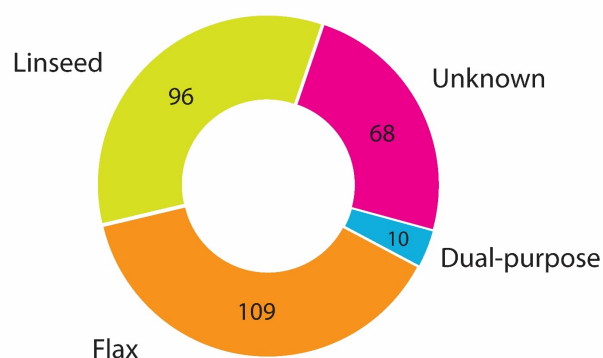
The seed average oil content (SOC) ranges from 35.4% to 47.9%, with a median value of 40.7%. The polyunsaturated fatty acid composition (PUFA, linoleic + linolenic acid) of the seed oil varies between 59.9% and 71.5% with a median of 65.4%. The ratio between polyunsaturated and saturated acids (PUFA/SFA) was in the 4.6–8.3 interval with a median of 6.2.

High temperatures typically exerted a detrimental influence on seed yield and seed oil content, while the fatty acid composition remained almost unchanged. A higher variability was observed in the response to rainfall, which, depending on the accession, exhibited both positive and negative effects on seed yield and oil content. This variability influenced fatty acids, particularly the monounsaturated fraction, which was predominantly reduced by rainfall.

Linseed adaptation for autumn planting was studied by comparing south Italian ecotypes rich in seed oil content and/or alpha-linolenic fatty acid (omega-3) with winter cultivars (Figure 7b,c). Autumn sowing, compared to spring sowing, increased seed yield by up to 79.4%, although oil content rose by only 1.6%. While saturated and monounsaturated fractions declined, the polyunsaturated fraction increased by a maximum of 13.1% (Fila et al, 2024).

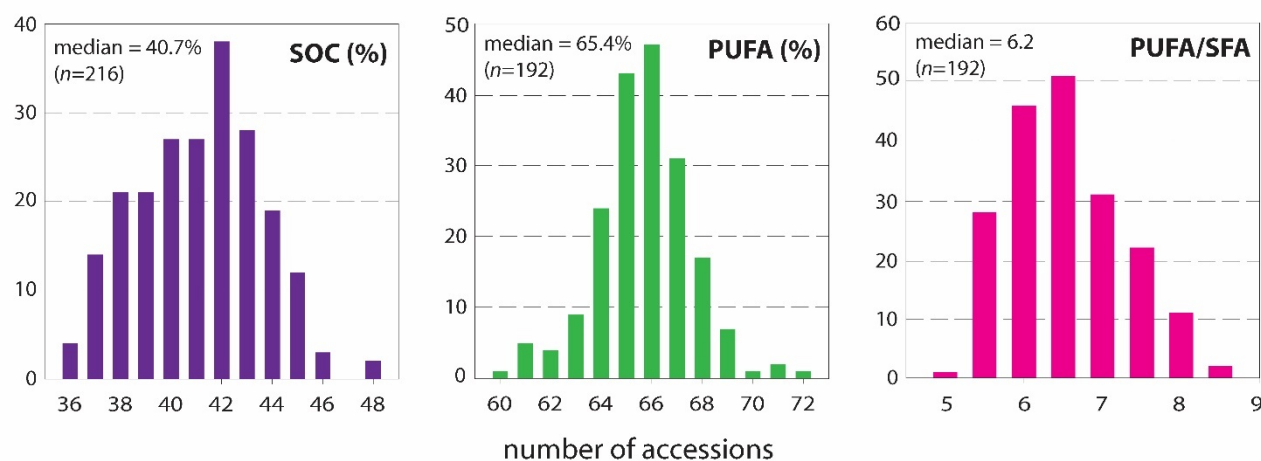
One of the tested accessions, considered a spring accession, consistently showed an omega-3 seed content exceeding 60% in autumn sowing (Tavarini and Angelini, 2016). After a mass selection, it was registered as 'Pepita' in the Italian National Variety List, the second cultivar of *L. usitatissimum* published by an Italian breeder (Figure 7a).

A 3-year field trial compared cultivars and southern Italian landraces and also evaluated climatic factors affecting linseed in our environment to identify genotypes suitable for quality-oriented dual-purpose cultivation for both seed/oil production and secondary fibres for the non-textile sector. The collection was effective in

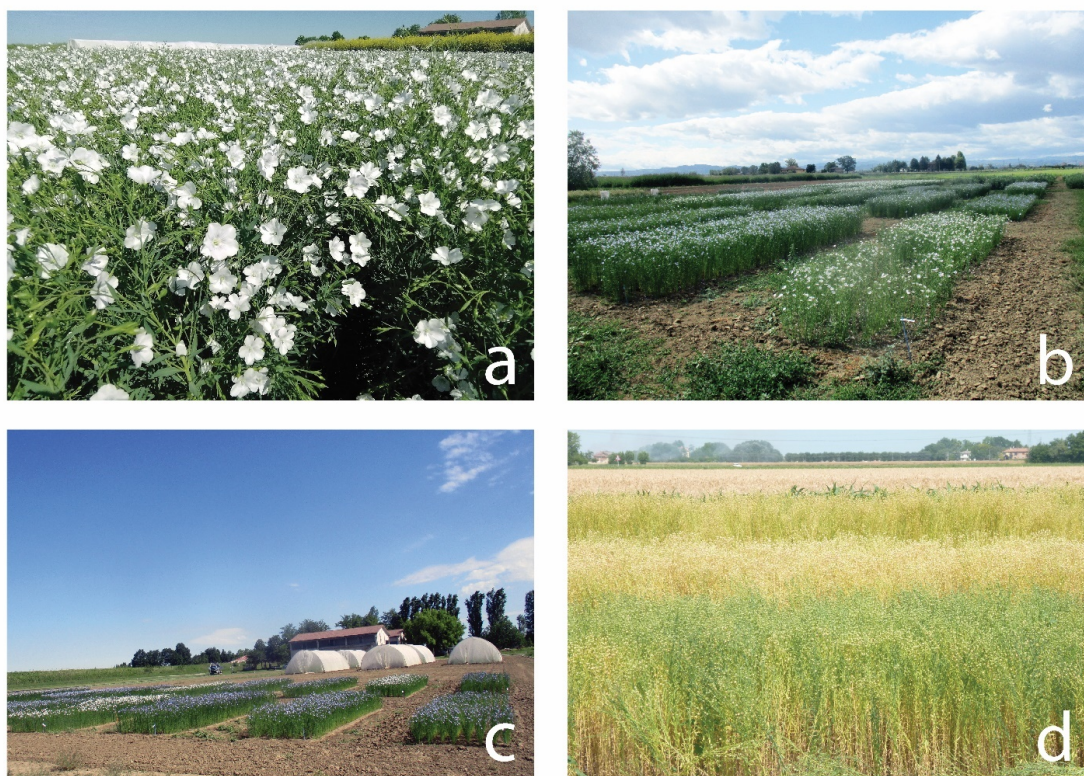


**Figure 5.** The CREA-CI collection includes the following accessions: 109 flax, 96 linseed, mostly ecotypes from Southern Italy, 68 unknown with uncertain suitability to the fibre or oil production and 10 designated as dual-purpose intended for both final outputs.





**Figure 6.** Distribution of accessions tested during 1989-2022 period in relation to seed oil content (SOC % w/w), polyunsaturated fraction (PUFA % w/w), and ratio of polyunsaturated to saturated fraction (PUFA/SFA, Elaborated from Fila *et al* (2024)).



**Figure 7.** a, ‘Pepita’, the new cultivar of *Linum usitatissimum* adapted to autumn sowing, with high omega-3 seed content; b and c, Different flowering times of several linseed accessions at the CREA-CI Experimental Farm of Anzola dell’ Emilia (Bologna); d, A 3-year field trial conducted in spring to identify accessions suitable for dual-purpose use. Flax cultivars, with long stems (upper portion of the image) were compared to linseed southern Italian landraces at different stages of stem maturity (lower portion of the image).

identifying a group of linseed accessions producing good seed yields (above 2t/ha), with a seed oil content of at least 40% and an alpha-linolenic content above 50% in northern Italy, and yielding significant amounts of fibre (0.3–0.44t/ha) and straw (2.5–3.0t/ha) (Figure 7d). Based on the intended cultivation purpose, this study provides guidance for selecting the best-performing cultivars from the accessions tested (Fila et al, 2018).

The reintroduction of flax/linseed in Italian environments as a low-input crop would be advantageous and appropriate for sustainable agricultural systems. This crop is easy to cultivate, requiring no specialized equipment, minimal water and chemical inputs, and having a short vegetative cycle. Data collected suggest that flax may provide interesting outcomes in terms of variety of fibre, oil and fatty acid content, enabling harvest quality to be tailored to the intended use.

Winter linseed cultivation, traditionally practiced in the south, was demonstrated to be feasible even at the study site in the north, thus expanding options for designing crop rotations and improving yields.

### Sunflower and castor bean collections

The CREA-CI experimental research unit in Osimo, belonging to the Bologna Research Centre and located in the Marche region, preserves a large amount of sunflower and castor bean accessions resulting from breeding programmes conducted since the early 1980s. Thanks to participation in the RGV/FAO Programme, in the last decade it was possible to undertake a serious recovery action which is essentially focused on two main objectives: (1) seed regeneration avoiding external pollen contaminations and using staggered sowings to elude the overlapping of flowering dates (Figures 8 and 10), and a morpho-phenological characterization of the collection, using descriptors that were specifically implemented according to UPOV or National Register of Varieties standards.

This multiplication activity began in 2011 with dedicated annual sowing and subsequent chemical analyses to measure oil content and fatty acid composition for both species. For the newly acquired lines, seeds were planted in a controlled environment to assess their phytosanitary status, adaptability and productivity, as well as to record their morpho-phenological characteristics.

Today, the collection maintains 95 accessions of sunflower and 18 of castor bean. Accessions are catalogued and stored in vacuum-sealed trilaminar aluminium bags. Bags are then stored in boxes and kept in a cold room at 4°C to extend the viability of the seed batches produced each year. This helps maintain acceptable germinability for seed batches for 5–8 years. At the same time, long-term storage in a dedicated freezer at -20°C is carried out.

### Sunflower

The starting material for sunflower breeding at CREA-CI consisted of Russian varieties of the Vniimk, Peredovik and Cerneanka types. After self-fertilization, homo-

neous base populations were selected and combinations of hybrids capable of improving yields in traditional sunflower-growing areas were generated (Kovacik and Skaloud, 1972; Fick, 1975). Pollinator maintainer lines (B) were selected, with the corresponding cytoplasmic male sterility (A) and other genes for the restoration of pollen fertility (R). Since 1996, several F2 populations, extracted from commercial hybrids, have been established annually and used for the selection of new R, B and A lines. This material not only provided a relatively inexpensive source of genetic variability but has facilitated the breeding activity because it was no longer necessary to use parental and related wild materials (DelGatto and Laureti, 2002; Laureti and DelGatto, 2004).

Subsequently, a study of general and specific combining ability (GCA and SCA) of the breeding lines was carried out (Serieys, 1994), to identify the testers for the following selection programmes, in addition to verifying the intrinsic value of experimental hybrids (Laureti and DelGatto, 2001). Some genotypes showed interesting productive performances with shorter biological cycle duration (DelGatto and Laureti, 1998; Laureti and DelGatto, 2000; DelGatto and Laureti, 2002). Nine of these genotypes ('Ausonia', 'Esperia', 'Kappa', 'Sigma', 'Tea', 'Mito', 'Gamma', 'Elly' and 'Lapo') were inscribed in the National Variety Register (Pirani et al, 1995) (see Supplemental Table 2 for in-depth descriptions).

In 1997, a breeding programme for high-oleic (HO) varieties was initiated, starting from F3 populations provided by the Sustainable Agriculture Institute of Cordoba (Spain) and from F2 commercial hybrid populations, with the aim to introgress the HO trait into conventional B and R lines. This allowed the identification of good individuals with interesting specific combinations Laureti and DelGatto (2001).

In 2003, F2 populations were extracted from HO commercial hybrids with good productive performances. After the selection of HO maintainer lines and the corresponding male sterility DelGatto and Laureti (2006), some lines, suitable for establishing valid hybrid combinations and used as testers in future evaluations, were identified (DelGatto and Laureti, 2005; DelGatto et al, 2005b,a).

This breeding activity released hundreds of experimental hybrid combinations, and a programme was carried out in several localities to verify their agronomic value (DelGatto and Laureti, 2005). In 2004 one of these HO hybrid, 'Crono', was registered in the National Register of Varieties (see Supplemental Table 3 for an in-depth description).

The collection subsequently was enriched with differential lines with varying resistance to *Plasmopara halstedii*: 9 of Hungarian origin and 12 provided by the US Department of Agriculture-Agricultural Research Service (USDA) Northern Crop Science Lab in Fargo. These accessions clearly distinguish the different races of the pathogen and provide a more comprehensive understanding of its spread in Italy.





**Figure 8.** Sunflower corollas of 25 different accessions of the CREA-CI Osimo collection.

Regarding the most recent activity, 91 sunflower lines were described, each accompanied by a significant photographic record, including images of the entire plant and close-ups of the inflorescence, as partially shown in [Figure 8](#).

For each line, 23 descriptors were recorded on leaf, flower, seed, plant architecture, etc. (for a complete list of descriptors and results, see [Supplemental Tables 4, 5, 6](#)).

In addition, analytical tests were carried out to: (1) measure oil content on dry matter using the nuclear magnetic resonance (NMR) method, and (2) create an acid profile of the extracted oil using a gas chromatographic analysis of the methyl esters of the fatty acids in the achene ([Supplemental Table 7](#)). A description of these analyses on a sub-sample of 53 accessions can be observed in [Figure 9](#).

The collection shows considerable variability in terms of biological cycle length, overall and in its sub-phases, plant height, flowering and maturation times, as well as seed and oil production. In particular, the variability in oil content expression in the achene is quite remarkable, with frequent high values for the species, contrary to what might be expected from inbred lines. The fatty acid content also shows significant variability, demonstrating interesting potential for future applications.

## Castor bean

The starting material for breeding at CREA-CI on castor bean is of US ('MC Nair 506', 'Pacific', 'Hale', 'Dale',

'Lynn', 'Cnesl'), Israeli ('H22') and French origin ('HD 912', 'H531', 'HD 913', 'Pronto'). All varieties introduced from abroad have shown an excessively long vegetative cycle in our climatic conditions. Therefore, it was necessary to reduce the life cycle duration and select crops suitable for mechanized agriculture ([Laureti, 1981, 1982](#)).

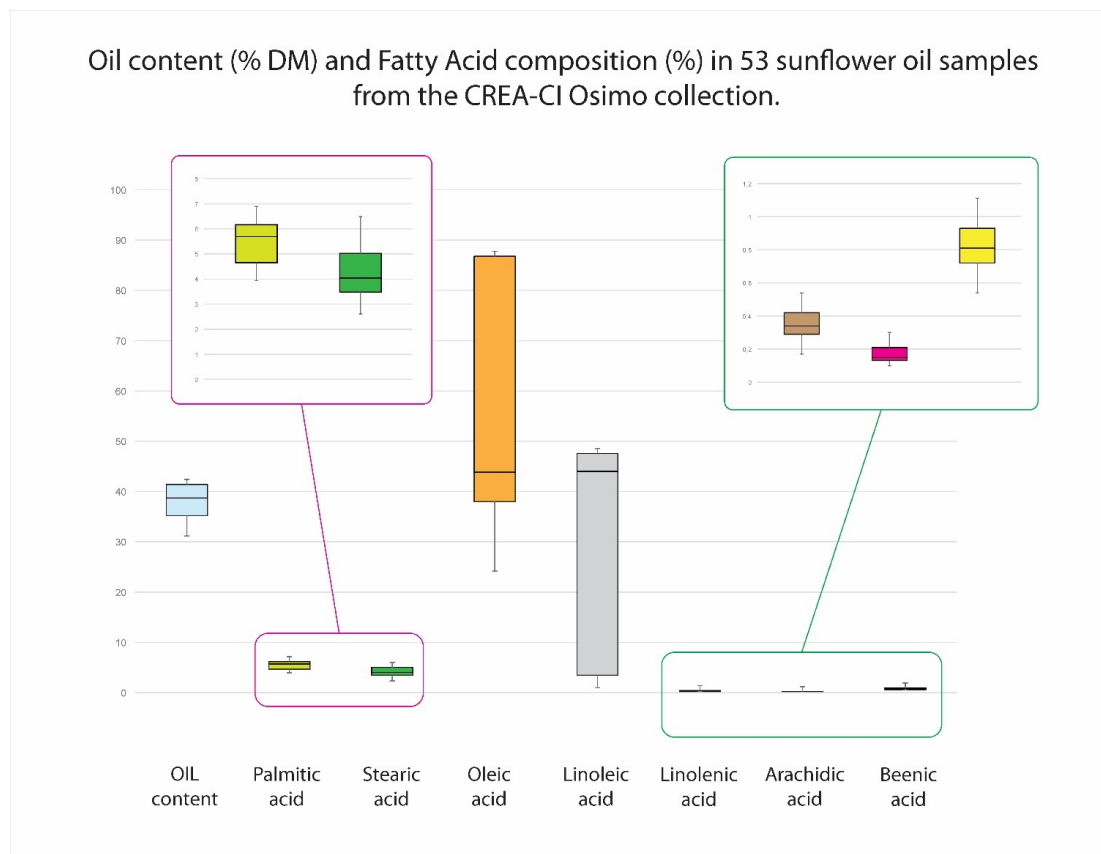
An ideotype appropriate for the Italian and European growing conditions was identified, with good productivity (more than 3.0t/ha) and, at the same time, a reduced size of the plant (about 1m). The development of hybrid cultivars required the selection of male-sterile (gynoecious) lines, and, over the years, 15 gynoecious lines were selected. At the same time, pollinator lines that would combine well with females were identified. Applying the general and specific combining ability, it was possible to identify about 50 monoecious inbred lines adapted to the Italian area and used to obtain experimental hybrid combinations ([Laureti, 1987](#)).

Since 1985, several experimental hybrids have been created and undergone agronomic evaluation in repeated trials over several years in both dry and irrigated environments ([Laureti, 1995](#)).

After an initial varietal comparison, two high hybrids, 'Castore' and 'Polluce', were released, both with excellent productivity and earliness traits, and two other low hybrids, 'Riscio' and 'Negus', were identified ([Supplemental Table 8](#)) ([Laureti, 1998, 2002](#)).

In 2023, the collection was enriched with 17 lines from the USDA germplasm bank ([Figure 10](#)).





**Figure 9.** Oil content (% Dry Matter) and fatty acid composition (%) in 53 accessions of the Osimo CREA-CI collection.



**Figure 10.** Characterization and regeneration of castor bean accessions at CREA-CI Osimo unit.

A photographic portfolio was also established to highlight the key traits of the plant's habitus and inflorescence. Additionally, the material was characterized based on 20 morpho-phenological specific descriptors (Supplemental Table 9).

Further studies would be interesting for determining the fatty acid content of the seed, an aspect that has not yet been explored.

## Hemp collection

Although some agronomic trials were carried out in Bologna before World War II, the establishment of the *Cannabis* germplasm collection, currently maintained both in Bologna and Rovigo, began in the mid-1950s. In 1953, the *Consorzio Nazionale Produttori Canapa* (National Hemp Producers Consortium) was established in Bologna at the Istituto di Allevamento Vegetale, now CREA-CI. In those years, several study missions were conducted across Europe, facilitating a rich exchange of plant material. Initially sourced from Germany, then from many other European countries, this exchange gave rise to the first nucleus of our collection (Ranalli and Casarini, 1998). The need to establish a collection of accessions from various origins and with wide genetic variability was prompted by the need to enrich the national varietal landscape, increase production performance and counterbalance the loss of hemp-growing areas.

Hemp is a naturally dioecious plant with predominantly anemophilous pollination. The traditional Italian varieties selected and released in Bologna have great intra-varietal genetic variability and can, therefore, be considered as populations (Allavena, 1961; Barbieri and Tedeschi, 1968). The establishment of new improved varieties was not sufficient to reverse the decline of the crop, which was mostly driven by competition with cotton and synthetic fibres.

The entire hemp supply chain almost disappeared during the 1970s and 1980s due to commercial and productive disinterest. However, the situation changed almost suddenly in the 1990s when renewed attention to the countless potential of this plant was observed, first in Europe and then in Italy (DiCandilo *et al.*, 2003). The research highlighted the new and different products derived from all components of the plant (stem, flower, seed) and their use in the most disparate production chains (pharmacological, automotive, food, and green building), thus bringing hemp back to the attention of the economic world not only as a fibre crop but as a multi-product plant that agronomically has interesting peculiarities due also to the low production inputs that its cultivation requires.

Collecting missions in various continents, as well as the exchange and purchase of seed from other European countries, where the study of this species had never been interrupted, revitalized the collection and reactivated subsequent studies and experimental activities (Faeti *et al.*, 1996; Forapani *et al.*, 2001; Mandolino *et al.*, 2002).

At the time of writing, the *Cannabis sativa* collection contains 90 accessions, both dioecious and monoecious, with different and multiple uses and genetically distinct chemotypes. This extraordinary ability to generate different uses of this species has led our research institute to activate two specific lines of research. One aims to characterize the germplasm for the production of terpenic substances for cosmetic, recreational and pharmaceutical uses (Pacifico *et al.*, 2008; Grassi and Partland, 2017; Pieracci *et al.*, 2021; Menga *et al.*, 2022), while the second line of research focuses on the characterization and development of hemp varieties for fibre/biomass or seed production in different Italian production areas (DiCandilo and Liberato, 2002; Ranalli and Venturi, 2004). Almost all of the materials in the collection are accessions from the latter industrial exploitation, and they are regenerated with funds from the ongoing RGV FAO 2023/2025 Programme (Vaccino *et al.*, 2024). The research on new germplasm material, along with its agronomic and chemical characterization, complements the activities undertaken.

The major requirement in the management of a collection of allogamous species such as hemp is great attention during the regeneration process to avoid any possible contamination by external pollen. To achieve this goal, different isolation strategies are used: (1) physical barriers when the genetic variability of the accession is restricted (Figure 11), i.e. the number of individuals of each population/variety is limited; (2) when seed regeneration is in very large fields, according to Italian regulations (Directive 2002/57/CE, EC (2002)) a distance of several kilometres between the different multiplications is required as the pollen of this species is very light and can fly over distances of up to a few kilometres.

As breeders of various varieties registered in the EU plant catalogue, we cannot forget the obligation to carry out a conservative selection according to different distinctive bio-morphological parameters during multiplication. Furthermore, molecular and/or chemical analyses are necessary to control the chemotype of the reproduced accession.

Since the 1990s, several varieties have been established for different industrial uses (see Table 2). The latest born is the dioecious variety 'Felsinea', which has recently been included in the UE Catalogue of Varieties (Figure 12). This cultivar was selected from a historical accession and has two very important characters – high fibre/biomass production and a block in the pathway of cannabinoids synthesis. This feature allows the accumulation of the Cannabigerol (CBG) cannabinoid, precursor to the synthesis of the two most common Cannabidiol (CBD) and Tetrahydrocannabinol (THC), thus reducing their synthesis. Several of our improved cultivars/lines selected for pharmaceutical use are also characterized by different blocks during cannabinoid synthesis, and for this reason, they must undergo the most stringent control tests during their multiplication.





Figure 11. Small isolators used in the reproduction of very low genetic variety hemp lines.

Table 2. Hemp cultivars selected by CREA-CI and included in the European Plant Variety Portal (EUPVP)

Cultivar name	Sexual determination	Use
Carmagnola	Dioicous	Fibre/Biomass
CS	Dioicous	Fibre/Biomass
Fibranova	Dioicous	Fibre/Biomass
Fibrante (ex Red Petiole)	Dioicous	Fibre/Biomass
Asso	Dioicous	Fibre/Biomass
Codimono	Monoecious	Seeds/Biomass
Carmaleonte	Monoecious	Seeds/Biomass
Eletta Campana	Dioicous	Fibre/Biomass
Felsinea	Dioicous	Fibre/Biomass



Figure 12. The new hemp cultivar ‘Felsinea’.



## Potato collection

The CREA-CI Bologna has a potato (*Solanum tuberosum* L.) collection maintained through *in vitro* culture. The conservation of potato germplasm has a primary goal – the maintenance of the health status of the propagating material, guaranteed by micropropagation of potato plantlets. For these reasons, for several decades, CREA has been preserving and enriching the *in vitro* collection with traditional varieties, local ecotypes, clonal selections and wild species, although complete phenotypic characterization has not always been possible. Currently, the collection consists of 45 potato genotypes (Table 3). Each genotype is preserved in quadruplicate in two climate-controlled rooms.

Over the years, the potato collection has provided also the basis for genetic improvement programmes with cross-breeding and field trials, obtaining new materials and varieties registered in the National Variety Register (RNV), characterized by the main useful traits for Italian potato cultivation: early maturity, adaptability to southern environments and fresh consumption, prolonged dormancy, low tendency to sweetness, adaptability to industrial processing, and resistance to pests, among others. As a result of the breeding activity, in the last ten years, the Bologna Center has registered ten potato varieties in the RNV.

A key role of the collection is also the preservation of recovering ecotypes from typical and marginal cultivation areas (Parisi *et al.*, 2022). Often grown at high altitudes, Italian potato ecotypes represent not only an important genetic pool to be preserved from the erosion typical of recent decades but also an economic opportunity for mountain communities. Indeed, in the last 30 years, the establishment and spread of modern varieties have led to the loss of many potato ecotypes. Since most ecotypes still cultivated locally are often multiplied without repeated virus-free plant purification processes, they exhibit viral deterioration with multiple infections, reduced plant vigour and low productivity. We recovered tubers from potato ecotypes in different regions of Italy and conducted serological and molecular tests to determine the putative presence of phytoviruses (potato virus Y (PVY), potato virus X (PVX), potato leafroll virus (PLRV)). Virus cleaning for ecotypes multiplied for decades on farm is a mandatory step before the insertion of these ecotypes in *in vitro* free stock plantlets collection. If the ecotypes test positive, a sanitation process must be conducted, which involves the extraction, insertion and maintenance of *in vitro* plants in the collection. These are subjected to meristem culture treatments and, if necessary, chemotherapy with ribavirin, regeneration, diagnostic checks and repeated treatments until complete sanitation is achieved. After regeneration, greenhouse acclimatization and tuberization, it is possible to produce healthy material. It is important to consider that, since the sanitation process can vary significantly in terms of duration depending on the quantity and quality of viruses detected in the serological investigation, the subsequent deployment of healthy tubers can take place

from a few months to several years after the sanitation activity.

In the last decade, CREA-CI Bologna contributed to the rediscovery, sanitation and multiplication of ten traditional ecotypes, including ‘Bianca di Starleggia’ and ‘Rossa di Starleggia’ (Lombardy), ‘Formazza’ (Piedmont), ‘Roti Oigje’ (Veneto), and ‘Crispa di Gavoi’ (Sardinia). ‘Riccione’ (Campania) was registered in 2012 in the National Register of Conservation Varieties (RNVC), becoming the first Italian potato ecotype to be listed in this specific register, aimed at regulating the reintroduction of local plant germplasm in the areas of origin, and its commercialization has also begun by the *O.P. Campania Patate Consortium*. The genetic profiles of 27 local Italian potato varieties, including those preserved in the CREA-CI potato collection, were determined. Their simple sequence repeat (SSR) profiles were compared among them, and with over 2,000 varieties belonging to EU Common Catalogue and SASA (Science and Advice for Scottish Agriculture) collection. Using 12 SSR markers we were able to discriminate all varieties, excluding known mutants (e.g. cultivars ‘Cara’ and ‘Red Cara’). Indeed, it is necessary to distinguish ecotypes unambiguously from the most used varieties, such as ‘Kennebec’, ‘Vitellotte’ and ‘Desiree’ in order to promote them properly and ensure their traceability (Mandolino *et al.*, 2015).

The potato collection also includes a huge variability in the composition and concentration (Pacífico *et al.*, 2024) of secondary metabolites (mainly steroidal glycoalkaloids and phenols). These metabolites have been shown to play a role in increasing plant ability to cope with environmental challenges, due to their biocide activity reported on insects, bacteria and fungi. They are also associated with health-promoting features, serving as nutraceuticals and pharmaceuticals (Calcio-Gaudino *et al.*, 2020), as well as additives for improving the shelf-life of fresh-cut fruits (Venturi and Bartolini, 2019).

Among the genotypes collected *in vitro*, the *Solanum tuberosum* x *Solanum berthaultii* advanced hybrid line, ‘Q115’, resulted particularly interesting as a putative source of genetic determinants of resistance to biotic stress, reduced or altered from the domestication. Thirteen advanced 4x-breeding clones derived from ‘Q 115-6’ and ‘Bionica’ crossing have been obtained at CREA-CI Bologna and some of those showed a good range of PTM (Potato Tuber Moth, *Phthorimaea operculella* Zeller) resistance. This resistance was measured as mortality during the early stages of larval development due to their skin content in caffeic acid and  $\alpha$ -chaconine (Pacífico and Musmeci, 2019).

Recently, from the biochemical characterization of peels of five potato genotypes present in the collection, we have demonstrated the great potential in the reuse or recovery of potato peel waste (PPW) from the agro-industrial potato processing. Raw extracts from the peel of ‘Lady Claire’, a processing variety conserved in the CREA-CI collection, proved to be the most suitable as a fungicide against fungal pathogens of cereals

Table 3. Overview of the potato collection at the CREA-CI Bologna

Accessions	Italian origin	CREA-CI selections	Anthocyanin rich	Carotenoids rich	Potato tuber moth tolerance
18 varieties	9	5	4	4	
12 clones	12	12	2	2	2
15 ecotypes	15				
45	36	17	6	6	2



Figure 13. Tubers of varieties and clones with different levels, distributions and types of anthocyanin content of skin and flesh.

(*Fusarium graminearum* and *Fusarium verticillioides*). Its effectiveness is mainly due to the activity of the phenolic fraction, which inhibited the tested fungi by up to 30% (Pacífico et al, 2024).

During the last decade, peel and flesh-pigmented potatoes (anthocyanins rich and carotenoids rich; Figure 13) were obtained, included in the collection and used in different research activities (Pacífico, 2018). Recently, some commercial potato varieties ('Bleuet'; purple skinned and fleshed tubers; 'Desiree', red-skinned and yellow-fleshed tubers and 'Kennebec', yellow-skinned and white-fleshed tubers), one advanced hybrid line ('98-11-1', purple parti-coloured skinned and fleshed tubers) and two Italian traditional ecotypes ('Bianca di Starleggia', yellow-skinned and white-fleshed tubers, and 'Rossa di Starleggia', red-skinned and yellow-fleshed tuber) were also tested at different altitudes grown either at the experimental farm of CREA, located in Budrio (Bologna area, 25 m.a.s.l.) and at Starleggia (Campodolcino, Valchiavenna, 1,560 m.a.s.l.). Preliminary results showed that the up-land environment influences the potato nutritional profile (Pacífico et al, 2022) and that potato antioxidant and antiinflammatory compounds, such as anthocyanins, could have

a preventive effect against LPS-induced inflammation in THP1 macrophages (Toccaceli et al, 2023)

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## Grain legumes collection

The research activity on Grain Legumes at the CREA-CI Bologna can be traced back to the 1960s with the first studies on *Phaseolus vulgaris* L. Breeding has always attempted to address problems associated with biotic stress, while also improving drought and high-temperature tolerance, quality, and optimizing the crop for various end-uses, including fresh consumption, dry grain production and the freezing industry. More than 40 common bean and pea varieties have been released over the past 50 years, and they have long served as Italian standard varieties (Ranalli, 1999; Ranalli and Parisi, 2000; Ranalli *et al*, 2004).

The grain legume collection at CREA-CI currently consists of 1,250 accessions with 27 species and 42 countries of origin represented (Figure 14). The most numerous species are common bean with 1,115 accessions, followed by chickpea with 30, runner bean with 23, lentil with 21. In the common bean collection, accessions can also be distinguished by their biological status: wild (285 accessions) and domesticated (830), traditional landraces (551) and modern cultivars (279); but also, further sub-clusters according to the type of product (snapbeans, Borlotto and Cannellino beans, black beans, kidney beans, etc.). As for the countries of origin, the most represented, in terms of the number of accessions, are: Guatemala (261), Italy and Mexico (201), Spain (119), USA (76), Colombia (61) and Portugal (39).

Over the past five years, the number of Italian landraces increased as a result of collecting trips to farms and local markets as well as the collaboration of regional institutions (e.g. in Liguria and Calabria, see Figure 15). The Iberian Peninsula has also substantially contributed to the collection, and together with Italy is the second largest centre of differentiation for this species since repeated crosses between Andean and Mesoamerican accessions have been shown to occur, facilitating the development of new genotypic and phenotypic diversity (Santalla *et al*, 2002; Angioi *et al*, 2010).

The last 25 years have seen a significant increase in the collection size, as marker-assisted selection (MAS) has facilitated characterization and accelerated pre-breeding. In addition, more cutting-edge techniques allowed the study of germplasm of different origins and

with much wider genetic variability (Rodriguez *et al*, 2016).

A significant example was the breeding initiative aimed at introducing different resistant sources for Root-Knot Nematodes (RKN) in common bean. Following the collection and characterization of resistant and tolerant accessions, markers associated with these resistances and new improved varieties and lines were developed (DelBianco *et al*, 2004; Carboni *et al*, 2004, 2005; DelBianco and Carboni, 2006; DiVito *et al*, 2007; DelBianco *et al*, 2007; Parisi *et al*, 2007). After a genomic analysis conducted on over 400 wild and 400 domesticated accessions of Mesoamerican and Andean gene pools, three wild and five cultivated accessions showing resistance were found.

A subsequent research project involved extensive phenotypic and genetic characterization of 192 genotypes, mostly landraces, with over 40 bio-morphological descriptors across two environments over two years. The data collected showed a high level of genetic diversity, especially for characters associated with flowering and 100-seed weight. A subsequent genome-wide association study (GWAS) enables: (1) the definition of the genetic structure of European germplasm, (2) the identification of markers and favourable alleles in genotypes that perform better under various environmental conditions, and (3) the identification of seven SNPs associated with flowering character control (Caproni *et al*, 2019; Raggi *et al*, 2019).

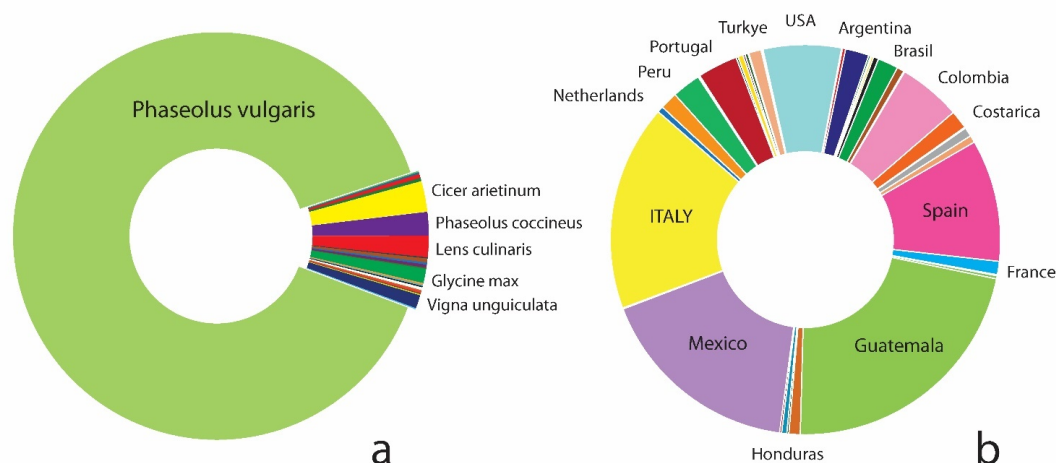
A distinguishing characteristic of the Leguminosae family is that it is a valuable source of plant protein, and the collection is constantly analyzed to determine the total protein content of the seeds (Figure 16).

These boxplots are based on the average values of at least two to three reproduction cycles for every accession and the data are summarized by different bean cluster or sub-cluster of commercial type. In particular, the first three boxplots Mesoamerica gene pool (MG), Andean gene pool (AG) and European landraces (EL) were calculated on homogeneous groups in number, 180 accessions each. The fourth boxplot, Italian and European cultivar (IE) could be subdivided according to different categories of market end-products: Borlotto type with 28 accessions, Yellow Romano type with 11 and Snapbean type with 40.

The MG's distribution is significantly more homogeneous than that of the AG group, and the South American group's 75th percentile is even lower than the 25th percentile of the MG group.

The graph also demonstrates that the higher protein content is found in the MG group, with a maximum of 35.62% dry matter, and in the EL group. The IE group has a protein content distribution similar to that of the AG group. A closer look at the IE group reveals that three distinct subclusters show different behaviours: the Borlotto type exhibits very low protein production values; the Yellow Romano type appears to show less genetic variability and a strong correlation between pod phenotype and high protein production; and the





**Figure 14.** a, Species and b, Countries of origin of the accessions maintained in the grain legume collection.



**Figure 15.** Examples of Italian agrobiodiversity maintained in the grain legume collection: a, b and c are Ligurian landraces (respectively 'Pisello nero di L'Ago', 'Fagiolana di Torza', 'Fagiolo di Mangia'); d, e and f are Calabrian landraces ('Russa Janca', 'Capomacchia', 'Cocò gialla').

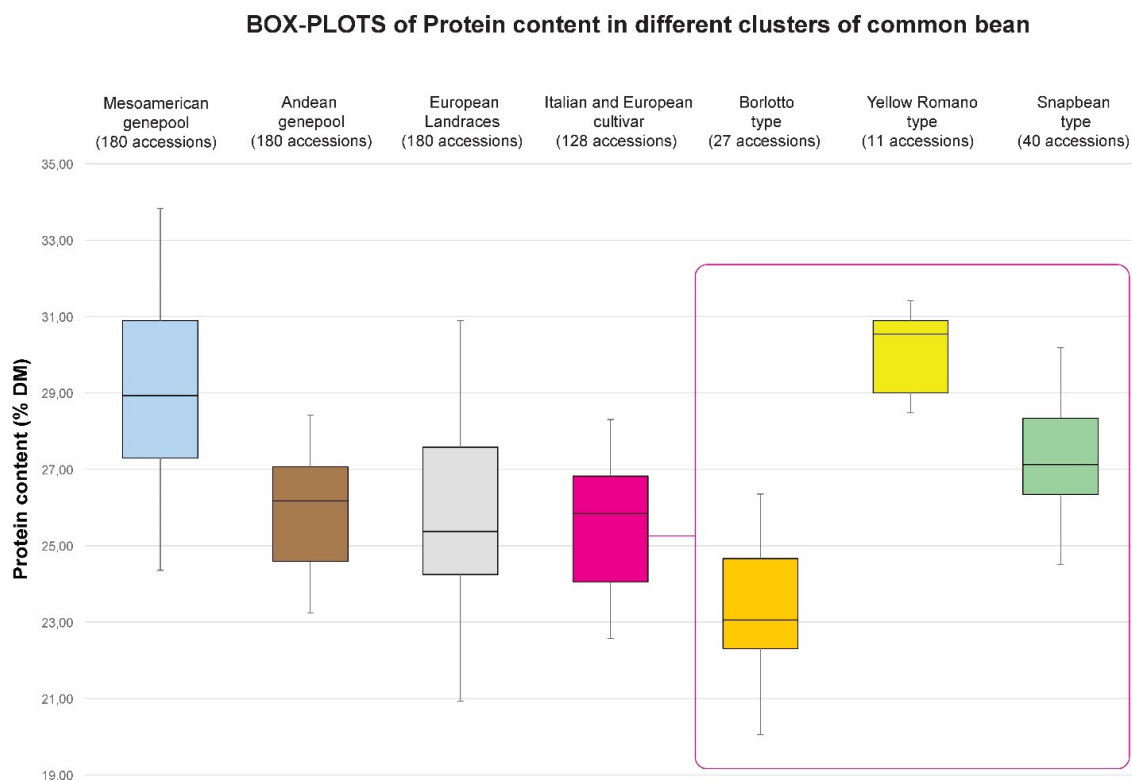
Snapbean type is intermediate and different from the other two.

It is interesting to note that traditional breeding, focused in the past mainly on resistance genes or macro-morphological traits of seed and pod quality, never evaluated the protein production trait. This bottleneck is evident comparing the Borlotto type with the other two classes of beans (Yellow Romano and Snapbean). In the case of Borlotto, the selection was traditionally carried out by looking at the brightness of the red colour of seed and pod, according to the preference of the Italian consumers or the freezing industry. Yellow Romano type and Snapbean, although not selected for

seed production, show significantly higher protein yields when harvested as dry seed than the Borlotto type. This is an example of how a well-characterized germplasm collection can become an essential tool for correcting unwitting genetic drift.

## Conclusions

More than 20 years ago, when the Italian Ministry of Agriculture, prompted by visionary colleagues such as Professor Carlo Fideghelli, called for action to better preserve the germplasm accumulated within its research



**Figure 16.** Box-plots of protein content (%DM, dry matter percentage) in different clusters of the CREA-CI common bean collection.

institutes, the scientific community initially struggled to understand the reason for this initiative.

For most of the older staff, who may have perceived the duty of sharing materials as a risk of losing possession of ‘their’ PGR and the associated knowledge, training activities and the need to regenerate stored accessions initially seemed disconnected from their daily research activity.

This process was gradual but necessary, making it possible to establish a ‘dispersed’ germplasm bank of 40,186 accessions (considering all CREA collections) and to create a network and a critical mass that is now more aware and active in germplasm preservation and exchange (Vaccino *et al*, 2024). However, such a radical change in perspective has not always been linear.

The diverse educational background of those working with PGR in Bologna and Rovigo is indicative of biodiversity itself: we are agronomists, geneticists, biologists and biotechnologists with distinct specializations. Yet, perhaps because of our different approaches to PGR, we have developed a level of teamwork that was not so obvious at the beginning but is now accelerating in an unexpected, engaging and more conscious way.

The 2,237 accessions preserved in Bologna and Rovigo are the result of the work of many researchers over more than 100 years. This collection functions as a living organism, having experienced challenges over

the course of its history: losses from different causes followed by frequent renewals.

These PGR are well characterized from different points of view: from phytopathological tests to the latest and innovative chemical and genomic analyses; from traditional agronomic trials to frontier pharmacological, nutraceutical, medical, food and industrial uses. These accessions aim to provide pollinating insects with food support or to supply essential secondary metabolites in an emerging ecologically friendly and sustainable agriculture. They are the outcome of selection aimed at mitigating the effects of climate change, which is forcing us to deal with drought and extreme temperatures as well as new biotic stresses.

The next crucial steps include adding further characterization data to the passport data that identify each accession Anglin *et al* (2018); Kumar *et al* (2024) as well as a greater openness to exchange PGR. Until now, these resources have only been made available through scientific collaboration agreements. Expanding access will be a key challenge in the coming years.

### Supplemental data

**Supplemental Table 1.** Description of the Brassicales collection: status, major glycosinolates, major fatty acid.

**Supplemental Table 2.** Sunflower hybrids registered in the National Variety Register.

**Supplemental Table 3.** Sunflower hybrids with high oleic acid content in the National Variety Register.

[Supplemental Table 4](#). Descriptive traits recorded on 46 sunflower lines in 2021.

[Supplemental Table 5](#). Descriptive traits recorded on 25 sunflower lines in 2022.

[Supplemental Table 6](#). Descriptive traits recorded on 22 sunflower restorer in 2022.

[Supplemental Table 7](#). Oil content and analysis of the fatty acid spectrum contained in sunflower lines.

[Supplemental Table 8](#). Description of the hybrids produced at Osimo CREA-CI Unit.

[Supplemental Table 9](#). Descriptive traits recorded on 14 castor bean lines in 2021.

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## Authors contribution

Andrea Carboni was responsible for the initial conception and design of the article, and wrote the draft of the introduction, discussion and conclusion. Each of the authors described the status of their collection for their respective species and can be contacted via e-mail for enquiries: Ilaria Alberti (ilaria.alberti@crea.gov.it) for sugar beet, Manuela Bagatta (manuela.bagatta@crea.gov.it) for Brassicales and flax, Andrea Carboni (andrea.carboni@crea.gov.it) for Grain Legume, Andrea Del Gatto (andrea.delgatto@crea.gov.it) for sunflower and castor beans, Massimo Montanari (massimo.montanari@crea.gov.it) for hemp, and Daniela Pacifico (daniela.pacifico@crea.gov.it) for the potato collection.

All authors read and approved the final manuscript.

## Conflict of interest statement

The authors declare that they have no conflicts of interest.

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# Safeguarding, evaluating and valorizing fruit tree genetic resources in Belgium: Insights from nearly half a century of unsprayed orchard management

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**Abstract:** In response to the rapid genetic erosion threatening Belgium's fruit tree cultivar heritage, the Walloon Agricultural Research Centre (CRA-W, Gembloux, Belgium) initiated nationwide prospection campaigns in 1975 with support from citizens. These campaigns aimed to collect and conserve the country's highly diverse fruit tree genetic resources (FTGR), including historically significant amateur-bred and landrace cultivars, for future breeding efforts.

Since then, the CRA-W has maintained a diverse collection – primarily apples (1,629 accessions) and pears (1,198 accessions), but also cherries (355 accessions), plums (236 accessions), grapes (98 accessions), and peaches (29 accessions) – in *ex situ* unsprayed repository and experimental evaluation orchards.

This approach makes it possible to assess these cultivars for multiple traits related to their tolerance and adaptability to biotic and abiotic stresses. This long-term evaluation method enables the identification of numerous quantitative traits and their impact on robustness and stress tolerance. Moreover, CRA-W has actively sought ways to promote the sustainable use of FTGR through partnerships with public institutions, private stakeholders and citizens. One key initiative was the gradual establishment of a nursery network governed by a participatory fruit tree quality charter, coupled with a traceability system for high-quality propagation material.

This initiative led to the release of 33 well-performing heritage cultivars, notable for their sufficient robustness and disease tolerance, for use in both amateur and professional orchards. A decade later, a dedicated apple pre-breeding and breeding programme was launched to harness the extensive FTGR collection as a source of quantitative disease tolerance, robustness and quality traits.

**Keywords:** *Malus x domestica*, *Pyrus communis*, disease tolerance, robustness, untreated organic evaluation orchard, participatory breeding, collaborative breeding, low-input organic farming

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## The origins of the fruit tree genetic resources collections at Gembloux

The establishment of the fruit tree genetic resources collection and its evaluation was initiated in 1975 (Populer, 1975) at the State Plant Pathology Station of the former Agricultural Research Centre of Gembloux (CRA, Gembloux, Belgium) under the leadership of plant pathologist Charles Populer.

Populer's initiative (Populer, 1979) stemmed from the observation that most cultivated apple (*Malus × domestica* Borkh.) and pear (*Pyrus communis* L.) trees offered by nurseries to both amateur and professional growers were highly susceptible to diseases such as scab (*Venturia inaequalis* on apples and *Venturia pirina* on pears). Additionally, the genetic diversity of these cultivars was quite limited, and most breeding programmes at the time focused on introducing monogenic resistance genes to improve apple tree resistance to apple scab.

It therefore seemed wise to begin collecting cultivars adapted to local climatic conditions that had been cultivated before the advent of modern fungicides (pre-World War II) and before the widespread use of Bordeaux mixture (late 19<sup>th</sup> century).

During the same period (between 1975 and 1980), several independent initiatives emerged across Western Europe, aiming to collect the remaining old fruit tree varieties, particularly apple trees. In 1975, Corbaz and Stoll began surveys in Switzerland (Corbaz, 1983). In France, similar projects started in 1979, including Leterme's work at the Landes Regional Park (Leterme, 1983), and in 1982, Stievenard (1999) initiated a programme to conserve and develop local and heirloom fruit varieties in northern France at Villeneuve d'Ascq. In the Netherlands, Blommers (1983) and in Spain, Dapena (1996), also organized surveys in 1974 and 1987, respectively.

At the Walloon Agricultural Research Centre (CRA-W), the first intensive survey period occurred between 1975 and 1985. Initially, efforts focused on visiting historical horticultural formal collections (1975–1980), where materials were collected based on criteria outlined in Table 1. Ten Belgian collections – five in the Flemish Region, four in the Walloon Region and one in the Brussels-Capital Region – were surveyed, resulting in the collection of 620 pear and 580 apple accessions. Thirty-four accessions were recovered from foreign national collections (England, Brogdale Farm, Kent, and France, INRAE, Angers), and 160 were collected from private citizens. This effort unexpectedly led to many historical collection managers dismantling their collections, arguing they were safeguarded at Gembloux.

The programme's next phase was significantly boosted by widespread public interest, driven by media coverage in the press, radio and television highlighting efforts to preserve fruit tree heritage (Populer et al, 1998). Between 1980 and 1987, over 2,000 individuals contacted the institute, reporting hundreds of endangered old fruit trees in gardens and orchard meadows and requesting assistance in preserving them. In response, intensive prospection campaigns were organized across the Walloon Region. During this period, numerous landraces and previously unknown apple, pear, plum, cherry and peach varieties were collected. By 1987, the collection had grown to 2,181 accessions.

Each collecting mission involved engaging with tree owners to learn about the varieties' qualities, traits and uses, and to gather valuable ethnobotanical knowledge.

Post-1987, the collection continued to expand through collaborations with institutions such as the *Proefstation voor de Fruitteelt* (Wilhelminadorp, The Netherlands), the *Centre Régional de Ressources Génétiques de Villeneuve d'Ascq* (France), the *Station d'Amélioration des Espèces Fruitières et Ornementales* (INRAE, Angers, France), the Long Ashton Research Station (University of Bristol, Great Britain), the University of Illinois (USA), the Institute of Experimental Botany (Prague, Czech Republic), and the Research and Breeding Institute of Pomology (Holovousy, Czech Republic), reaching 2,526 accessions by 1997. About one-third of these accessions came from partner collections, while two-thirds were sourced from the countryside with citizens' assistance.

This extraordinary public engagement attracted the attention of the European Cooperative Programme for Plant Genetic Resources (ECPGR) and was presented at its Second Steering Committee Meeting in Oeiras (Portugal) in 1984 titled: 'Mobilization of Public Opinion (Including Practical Involvement of the Public) in the Preservation of Fruit Tree Genetic Resources' (ECPGR, 1984).

Regarding the selection of plant material (budwood) of cultivars to be introduced in a fruit tree collection (genebank), curators must prioritize based on objectives and available resources. Table 1 outlines the main criteria used for introducing varieties into the Walloon Agricultural Research Centre (CRA-W) collection.

Currently, collecting activities have slowed and are primarily driven by public requests for pomological consultations. Each year, dozens of fruit identification requests are received through a standardized template that includes contact information, sampling location and details on tree characteristics, fruit traits, uses and history. Annually, 300 to 900 fruit samples (mainly apple and pear) are submitted, though only a few are selected for inclusion in the collection. Depending on selection outcomes and consultation context (e.g. local survey for developing a regional repository orchard), budwood may be requested for propagation to be introduced into the collection or planted in local repository orchards. Upon receiving budwood, labels and passport data are

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**Table 1.** Criteria for selecting varieties introduced into the fruit genetic resources collection of CRA-W (Populer, 1980; Lateur and Populer, 1996)

Accessions collected from historical horticultural formal collections (1975-80)	Accessions collected with the help of the public from private gardens and orchards (ongoing process)
Varieties of Belgian origin	Named varieties, of local origin and with a local history and use
Varieties from neighbouring countries with similar climates to Belgium.	Varieties, even unnamed, that perform well against the main pests and diseases and/or abiotic stresses, which express good robustness.
Varieties dating back to before fungicide use (before 1850).	Varieties that significantly enhance existing diversity (hardiness, quality, storage ability, etc.).
Varieties noted in literature for good disease resistance/tolerance.	
Varieties at risk of extinction and absent from other ECPGR collections.	

recorded, initiating a traceability process from storage and propagation to nursery monitoring, inventory, and eventual tree lifting and planting.

A key principle of this programme has been to offer donors a young tree after successful propagation. This win-win approach acknowledges contributors by providing one or two young trees of the conserved variety and supports pragmatic on-site conservation by replanting original varieties in their native locations.

### Definitions and categories of ‘Old Fruit Varieties’

The main hypothesis of the CRA-W Biodiversity and Plant & Forest Breeding Research Unit is that cultivars selected and propagated before the widespread use of fungicides underwent stronger selection pressures, leading to natural selection of more robust varieties that could thrive even without phytopharmaceutical treatments. This makes them more likely to exhibit greater tolerance to fungal diseases. Similarly, cultivars selected and released prior to the significant shifts in agricultural practices following World War I and World War II – and before the extensive use of mineral fertilizers – are presumed to be more resilient and better suited for low-input organic agriculture.

To support this hypothesis, we propose a classification system for pome fruit cultivars based on the period when they were first documented (Figure 1):

- Cultivars mentioned before 1760 are categorized as ‘ancient’
- Cultivars mentioned between 1761 and 1850 are classified as ‘very old’
- Cultivars first mentioned between 1851 and 1914 are designated as ‘old’
- Cultivars mentioned between 1915 and 1945 are labelled as ‘pre-modern’
- Cultivars mentioned after 1945 are categorized as ‘modern’.

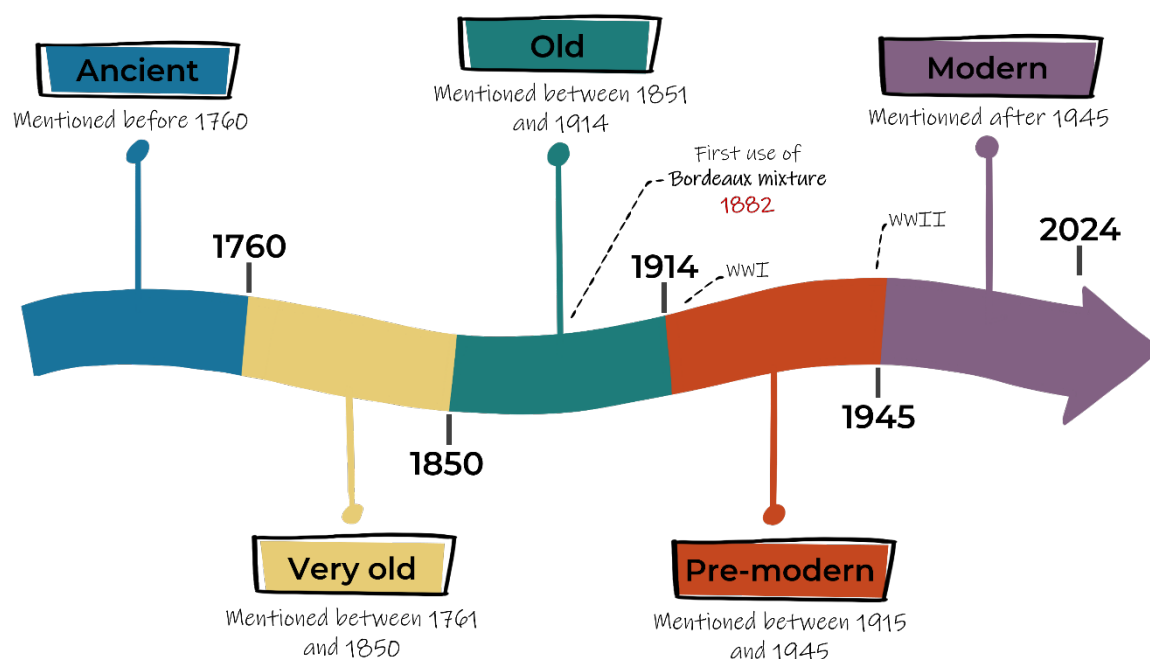
This classification framework helps to contextualize the historical development and adaptive traits of apple and pear cultivars across different agricultural eras.

### Defining the concept of landraces for fruit trees

The concept of landraces, introduced by von Rümker (von Rümker, 1908), originally referred to locally grown cultivars that were not consciously selected. Camacho-Villa *et al* (2005) further defined this concept highlighting the evolution of a genetically diverse and dynamic population. However, this definition primarily applies to seed-propagated crops. Negri *et al* (2009) expanded the definition of landraces for seed-propagated crops as follows: “A landrace of a seed-propagated crop can be defined as a variable population, which is identifiable and usually has a local name. It lacks “formal” crop improvement, is characterized by a specific adaptation to the environmental conditions of the area of cultivation (tolerant to the biotic and abiotic stresses of that area) and is closely associated with the traditional uses, knowledge, habits, dialects, and celebrations of the people who developed and continue to grow it”. However, perennial plants like fruit trees are predominantly propagated vegetatively, resulting in clonal populations (e.g. groups of trees that all have the same genome because they have been vegetatively propagated e.g. by grafting). Therefore, the concept of landraces must be adapted and redefined for these specific crops.

Historically, farmers propagated pome fruit from open-pollinated seedlings collected in their surroundings. Through mass selection, some of these seedlings (known as ‘chance seedlings’) occasionally gave rise to new landraces. The most promising ones were propagated vegetatively within limited areas. The less interesting ones were used as rootstocks and grafted with landraces to make high-stem trees for orchard meadows. The landrace cultivars propagated by rural communities were usually well-adapted to local needs, uses and environmental conditions, including biotic and abiotic stresses.

Charles Populer (Lateur, 2001) provided a more nuanced definition using pear trees as an example. According to those authors, pear landraces differ from amateur-bred cultivars in several key aspects (Table 2). These criteria are instrumental in distinguishing the



**Figure 1.** Classification of pome fruit (apple and pear) cultivars based on the period of their first documented mention. WWI, World War I; WWII, World War II

historical origin of pear cultivars within our collection (landraces or amateur-bred cultivars).

Nevertheless, since synonyms for cultivar names, mislabelling of material and errors are frequent in fruit tree genebanks (Oger and Lateur, 2004), it is essential to remain proactive in determining which material is true-to-type by cross-checking information, i.e. historical descriptions, accession evaluations and characterization data, expert knowledge and finally, genotypic data (e.g. molecular markers such as microsatellites and single-nucleotide polymorphisms).

Once materials have been carefully selected and ethnobotanical information has been gathered, a series of stages and activities follow over time, involving the active collaboration of multiple stakeholders. These steps and activities are illustrated in Figure 2 and are described in detail below.

### **Vegetative fruit accession propagation: an experimental organic nursery**

After encountering challenges with local nurseries tasked with propagating our initial collected accessions, we established our own experimental nurseries in 1980. Currently, the area dedicated to fruit tree propagation covers approximately 1.5ha per year. Virus-free rootstocks are ordered from specialized professional nurseries. A decade ago, our nurseries transitioned to management under organic farming system regulations (EU, 2018), and for the past six years, they have been officially certified for organic production.

Each accession is grafted onto dwarfing or semi-dwarfing rootstocks. For apple trees, we primarily use

‘M9’ rootstocks, and more recently, the ‘GENEVA® G11’. Due to frequent incompatibility or partial incompatibility between many pear accessions – particularly landraces – and quince (*Cydonia oblonga* Mill.) rootstocks, we traditionally used ‘Quince A’ grafted with a ‘Beurré Hardy’ used as ‘interstock’ before grafting the desired accession. To simplify propagation procedures, over the past ten years, we have progressively transitioned to using the ‘Pyrodwarf’ pear rootstock. This semi-dwarfing rootstock (about 20% more vigorous than ‘Quince A’) has a relatively short juvenile phase and, most importantly, is compatible with all pear varieties.

For European plum and cherry trees, we use the semi-vigorous rootstocks ‘St. Julien A’ and ‘Gisela-5’, respectively. Recently, the ‘Rubira’ rootstock has shown promising results in propagating our peach accessions. Nearly all grape accessions of our collection are propagated directly from cuttings.

To ensure proper conservation and evaluation of the accessions in our collections, we aim for a minimum of two trees per accession in the *ex situ* repository orchard and one in the evaluation orchard. Therefore, we routinely plan to graft at least five rootstocks per accession in order to get at least three trees per accession.

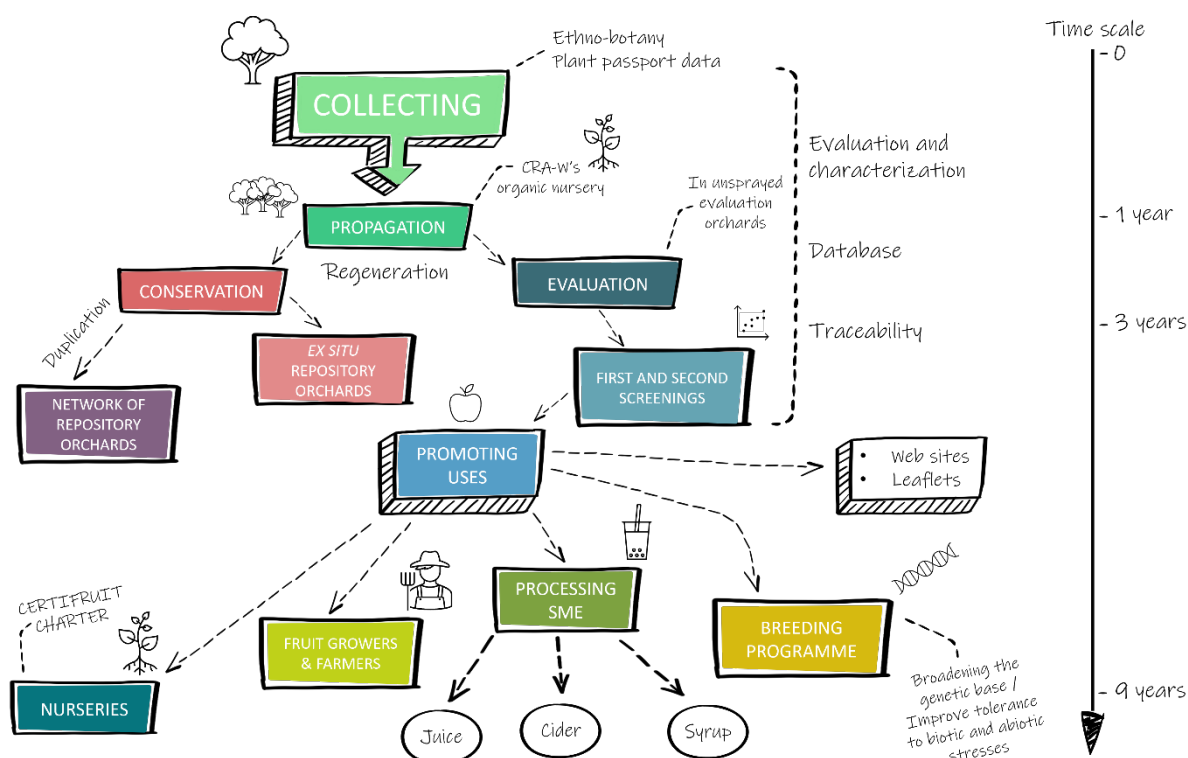
### **Organization of Belgian fruit tree genetic resources conservation**

#### **Repository orchards used as *ex situ* collections**

Our first repository orchards were established during the 1978–1979 period, primarily focusing on apple

**Table 2.** Criteria that differentiate the historical origins of very old pear cultivars in two main classes: ‘landrace’ and ‘amateur-bred’, also known as ‘Bourgeois’ cultivars (Lateur, 2001).

	Landrace cultivars	Amateur-bred or ‘Bourgeois’ cultivars
1	No acquisition date	Date of the acquisition is very often known
2	Name of the breeder and location are usually unknown.	The name of the breeder is very often referenced. Breeders were often belonging to higher social classes (aristocracy, bourgeoisie, artisans, clergy – never female breeders).
3	‘Chance seedlings’. Discovered by anonymous peasants, propagated by rural non-profit users.	Often from deliberate crosses, clonal seedlings or named (re-named) ‘chance seedlings’, propagated and released by historical well-known professional nurseries
4	Often distributed regionally or locally.	Distribution of the variety in larger areas, often international
5	Rural/dialectic cultivar name (cvs. ‘Poire de Gros’, ‘Poire de Malades’, ‘Pwèr di Fièr’, etc.)	The name of the cultivar usually refers to the breeder and their entourage, historical figures, or the fruit itself, often using a high lexical style (‘Souvenir de la Reine des Belges’, ‘Hélène Grégoire’, ‘Napoléon Savinien’, ‘Nec Plus Meuris’).
6	Almost never described in pomological historical literature nor in catalogues, Information sharing nearly always linked with oral transmission.	Well described in the literature. Can be found in old historical nursery catalogues. Often found abroad in many collections.
7	Fruits mostly selected for their long storage abilities or in order to enlarge the period of fruit consumption (from extremely early to extremely late).	Fruits usually selected for their taste or attractive visual appeal.
8	Mainly ‘survival’ uses, coarse texture and/or astringent used as cooked and or processed fruit for local products (e.g. ‘Sirop de Liège’ or oven-baked pear).	Mainly dessert fruit, buttery and smelting flesh.
9	Usually show better tolerance to pests and diseases and better robustness.	Not particularly selected for their robustness traits.
10	Grafted on seedling vigorous rootstocks and grown traditionally on high-standard trees in orchard meadows.	Grafted and grown on dwarfing rootstocks such as quince (very often as espaliers, counter-espalier).
11	Often graft incompatibility symptoms when grafted on quince.	Exceptionally rare graft incompatibilities on quince.

**Figure 2.** General overview of the steps and activities at the CRA-W research programme aimed at safeguarding and promoting the sustainable use of biodiversity in old fruit varieties.



and pear accessions. Due to limited land availability, a high-density planting system was employed, with 0.5m spacing between vertical cordons and 2.5m between rows, resulting in a density of 8,000 trees per hectare. Each accession was grafted on two trees planted side by side. Separate blocks were designated for apple and pear collections, planted adjacent to one another. These orchards were monitored but never sprayed against pests and diseases. To manage weeds, as dwarfing rootstocks were used, herbicides were periodically applied very locally in a narrow strip between grass and trunks at the base of the trees; however, we completely stopped using herbicides in 2000.

The repository orchard for plum accessions was established with a lower-density planting system of 6m × 5m, with one tree per accession.

Thirty-five years later, the need to regenerate trees planted at such high densities prompted the development of a second generation of *ex situ* repository orchards. This new design aimed to reduce the risk of losses in unsprayed conditions and to accommodate mechanical weed control.

Key improvements in the second-generation repository orchards included:

1. Separate locations for apple and pear collections: apple and pear repository orchards were planted in different locations to minimize the risk of disease epidemics, such as fire blight (*Erwinia amylovora*), which often spreads from pear to apple.
2. Dividing apple collections: the apple collection was split into two blocks located 1.5km apart, with a minimum of two copies but with one copy tree per accession planted in two different blocks, reducing the risk of total loss.
3. Increased spacing: spacing between trees and rows was increased to 1m × 3.5m for both apple and pear orchards. This adjustment reduced the risk of pest and disease spread, improved light penetration and ventilation, and allowed for better adaptation to mechanical weed control machinery.
4. Integrated hedgerows for pear orchards: in the new pear repository orchard, additional improvements included the introduction of multi-species hedgerows (excluding members of the Rosaceae family) planted every seven rows of pear trees. These hedgerows act as natural wind barriers, reduce disease dispersal, and serve as banker plants by attracting beneficial insects and fauna. They provide alternative nectar and pollen sources as well as reproductive habitats.

This innovative approach ensures the safe conservation and sustainable management of Belgian fruit tree genetic resources while promoting ecological balance and minimizing chemical inputs.

### The *in horto* pear collection

During the early 1990s, outbreaks of fire blight (*Erwinia amylovora*) – one of the most devastating diseases

affecting pear and apple trees – posed a significant threat in Belgium. Concerned about the potential loss of the pear collection, the most valuable cultivars were grafted onto ‘Quince A’ rootstock and preserved in containers within an insect-proof greenhouse. Remarkably, this pear collection has been maintained *in horto* as bonsai for over 30 years (Figure 3).

### On-farm safe duplication orchard network

Since 1999, through collaboration with numerous partners, CRA-W established the ‘Walloon Repository Orchards Network’ (WRON) (Villette et al, 2003). This initiative aimed to enhance the safe conservation of heritage diversity by dispersing it throughout the region. The network partners include farmers, local authorities, nature parks, regional administrations, associations, schools, universities and private owners dedicated to conserving and promoting local fruit tree heritage.

The primary objectives of this network are to:

1. Actively involve local stakeholders in safeguarding, conserving and developing their fruit tree heritage
2. Coordinate the duplication of rare endangered local varieties identified in their areas of origin, as well as the true-to-type landraces from the *ex situ* CRA-W collection in Gembloux
3. Reintroduce true-to-type old local varieties into their sub-regions of origin by increasing the number of genetic conservation sites.

The spirit of this multi-partner conservatory orchard network is to reintroduce and duplicate the great diversity of true-to-type old varieties collected at CRA-W, particularly the rarest and most threatened varieties, back to their places of origin. This approach counters decades of fruit tree diversity erosion while fostering a participatory dynamic in conserving fruit tree heritage. The involvement of local partners in the conservation orchards is vital for success, relying on integrating new local surveys of existing old orchards and trees, and maintaining a network of local partners to manage the primary conservation actions.

We coordinate a collaborative and interactive network that enables enthusiasts of old fruit tree varieties to develop synergies and revive this fruit tree heritage. The network aims to coordinate actions, share expertise and develop strategies to enhance the value and uses of this diversity. Expanding the range of species and varieties is crucial for expressing the best adaptive traits to climate change and countering biodiversity loss. Bellon et al (2015) explored this concept and conducted an insightful study on the benefits and challenges of on-farm conservation.

Since 2019, a significant portion of on-farm repository orchards and their trees have been geo-referenced and monitored for health. Technical support is provided to orchard owners to ensure the long-term viability of the trees. In the latest update (2024), WRON includes 93 orchards covering 154ha for a total of 8,000 stan-



**Figure 3.** View of the *in horto* pear cultivars repository collection.

dard fruit trees representing approximately 3,000 accessions. According to our 2023 inventory, apple trees constitute 63% of the total, pears 21%, cherries 6%, plums 8%, and other species (peach, quince, walnuts, etc.) 2%. Our ongoing goals are to continue planting new on-farm repository orchards, provide stakeholders with appropriate support, and involve local organizations in the inventory, conservation, utilization and public awareness efforts to maintain this heritage.

### Current status of our *ex situ* fruit tree genetic resources collections

CRA-W *ex situ* field collections currently comprise 1,629 cultivated apple tree accessions, alongside 172 indigenous *Malus sylvestris* (L.) Miller unique genotypes forming a Belgian ‘core collection’ (Keulemans *et al*, 2007; Jacques *et al*, 2009). The collections include also 1,198 cultivated pear tree accessions and 203 wild *Pyrus pyrausta* Burgst. indigenous unique genotypes. Additionally, we maintain 317 sweet cherry (*Prunus avium* (L.)), 38 sour cherry (*Prunus cerasus* (L.)), and 107 botanical/ornamental cherry tree (*Prunus* spp.) accessions. Other species in the collections include 236 European plum tree (*Prunus domestica* (L.)) accessions, 98 table grape (*Vitis* spp.) accessions, 29 peach (*Prunus persica* (L.) Batsch) accessions, and six local walnut

(*Juglans regia* (L.)) tree accessions (Table 3). Based on our latest data update, Table 3 gives also a preliminary estimation of unique accessions and landraces per fruit species.

### Management of unsprayed evaluation and repository orchards

For each accession, one tree is planted in one of our evaluation orchards. The spacing is 2m × 4m for apple and pear, while plum trees are spaced 5m × 6m and trained as half-stem. Additionally, two trees of each accession are systematically planted at distinct sites within our conservatory orchards (1m × 3.5m). Similar conservation strategies are defined by other institutions, such as the German Fruit Genebank (GFG) (Höfer *et al*, 2019; Reed *et al*, 2004) and the USDA-ARS-NPGS apple field collection managed by the Plant Genetic Resources Unit in Geneva, NY (Bramel and Volk, 2019; Volk *et al*, 2015). However, we do not employ alternative preservation methods such as *in vitro* or cryopreservation but – at least for pome fruits – we plan to implement the concept of storing dried open-pollinated seeds from diploid pome fruit accessions at low temperatures as a complementary safety conservation tool.

**Table 3.** Summary of the number of accessions and cultivars per crop/species currently preserved in the CRA-W *ex situ* collections. <sup>1</sup>, Total number of entries present in the collection; <sup>2</sup>, Estimated number of different cultivars/genotypes among the accessions. As several accessions can be synonyms of the same cultivars or different origins of the same cultivars, we have fewer cultivars than accessions; <sup>3</sup>, Estimated number of cultivars that are classified as landraces among all the accessions; <sup>4</sup>, Total number of cultivars (all species combined) and percentage of accessions that represent different cultivars; <sup>5</sup>, Total number of landraces (all species combined) and percentage of cultivars that are classified as landraces.

Crops	Species	No. of accessions <sup>1</sup>	Estimated no. of cultivars/genotypes <sup>2</sup>	Estimated no. of landraces <sup>3</sup>
Apple	<i>Malus × domestica</i> Borkh.	1,629	1,061	295
Wild apple	<i>Malus sylvestris</i> (L.) Miller	172	172	-
Pear	<i>Pyrus communis</i> L.	1,198	730	121
Wild pear	<i>Pyrus pyrastrer</i> Burgst.	203	203	-
Sweet cherry	<i>Prunus avium</i> L.	317	270	76
Sour cherry	<i>Prunus cerasus</i> L.	38	28	5
Botanical and ornamental cherry	Botanical & ornamental <i>Prunus</i> spp.	107	107	-
European plum	<i>Prunus domestica</i> L.	236	135	77
Peach	<i>Prunus persica</i> (L.) Batsch	29	29	8
Grapes	<i>Vitis</i> spp.	98	89	-
Walnuts	<i>Juglans regia</i> L.	6	6	6
<b>TOTAL</b>		<b>4,033</b>	<b>2,830 (70.2 %)<sup>4</sup></b>	<b>588 (20.8% )<sup>5</sup></b>

The total land area dedicated to fruit tree genetic resources (FTGR) research and management spans approximately 20ha across seven sites, all located within a 7km perimeter. Figure 4 depicts the principal apple, pear, grape and plum evaluation orchards situated near the main building. These orchards are managed in natural conditions without irrigation systems or crop protection measures such as hail netting.

Our philosophy emphasizes evaluating cultivars under the conditions they are expected to thrive in, particularly low-input, organic and regenerative agricultural systems. For this reason, our orchards, though certified for organic production, have never received plant protection treatments. In rare and exceptional cases, where pest damage threatens tree survival, we employ control methods that comply with organic production guidelines.

To create a favourable micro-climate, protect trees from strong winds and enhance biodiversity, the orchards are surrounded by highly diverse hedges. The inter-rows are grassed, featuring a central flower strip, and include additional ecological enhancements such as nest boxes, bat boxes and insect boxes.

Since 2013, the oldest apple and pear evaluation orchard (established in 1978–79 and grafted on ‘M9’ and ‘Quince A’ rootstocks, respectively) has been grazed by Shropshire sheep at a density of four to five females per hectare (Figure 5). This practice has proven effective in fostering synergies between livestock and fruit trees. Sheep trample and compact vole galleries, fertilize the orchard, graze on grasses which also helps birds of prey to hunt voles, and manage weeds that compete with trees. They also consume fallen diseased leaves and fruits, while benefiting from abundant food, natural shelter from sun and wind, and a secure environment.

Consequently, the approach has been extended to a plum evaluation orchard (grafted onto ‘St Julien A’ rootstock) and a 30-year-old apple repository orchard (grafted onto ‘M9’ rootstock).

### Evaluation and characterization process of genetic resources fruit tree accessions

The continuous adaptation of agriculture to ensure food security, through improvements in disease and pest resistance, tolerance to biotic and abiotic stresses induced by climate change, and other agronomic traits, relies directly on the genetic diversity of our genetic resources. These traits can only be effectively utilized if properly identified through evaluation activities. As Stalker and Chapman (1989) aptly noted: “A collection is of virtually no practical use until it has been properly evaluated and the data organised so that the content of the material collected can be known. Otherwise, it could be compared to a library whose books are neither sorted nor catalogued”.

Evaluation data is therefore the most critical component, as it determines how and which parts of the collections can be utilized and improved. For this reason, our primary focus has always been on evaluation activities, with characterization being of secondary importance. Table 4 delineates the differences between evaluation and characterization activities.

Given the significant size of genetic resource collections and the low probability of finding desirable traits in a single genotype, the evaluation process is typically conducted in stages. These stages are outlined in Table 5. This structured approach is essential for broadening the selection base across numerous accessions.

An important aspect of this process involves performing a preliminary evaluation before collecting a new





**Figure 4.** View of part of the evaluation orchards (apple, pear, grapes and plum) near the main building at the CRA-W (Gembloux, Belgium). (Courtesy of Emilie Mulot, 2024).



**Figure 5.** View of the oldest plum evaluation orchard (established in 1983–87) grafted on ‘St-Julien A’ (left) and apple and pear evaluation orchard grafted on ‘M9’ and ‘Quince A’ respectively. Both orchards are grazed by Shropshire sheep. Photo: CRA-W (Gembloux, Belgium).

accession. This initial step allows for an informed selection based on priorities established by the collection manager. This general evaluation process also applies to assessing quantitative disease resistance traits, ensuring that the traits prioritized for future breeding programmes are thoroughly identified and understood.

### Experimental growing conditions

Since the inception of the Plant Pathology Station, the evaluation of tolerance to pathogens and pests has been the primary research focus. From the outset, both our evaluation orchards and our nursery have been managed according to organic farming practices. However, our approach goes far beyond standard organic requirements i.e. we never use fungicides, and

the application of organic insecticides is exceptionally rare. This unique approach serves two critical objectives:

1. Accurate evaluation of cultivar robustness: for an important part of the accessions we have more than 25 years of collected evaluation data which allows for a reliable and/or non-parametric assessment of each cultivar's resilience in the absence of phytosanitary plant protection.
2. Preservation of pathogenic and beneficial diversity: Maintaining a diverse population of both pathogens and beneficial organisms in our long-term non-sprayed evaluation orchards ensures that new breeding cultivars are tested under high and varied selection pressures, providing a robust evaluation of their adaptability to biotic stresses prior to release (Lateur *et al*, 2000).

**Table 4.** Description of evaluation and characterization activities applied to plant genetic resources collections (Lateur, 2001).

	Characterization	Evaluation
<b>Definition</b>	Description of the most stable traits with respect to interaction with the environment	Study of traits for which the degree of expression is influenced by environmental factors.
<b>Main objective</b>	Distinguish and identify genotypes	Improve knowledge of the potential of the accessions
<b>Traits</b>	Mostly qualitative	Mostly quantitative
<b>Time required</b>	Relatively short for trait stability validation	Relatively long to be able to define the extent of variability of traits depending on the interaction with the environment
<b>Experimental protocols</b>	Relatively simple, based on standardized descriptors	Experimental conditions need to be well defined and, for a given collection, should initially be relatively stable
<b>Descriptors</b>	Yes - Qualitative Nominal variable scales or binary categories	Yes - Quantitative Often ordinal variable scales – need of reference cultivars.
<b>Examples</b>	Specific descriptive fruit traits (fruit shape, presence of ribs, fruit crowning at apex, aperture of eye, length of stalk etc.)	Agronomic features, disease, pest or abiotic stress tolerance/resistance, flowering period etc.

### Descriptors used

Aligned with ECPGR goals, considerable effort has been dedicated to developing harmonized and standardized protocols for evaluating and characterizing plant genetic resources. These efforts have been especially collaborative within the ECPGR *Prunus* and *Malus/Pyrus* Working Groups, leading to the creation of comprehensive descriptor lists (Lateur and Populer, 1996; Lateur et al, 1999; Lateur, 1999a; Lateur et al, 2002; Lateur, 2010; Kellerhals et al, 2012). Most recently, this work culminated in the updated ECPGR Characterization and Evaluation Descriptors for *Malus* and *Pyrus* Genetic Resources (Lateur et al, 2022a,b)

Apple and pear accessions are systematically evaluated for a wide range of traits, including fruit and tree characteristics, agronomic performance, fruit quality attributes and tolerance to biotic and abiotic stresses. A notable recent development is the introduction of a global foliage quality descriptor. This integrated trait provides an overall assessment of tree health and toler-

ance to various stresses by combining multiple individual indicators.

In the context of the European InnOBreed project, new descriptors are being developed to enhance the characterization and evaluation of fruit tree genetic resources' tolerance to abiotic stresses associated with climate change (drought tolerance, sunburn and flower frost tolerance).

### Direct valorization of best-performing cultivars through public-private partnerships

A vital strategy for increasing public awareness of FTGR is to allow the public to visit the evaluation orchards. The orchards serve as open spaces where the public, professionals and policymakers can periodically visit to taste fruits, discover heirloom varieties and learn about their historical significance.

Although the programme initially focused on safeguarding FTGR and utilizing them as breeding material, several cultivars naturally exhibited desirable traits suit-

**Table 5.** General stages in the evaluation process of genetic resource collections (Dotlacil et al, 1994; Horvath and Szabo, 1997)

Stages	Description, objectives (O) and responsibilities (R)
A Preliminary evaluation	(O) Simple evaluation carried out before collecting the material in order to avoid accessions that are (1) virus-infected and/or (2) not adapted to the soil and climate conditions, (3) duplicates and (4) accessions that are too susceptible to pests and diseases or to abiotic stresses under <i>in situ</i> conditions. (R) Fruit tree genetic resources managers.
B Basic primary evaluation	(O) The first evaluation is carried out during a strict minimum of a 5-year period under experimental harmonized conditions and using standardized protocols, but the experimental setup is simple because it must be applied to a large number of accessions. (O) Initial screening of accessions to highlight the most interesting material (best performing for the traits of interest). (R) Fruit tree genetic resources managers and/or interested potential users.
C Secondary and specific evaluation	(O) More accurate experimental design involving a sufficient number of replicates; multi-location trials; in the case of disease resistance, possible use of well-characterized pathogenic strains and artificial inoculation techniques. (O) A more detailed evaluation of the material that was pre-selected during the basic evaluation. (R) Potential users such as breeders and multidisciplinary teams.



able for direct propagation. These cultivars were propagated by a network of small family-run nurseries and offered to amateur gardeners and farmers for cultivation in unsprayed high-stem orchard meadows.

Since 1985, CRA-W has actively promoted these outstanding old varieties, recognizing their excellent balance of disease tolerance, agronomic performance and unique qualities suitable for cultivation without plant protection products. This initiative led to the creation of a new range of varieties and a production chain through partnerships with Belgian nurseries. These 33 exceptional heirloom varieties have been reintroduced to the market under the collective name 'RGF-Gblx Varieties', an abbreviation for *Ressources Génétiques Fruitières de Gembloux*. These are typically endangered, original varieties that align with the growing public interest in heritage and sustainable fruit cultivation.

The varieties progressively released under the 'RGF-Gblx' label are primarily old, forgotten or neglected local varieties, often landraces or selections from former amateur breeders that had disappeared from the market. These varieties, once common in the Belgian countryside, were rediscovered through survey campaigns and subsequently evaluated for a minimum of ten years in untreated orchards. This evaluation focused on their tolerance to diseases, pests and climatic stresses, their agronomic characteristics, quality and uses, and their adaptability to different rootstocks and regions.

These varieties also stand out for their originality compared to the classic commercial range, offering a rich diversity of taste profiles, forgotten aromas and various uses – both for fresh consumption and processing. They feature staggered ripening periods, easy tree management, and a strong level of robustness, meaning a better overall ability to adapt to various stresses and efficient nitrogen use.

More recently, new varieties resulting from the CRA-W breeding programme have been introduced. At least one parent of these new cultivars is an old local variety known for its polygenic resistance to scab and robustness traits. These selections must demonstrate long-lasting and sufficient tolerance to major diseases, mainly scab-robust agronomic traits suited for amateur cultivation and untreated high-stem orchards, original qualities and diverse uses, and solid adaptation to different rootstocks and regions, all evaluated over a minimum 10-year period in untreated orchards.

Currently, the range of old fruit varieties released to nurseries under the 'RGF-Gblx' label includes 18 apple varieties, 7 pear varieties, 4 European plum varieties, 3 cherry varieties, and 1 peach variety (Figure 6). Some of these are described in Figure 7.

The successful valorization of our FTGR is mainly due to the establishment of organized distribution channels through public-private partnerships. To support this, we outsourced the distribution of budwood and the associated phytosanitary monitoring activities to the



**Figure 6.** Promotional poster for the CERTIFRUIT 'RGF-Gblx' cultivars available on CERTIFRUIT nurseries and resellers.

Ormeignies nuclear stock managed by the regional *Centre d'Essais Horticole de Wallonie* (CEHW).

### The CERTIFRUIT quality charter and the associated nursery network

To ensure better traceability and guarantee the true-to-type identity of varieties for customers, the CERTIFRUIT quality charter and label (Figure 8) were developed for the 'RGF-Gblx' old varieties of merit. This initiative was created through a participatory approach in collaboration with a group of volunteer nurserymen.

The CERTIFRUIT charter (available at [www.certifruit.be](http://www.certifruit.be)) certifies:

1. A carefully selected assortment of more robust and disease-tolerant varieties
2. The guaranteed origin and identity of the propagation material, including cultivar, rootstock and any inter-stem
3. The superior quality of the nursery trees
4. Local and artisanal production methods.

Additionally, the CERTIFRUIT nursery network ensures high-quality advice and expertise from certified nurserymen. In 1991, a CEHW nuclear stock (3.3ha) was established – initiated by nurserymen and the CEHW,





Figure 7. Short description of a selection of ‘RGF-GbLx’ apple varieties. CRRG, Centre Régional de Ressources Génétiques des Hauts de France

with funding from the Ministry of the Walloon Region – to distribute certified budwood to nurseries. Currently, about half of the ‘RGF-Gblx’ released cultivars are certified as ‘virus tested’, while the remaining cultivars are distributed under EU CAC regulations (EU, 2008, 2014).



Figure 8. The CERTIFRUIT logo

### Promoting fruit tree heritage through the Diversifruits Association

One of the key objectives of CRA-W is to encourage the use of its extensive fruit tree collection gathered over the years. Among several initiatives, the Diversifruits Association ([www.diversifruits.be](http://www.diversifruits.be)) was established in 2018, driven by CRA-W and the *Fédération des Parcs Naturels de Wallonie*.

The association, managed by volunteers and supported by two publicly funded project managers, brings together around 150 members. Its mission is to unite the public and stakeholders in safeguarding and promoting this valuable fruit tree heritage. This is achieved through the planting of high-stem unsprayed orchard meadows and various agroforestry projects.

Diversifruits offers guidance in selecting the most suitable cultivars and support in orchard management. It is also involved in developing the economic sector related to both the direct sale of fruits and the production of processed goods (such as juice and cider) through its ‘Wal4Fruits’ project. Over the past decade, more than 500ha of orchard meadows have been planted by farmers with the association’s support. Each year, Diversifruits organizes approximately 70 activities, including conferences, training sessions and awareness events for both the general public and professionals.

To further promote and distinguish locally grown fruits – such as apples, pears, plums, cherries, walnuts and chestnuts – produced through this extensive and organic farming model (Figure 9A), the association created the ‘Vergers Vivants’ label. This certification guarantees that fruits are cultivated in non-sprayed orchard meadows. Officially recognized by the Walloon Region, the label also advocates for the fair remuneration of farmers.

These extensive orchards (Figure 9B, C, D) improve the ecosystemic services and provide fruits of superior quality.

### Pre-breeding and breeding programme using fruit tree genetic resources

The breeding programme at CRA-W was initiated in 1988 with the primary objective of developing cultivars exhibiting polygenic resistance to apple scab (*Venturia inaequalis*) and other biotic stresses, aiming for commercial production. This programme leverages both ancient and modern cultivars, using extensive phenotypic data collected over the years on our FTGR to select parent plants for crossing. The chosen parents possess complementary traits that help mitigate each other’s weaknesses (Lateur, 1999b).

The initial phase of seedling evaluation (Figure 10) focuses on assessing tolerance to apple scab. At the 3-4 leaf stage, seedlings are sprayed with a mix of *V. inaequalis* strains with a defined concentration of spores using a pulverization bench. After a controlled incubation period, the seedlings are rated for apple scab tolerance using a simplified scale based on the percentage of leaf surface affected by lesions. Our selection is not limited to fully resistant seedlings; those with up to 25% – and occasionally up to 50% – leaf damage are also retained for further evaluation.

Once transplanted to our nursery and evaluation orchard, these young trees undergo comprehensive assessments for various traits. This includes tolerance to apple scab, powdery mildew, European canker, anthracnose (*Elsinoë piri*), and apple rosy aphid. Elite cultivars that perform well are subsequently grafted and further evaluated for fruit production and quality traits.

This comprehensive, multi-stage selection process ensures the development of robust cultivars that combine resilience to biotic stresses with desirable agronomic and fruit quality traits, supporting sustainable and low-input fruit production systems.

### Novafruits: a transborder participatory breeding programme

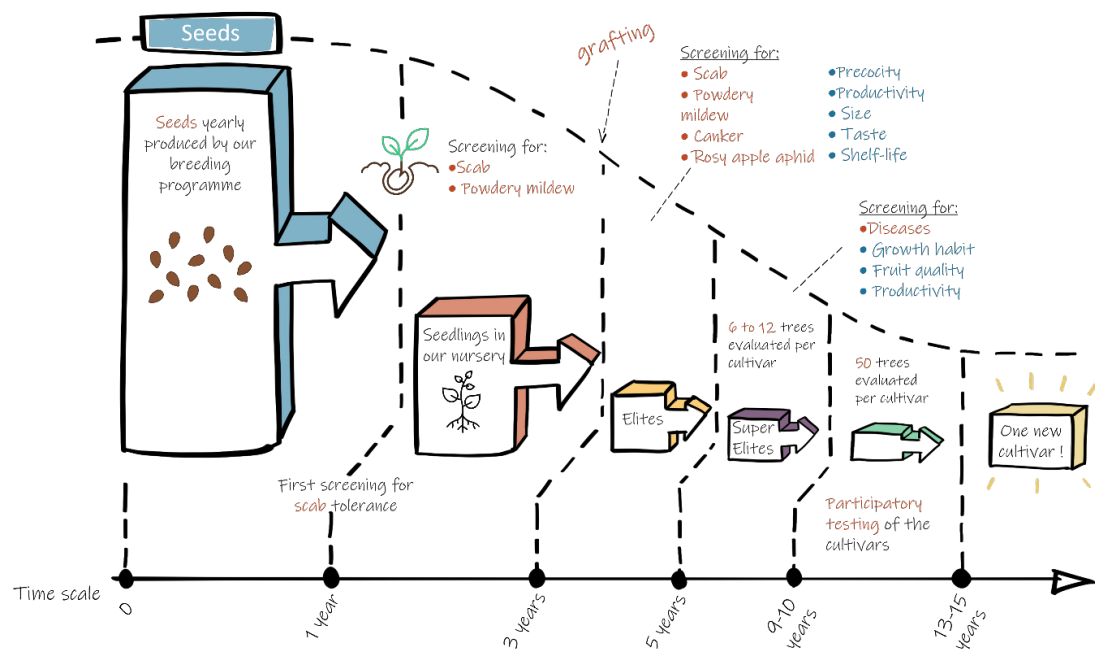
Since 2014, our breeding activities have primarily focused on participatory breeding within the framework of a public–private partnership. This programme involves two distinct growers’ associations, with the Novafruits association serving as a transborder collaboration between partners from northern France and southern Belgium (Wallonia). Novafruits brings together 31 organic fruit tree growers, two regional public institutes – the *Espaces Naturels Régionaux* (ENRx) and CRRG – and CRA-W, along with both the GAWI and the *Chambre d’agriculture de Normandie* organic fruit extension services.

Through this partnership, elite cultivars selected by CRA-W and CRRG are planted by organic growers under professional cultivation conditions. Each year, Novafruits members convene to evaluate the traits of the fruits and corresponding cultivars. Since many growers sell their products directly to consumers, they gather and share public feedback on fruit quality and performance.





**Figure 9.** A, Fruit harvest of one conservatory orchard; B, High-stem orchard meadow at the harvesting period; C, Visit to a young high-stem orchard meadow; D, An old high-stem orchard meadow.



**Figure 10.** Illustration of the different steps of our breeding programme selection process.



Additionally, since 2012, CRA-W and CRRG have been officially linked by a collaboration agreement through which both institutes are: (1) developing shared database facilities, (2) pooling expertise in the identification of local fruit cultivars, (3) rationalizing and sharing responsibilities for cross-border fruit tree genetic resources, (4) mutualizing high-quality plant propagation material, (5) co-steering participatory apple and pear breeding activities (e.g. through the cross-border Novafruits association, including planning common breeding objectives and sharing breeding material and offspring), and finally (6) jointly managing two organic variety and elite testing experimental apple and pear orchards.

This collaborative approach has led to the release to cross-border organic fruit growers of several cultivars, the most recent being ‘Ducasse’ (OCVV/CPVO), a cross between ‘Reinette Libotte’ and ‘Rubinola.’

### Perspectives

The emergence of new pathogens, such as apple blotch disease (*Diplocarpon coronariae*) and anthracnose (*Elsinoë piri*), along with the increasing frequency of abiotic stresses like sunburn, prolonged drought, and to a lesser extent, partial lack of chilling requirement, have become evident through the monitoring of our collections. This situation compels us to develop new descriptors to better assess the individual tolerance of our cultivars to these emerging threats and to enhance parent selection in our organic breeding programme.

Another ambition of the CRA-W collection is to broaden the diversity of cultivars for certain species, including peaches and table grapes, and to introduce new fruit species such as persimmons and fig trees. These species may prove suitable for cultivation in Belgium, offering organic farmers both increased resilience to climatic variability and opportunities for income diversification.

We firmly believe that the success of robust organic fruit farming relies on a systems-based approach. This approach integrates the use and selection of more robust, more resilient and well-adapted cultivars such as recently described by Serrie *et al* (2024) with agricultural practices that promote biodiversity, soil health, and the regeneration of agroecosystems. Consequently, we are also exploring the impact of several practices – such as fruit hedges, grazed orchard meadows, agroforestry and successional agroforestry – alongside measures designed to enhance functional biodiversity on fruit production and crop health.

Managing and monitoring our collection is both time-consuming and complex. To address this, we are investing in digital tools that streamline data acquisition and analysis, making data collection more efficient. Additionally, we are adopting sequencing technologies to deepen our understanding of the genetics within our collections, thereby guiding our breeding strategies.

As emphasized in the ECPGR report dedicated to strengthening the ‘AEGIS’ European strategy (Engels

*et al*, 2019), we believe that it is essential for collection managers to use a standardized and common tool for genotyping their germplasm, such as the set of apple 16 SSR markers (linked to the *Malus* UNiQue genotype codes, MUNQ) developed for apple (Muranty *et al*, 2020) and similarly on pear (Durel *et al*, 2023), or the recently proposed SNP-based MUNQ system based on a set of 96 SNPs (Muranty *et al*, 2024). These valuable tools help eliminate duplicates from collections, verify that accessions are true-to-type, and facilitate comparisons between collections at national and international levels. This enables the identification of common and unique accessions across collections, thereby supporting the development of a robust conservation strategy for the most valuable genotypes.

The challenges ahead for low-input organic farming and integrated fruit production are significant. Addressing these challenges will require strong collaboration and synergy between genetic resource collection curators and research institutes. To this end, we are actively working toward and advocating for the establishment of participatory organic breeding programmes, supported by European research initiatives such as InnOBreed (<https://innobreed.eu/>, grant agreement no. 101061028) and FRUITDiv (<https://fruitdiv.eu/>, grant agreement no. 101133964).

### Conclusions

Low-input organic fruit production and integrated fruit production face numerous challenges: (1) the emergence or the increased impact of new pathogens on crops, (2) the rise of abiotic stresses linked to climate change, (3) evolving restrictions and standards requiring the development of innovative, environmentally friendly control techniques, and (4) the accelerating erosion of genetic diversity in cultivated plants.

In this context, the conservation and valorization of FTGR have become increasingly important. The collection and conservation efforts initiated nearly 50 years ago at CRA-W underscore the enduring importance of the preservation and valorization of plant genetic resources. It is essential to continue expanding their collection, not only by increasing the number of accessions but also by integrating new species that may demonstrate promising adaptation to changing environmental conditions. Additionally, there is a critical need to improve the characterization and evaluation of existing genetic resources, focusing on their tolerance to emerging biotic and abiotic stresses and deepening our genetic understanding of these resources.

This article presented our diverse approaches and experiences in managing and promoting the use of FTGR collections. A pivotal aspect of this work is the systematic, long-term evaluation of varieties under unsprayed conditions. This process identifies superior-performing cultivars with valuable traits such as enhanced disease tolerance and greater overall robustness – qualities that are increasingly vital in the

face of climate change. Evaluation data are crucial for releasing robust old varieties directly for use through public–private partnerships, enabling their marketing to individuals and farmers via the Certifruit and Diversifruits associations. Moreover, these evaluations support the development of participatory breeding programmes, exemplified by the Novafruits association, which aim to introduce new fruit varieties with broader genetic diversity, improved robustness and better adaptation to low-input organic and integrated fruit production systems.

Our overarching goal is to sustain more durable production systems with fruit tree cultivars that exhibit greater resistance and adaptability. The conservation, deeper understanding and promotion of agrobiodiversity and fruit genetic resources depend on collaborative efforts among research institutes, farmers, small family-run nurseries, NGOs and the general public (Lateur, 2003). This work would not have been possible without the support and interest of the public. Therefore, it is crucial to return to citizens the best cultivars we have safeguarded, along with the new varieties developed from crosses using these valuable genetic resources.

### Authors' contributions

Marc Lateur and Baptiste Dumont wrote this manuscript. The other authors contributed to the review of this manuscript.

### Conflict of interest

The authors declare no conflict of interest.

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# A significantly enhanced role for plant genetic resource centres in linking *in situ* and *ex situ* conservation to aid user germplasm access

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**Abstract:** Plant genetic resources (PGR) serve as the cornerstone for global varietal enhancement and food security. However, these resources face significant threats, including diversity erosion and extinction, are often inadequately conserved, and frequently remain inaccessible for practical use. Traditionally, PGR have been primarily conserved through population seed samples stored *ex situ* in genebanks. In contrast, complementary *in situ* techniques – whether involving crop wild relatives (CWR) in genetic reserves or crop landraces (LR) on-farm – have largely remained experimental. The demand from breeders for a broader diversity is driving a more integrated approach that combines *ex situ* and *in situ* methods. This paper posits that such an integrated strategy would be mutually advantageous for PGR, biodiversity, and farmer-based conservation communities. As a foundation for future PGR science, we propose the three ‘Principles of PGR Conservation and Use Congruence’ and outline the practical processes involved in *in situ* and on-farm conservation. We also review the challenges associated with integrating *ex situ* and *in situ* conservation, specifically addressing how collaborative resource management can be established, how potential resource users can access *in situ* and on-farm conserved PGR, how to promote user access to *in situ* conserved populations, and the progress made thus far in integrating *in situ* and *ex situ* efforts. While it is acknowledged that full integration may be unrealistic without adequate resources for Genetic Resource Centres and the rectification of skill gaps, the potential to significantly enhance the long-term, sustainable conservation of PGR diversity holds profound existential benefits for humanity in the 21st century.

**Keywords:** crop wild relatives, *ex situ*, genebank, genetic reserves, *in situ*, landraces, on-farm conservation, integrated conservation

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## Introduction

Plant genetic resources (PGR) conservation is unique among conservation methods as it aims to preserve biodiversity while also utilizing conserved resources (Maxted *et al.*, 1997a). This process involves several steps: identifying genetic diversity across plant species, prioritizing target taxa, planning and implementing conservation actions, and characterizing, evaluating and utilizing resources by farmers, breeders or researchers. Clarity and expediency in this model's application are essential for global, regional, national and local initiatives focused on food security, poverty reduction, and enhancing human well-being, thereby supporting many UN Sustainable Development Goals (UN, 2015).

PGR conservation employs two main strategies: *in situ*, where resources are conserved in their natural habitats, and *ex situ*, where resources are relocated to safer environments for conservation and accessibility (see definitions in Supplemental Table 1). It is widely accepted that *in situ* and *ex situ* actions should complement each other, enhancing overall conservation effectiveness (FAO, 1996). Historically, formal PGR conservation and germplasm application for orthodox-seeded species have relied heavily on *ex situ* seed storage in genebanks and, latterly, cryogenic preservation, with field genebanks and tissue culture techniques primarily used for recalcitrant-seeded species and clonally propagated crops. Genebanks can secure long-term viability at low cost and have successfully made this diversity available to plant breeders and researchers (FAO, 1998, 2011). However, *ex situ* approaches alone do not fully address the growing demand for broader diversity in a rapidly changing environment.

The science of *in situ* and on-farm PGR conservation has advanced significantly, with refined techniques and a solid evidence base (Maxted *et al.*, 1997c, 2002, 2020; Brush, 2000; Eyzaguirre and Linares, 2004; Heywood and Dulloo, 2005; Jarvis *et al.*, 2007, 2016; Iriondo *et al.*, 2008, 2021; Veteläinen *et al.*, 2009; Hunter and Heywood, 2011; Hunter *et al.*, 2017). Initially, *in situ* and *ex situ* techniques were viewed as independent, even competitive (Ford-Lloyd and Maxted, 1993), but the case for their complementarity is now widely accepted, though their practical integration remains incomplete (Maxted *et al.*, 1997a, 2020; van Hintum *et al.*, 2021). Lack of integration limits conservation effectiveness, resulting in unconserved resources being unavailable to users and preventing their potential utilization. The challenge of increasing food production to feed a growing human population while mitigating climate change impacts on agriculture is escalating for the PGR and breeding communities (FAO (2010, 2012). Lack of breadth and access to conserved genetic diversity is now a barrier to crop improvement (McCouch *et al.*, 2013; IPCC, 2014; Dempewolf *et al.*, 2017; Zhang *et al.*, 2017).

There is an opportunity to better serve farmers and breeders by integrating *in situ* conservation, genebanks, and germplasm use into a cohesive continuum that could significantly enhance breadth and access to diversity for users (Maxted and Brehm, 2023). Failure to integrate these activities reduces the potential role of genebanks in leading PGR conservation and meeting user demands. Maxted *et al.* (2016) suggested that expanding the role of genebanks to include both *ex situ* and *in situ* conservation was logical and required change to the PGR paradigm and would warrant their renaming as Genetic Resource Centres (GRC), as the term 'genebank' implies a more restrictive focus.

To explore this enhanced GRC role, a questionnaire was prepared in 2024 on European genebank activities for the Horizon Europe project 'Promoting a Plant Genetic Resource Community for Europe' (PRO-GRACE - <https://www.grace-ri.eu/pro-grace>). The results indicated that 76% of genebanks (13 of 17 respondents) were interested in adopting complementary *in situ*/on-farm roles alongside traditional *ex situ* activities. Genebanks have historically succeeded in supporting breeders and farmers while maintaining the PGR foundation for diverse crop varieties, but human population increase and climate change's impact on crop production and food security are forcing a change of practice. Although some GRC may face limitations in skills and resources, with appropriate support, their roles could evolve to become even more critical for humanity's future.

This discussion focuses on how to better integrate *in situ*, *ex situ*, and user access in PGR conservation to provide greater diversity. We highlight current opportunities to: (1) clarify PGR conservation aims through proposed Principles of PGR Conservation and Use Congruence; (2) summarize practical processes for *in situ* and on-farm conservation; (3) promote resource management collaboration; (4) enhance user access to *in situ* and on-farm conserved PGR populations; (5) facilitate access to *in situ* conserved populations via the European Search Catalogue for Plant Genetic Resources (EURISCO, <http://eurisco.ecpgr.org>); and (6) identify future ways to better integrate *in situ* and *ex situ* conservation. For PGR actors and germplasm users, the clear advantage lies in addressing current challenges and ensuring greater diversity availability, with an enhanced role for genebanks or GRC at the core, ultimately leading to increased sustainable food production and long-term food security.

## The Principles of PGR Conservation and Use Congruence

The aim of PGR conservation may be summarized in three fundamental principles, to ensure: (1) long-term, sustainable maintenance of PGR<sup>1</sup> diversity, (2)

<sup>1</sup> The scope of PGR found outside of GRC, or breeding collections is commonly focused on crop wild relative (CWR) and landrace (LR) diversity both of which are highly threatened.

active<sup>2</sup> conservation and characterization of crop, varietal and related wild taxon diversity using complementary<sup>3</sup> techniques and (3) conserved resource documentation and availability for utilization within the applicable legislative context. The use of complementary techniques provides additional security by employing multiple, diverse approaches to conserve these resources, ensuring greater security as each technique backs up and supplements the others. There could as well be other subordinate objectives, such as maintaining seed viability, phenotypic and genotypic characterization and evaluation of conserved resources, and ensuring standard material transfer agreement (SMTA) enforcement, but the three fundamental objectives should hold true for whatever form of conservation strategy is applied. Together, these objectives may be referred to as the *Principles of PGR Conservation and Use Congruence*; overall, conservation should, in the long-term, maintain the full breadth of genetic diversity, employ multiple conservation techniques, and make the conserved resources available to actual or potential users.

These three objectives are met for most *ex situ* holdings (except for the requirement to link to complementary *in situ* conservation). *Ex situ* PGR conservation and use is well tested, and we know it already ‘works’, but there is now an urgent need to further develop *in situ* conservation approaches. Hawkes (1991) commented in the early 1990s that *in situ* techniques were in their “infancy”, and although advances in this area have been made (Maxted et al, 2020), *in situ* and on-farm conservation is still largely experimental and not based on more than 60 years of practice and the associated extensive evidence-base available for *ex situ* conservation. Additionally, effective standardization of *in situ* conservation techniques is itself challenging, as their application occurs in natural or semi-natural environments, or in on-farm locations, where diverse environmental, socioeconomic and cultural factors impact the target taxa, and effective PGR population managers (e.g. farmers, foresters, estate managers, etc.), may not be professional conservationists or have the necessary skills to maintain intrinsic genetic diversity. This is not to devalue the efforts of farmers or other landrace (LR) maintainers, or landscape managers, who have retained crop wild relative (CWR) populations on the estates they manage for extended periods of time. However, if *in situ* PGR conservation is to function as intended and be appropriately resourced, it must meet all three principles and objectives, as do *ex situ* approaches. Populations and diversity of *in situ* resources must be maintained in the long term via the application of complementary techniques, and the conserved resources must be

available to users. If *in situ* PGR conservation does not ensure availability of the conserved resource, it will not meet the Principles of PGR Conservation and Use Congruence and it is unlikely ever to be seen as truly complementary to *ex situ* conservation.

It should also be noted that the third principle, which conserved resources are available for use, may not always be achievable, for example, when the *in situ* conserved populations are rare or threatened, and few, or an *ex situ* conserved accession has limited seed numbers and low viability. In both cases, the sample may need to be multiplied or regenerated before it can be made available to users. The principle remains that resource availability is paramount, and any periods of unavailability should be temporary until germplasm can be offered.

### Practical processes of *in situ* and on-farm conservation

To identify potential opportunities for integration, we need first to summarize and understand how *in situ* and on-farm conservation operate. The conservation–utilization continuum for *in situ* conservation is divided into four component steps and summarized in Figure 1 (adapted from Maxted et al (2020)):

1. **Conservation planning.** This involves: (i) selection of target conservation units, either CWR or wild food plant (WFP) taxa or crop LR (Maxted et al, 1997c; Brehm et al, 2017); (ii) prioritization, usually based on potential use value, relative crop value and threat, identifying an easily implementable inventory of highest priority CWR, WFP or LR (Brehm et al, 2017; Nilsen et al, 2017; FAO, 2019b); (iii) ecogeographic and gap analyses to identify concentrations of the conservation units and predict which sites with target populations (Maxted and Kell, 2008; Maxted et al, 2012b; FAO, 2019b); and (iv) field exploration to check the validity of the previous prediction and establish where the target diversity will be conserved in genetic reserves, other effective area-based conservation measures (OECM), on-farm, or in home garden.
2. **Conservation technique implementation.** Conservation targets are actively managed either in nature for CWR or WFP or cultivated on-farm or in-garden for LR diversity. This involves: (i) selection of sites with targeted resource diversity (Hawkes et al, 2000; Maxted et al, 2002; Dulloo et al, 2008; Veteläinen et al, 2009; Iriondo et al, 2021); (ii) formulation of the management plan, a detailed plan for how the population(s) of the target taxa/crop are to be maintained and enhanced (Maxted et al, 2002, 2008; Dulloo et al, 2008; Veteläinen et al, 2009; Iriondo et al, 2021); (iii) implementation of the management plan, including the site interventions, implementation of which is likely to be experimental initially until tar-

<sup>2</sup> Active conservation implies targeted management and monitoring of conserved CWR or LR populations, as opposed to passive maintenance of CWR or LR populations, where there may be a conservation ethos but no targeted management and monitoring.

<sup>3</sup> Complementary conservation implies the use of both *ex situ* and *in situ* techniques to conserve CWR or LR populations.

get population retention is sustainable (Veteläinen *et al*, 2009; Iriando *et al*, 2021); (iv) resource monitoring at set time intervals to check the success the management regime (Veteläinen *et al*, 2009; Iriando *et al*, 2021); and (v) formation and upkeep of partnerships essential for *in situ* and on-farm conservation of the genetic resources to occur.

3. **Conserved resource description.** The pre-utilization stage will involve characterization and evaluation (Maxted *et al*, 2020). These data may be uploaded alongside passport data in EURISCO to facilitate germplasm selection.
4. **Conserved resource utilization.** The *in situ* conserved resource should be available for use by breeders, farmers, researchers and other potential bona fide users. Forms of traditional utilization should be encouraged, provided it is not detrimental to the target taxon or taxa, thus fostering local support for conservation actions.

### Proposed resource management collaboration

It is important to clarify not only how the target populations are managed, but also (1) who should provide oversight of the networks of *in situ* or on-farm sites and populations, and (2) who should practically implement the management interventions of individual *in situ* or on-farm sites and populations. There are several potential communities that might fulfil these roles: existing population managers, national GRC staff and other diverse PGR stakeholders (including allied non-governmental organizations (NGOs), research centres and universities). As noted above, given that often the conserved *in situ* or on-farm genetic resources have been managed by the reserve/protected area (PA) manager, landowner, farmer or gardener for extended periods, one might assume they are the most appropriate to play both roles. While existing *in situ* and on-farm site managers should continue their successful management of individual *in situ* or on-farm sites and populations, the question is: do they have the necessary skills, tools and resources to provide oversight of the network(s) of *in situ* or on-farm sites and populations established?

It can be argued that it would be impractical for individual *in situ* and on-farm site managers to provide oversight of the network(s) of *in situ* or on-farm sites and populations given they: (1) are unlikely themselves to use trait diversity from the conserved CWR or WFP populations; (2) lack skills and expertise in international and national policy and legislation; (3) lack skills and expertise in field trials or genomic analysis; (4) lack access to a PGR information system to aid *in situ* population management and transfer of germplasm to the end user; and (5) already have an existing heavy core load of activities in managing biodiversity populations or producing food and their scope to adding a significant additional activity is limited. Therefore, it would seem appropriate that national GRC staff (or other appropriate national PGR agency or PGR-focused

NGOs) would be better placed with the necessary skills, tools, resources and long-term experience from *ex situ* PGR applications, to provide multi-site PGR governance and overall oversight of the networks of *in situ* or on-farm sites and populations, including overall monitoring of natural reserves, other effective area-based conservation measures (OECM) sites or on-farm systems to prevent population losses (Maxted *et al*, 2016).

However, it is important to stress that national GRC staff cannot work in isolation. The PGR conservation goal of maximum PGR diversity conservation and availability can only be achieved by the three communities working in integrated collaboration, with national GRC staff providing national PGR leadership and oversight, individual PGR field population maintainers (i.e. reserve/PA manager, landowner, farmer or gardener) managing the genetic resources under their responsibility, and other PGR stakeholders (allied NGOs, research centres and universities) providing the necessary additional support. Furthermore, as the *in situ* or on-farm resource is maintained outside of a controlled unit, like a GRC, the local community within the vicinity of the *in situ*/on-farm resource site should also be involved in the conservation project management and associated decisions. Individual roles will vary depending on multiple factors (e.g. taxa included, whether wild or cultivated, resources available, value of resource conserved, etc.), therefore stakeholder discussions and negotiations will form part and parcel of the process of defining the roles of each actor, however it can be safely stated that the key expertise and areas of responsibility are likely to include those presented in Table 1. To aid clarity, Figure 2 highlights those components managed by GRC staff, and *in situ* site maintainers alone, and which may be managed jointly. Collaboration between the three communities would be critical and involve periodic meetings of a PGR *In Situ* Population Management Committee.

Such an integrated approach to *in situ* and *ex situ* collaboration would extend each communities roles and responsibilities. However, for those maintaining PGR populations (PA, OECM or on-farm field maintainers) and given the target populations were selected because of their 'health', the additional workload is not foreseen as being significant, at least initially, as it would primarily involve monitoring target populations, while the provision of additional ecosystem and food services from the site would underpin the public good value of maintaining PGR populations. Furthermore, in some countries, additional targeted PGR conservation could generate additional subsidies or added income for the site maintainers/owners through government funding (such as payments for ecosystem services, subsidies for farmers who cultivate and conserve landraces that suffer from genetic erosion), so the benefit to PGR field population maintainer could be substantial. The proposed changes outlined for the national GRC would also be significant, possibly requiring additional staff with *in situ* expertise and



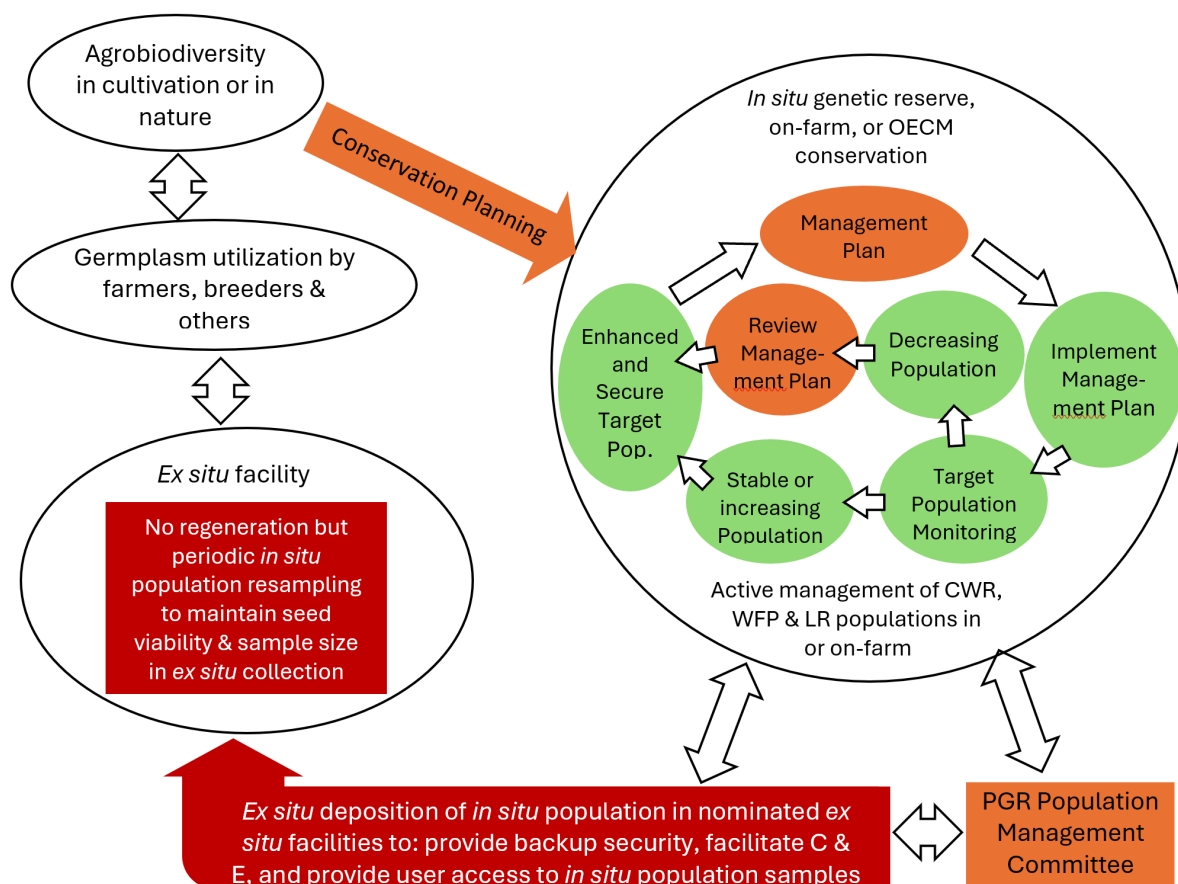


As a final point, the collaboration as outlined in this document, involves the transfer of *in situ* or on-farm samples from their original locality to a nominated *ex situ* GRC for backup and to facilitate access for

germplasm users. This means that the provisions emanating from the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) (FAO, 2001) and the Convention of Biological Diversity (CBD Nagoya Protocol (CBD, 1992, 2011)) are triggered and there is the need for an SMTA or Internationally Recognized Certificate of Compliance (IRCC) respectively, between the *in situ* maintainer and the recipient nominated GRC. This would need enacting even if the GRC had no intention to utilize the germplasm itself, but simply to conserve the *in situ* or on-farm sample and make it in turn available to more active users. By virtue of the relationship between the *in situ*/on-farm source, the GRC and the end user, the involved actors would be required to address the requirements to ensure fair and equitable sharing of benefits arising from the sample's potential final utilization, depending on the terms established under national regulations. The actual scope of the three-way (source, GRC and end user) relationship would require expert deconstruction and is therefore beyond the scope of this document but must be resolved before any germplasm transfer occurs.

**Table 1.** Collaborative activities of national Genetic Resource Centre (GRC) staff, plant genetic resource (PGR) population maintainers and other stakeholders. CWR, crop wild relatives; LR, landraces; WFP, wild food plants

CWR, WFP or LR <i>in situ</i> population conservation		
National GRC staff's role	PGR population maintainer's role	Other stakeholder's role
Lead preparation and periodic revision of National PGR Strategy and Action Plan, including <i>in situ</i> site selection and management plan production.	Contribute to preparation and periodic revision of National PGR Strategy and Action Plan, lead <i>in situ</i> site selection and management plan production.	Contribute to preparation and periodic revision of National PGR Strategy and Action Plan, and <i>in situ</i> site selection and management plan production.
Lead national PGR <i>in situ</i> conservation site network management.	Contribute to PGR <i>in situ</i> conservation site network management.	Contribute to PGR <i>in situ</i> conservation site network management.
Assist with implementing the site's individual management plan.	Lead implementation of individual site management plan.	Assist with implementing of individual site management plan.
Assist with periodic monitoring of target populations and analysis of demographic and genetic trends.	Lead periodic monitoring of target populations and analysis of demographic and genetic trends.	Assist with periodic monitoring of target populations and analysis of demographic and genetic trends.
Assist with periodic revision of individual site management plan and building evidence base.	Lead periodic revision of individual site management plan and building evidence base.	Assist with periodic revision of individual site management plan and building evidence base.
Target population characterization and evaluation. Ensuring user access to <i>in situ</i> conserved resources (via <i>ex situ</i> backup samples).	Periodic collection of target populations for <i>ex situ</i> representative backup samples.	Diverse research projects focused on aiding effective PGR diversity conservation and use.
Ensure integration of <i>in situ</i> and <i>ex situ</i> conservation activities and support tools and applications to aid <i>in situ</i> conservation site network management, e.g. national inventories, management and monitoring, germplasm access, characterization and evaluation databases and information management.	Collation of site and PGR population data, and integration with national PGR databases, activities assisted by network tools and applications.	Diverse research projects supporting national PGR population management and use, and associated tool and application development.
Promotion of national integration into international PGR community.	Promotion of PGR integration into the broader biodiversity community.	Participation in national and international research actions.
Lead and participate in the National PGR <i>In Situ</i> Population Management Committee.	Participate in the National PGR <i>In Situ</i> Population Management Committee.	Participate in the National PGR <i>In Situ</i> Population Management Committee.



**Figure 2.** Schematic description of key elements of *in situ* conservation, highlighting Genetic Resource Centre (GRC) staff (dark red), *in situ* populations manager (green) and joint (orange) responsibilities. CWR, crop wild relatives; LR, landraces; OECM, other effective area-based conservation measures; WFP, wild food plants.

### User access to *in situ* and on-farm conserved PGR populations

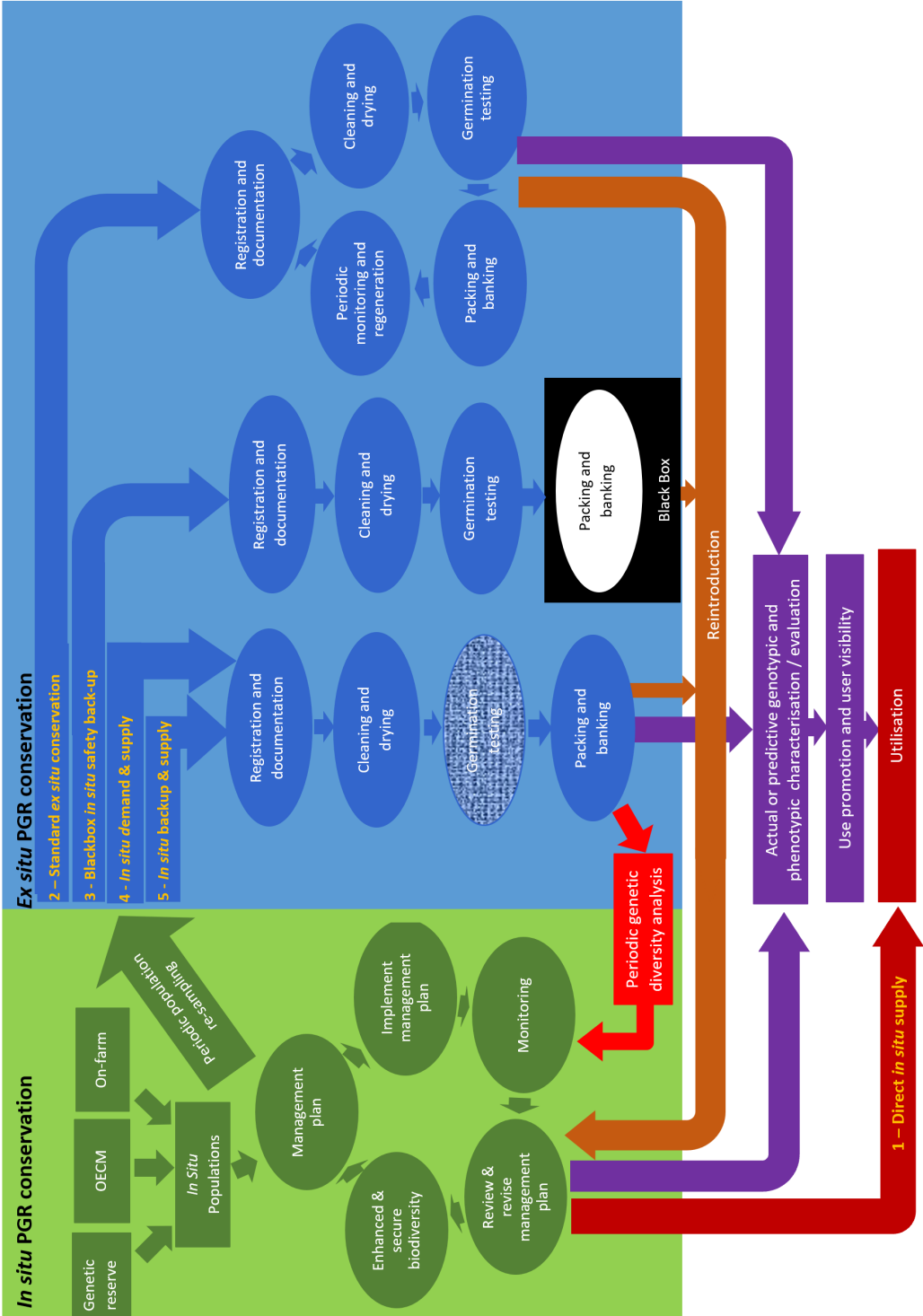
The endpoint of PGR conservation is not conservation itself but ensuring that conserved germplasm is available for present or potential future utilization (Maxted et al, 1997a). The pathway of use for *ex situ* conserved PGR is tried and tested, but, apart from the positive activities of farmers and farming NGOs focusing on PGR diversity and farming systems, the *in situ* pathway to utilization has yet to be established. Without effective *in situ* conservation-to-use linkage, it is doubtful whether *in situ* conservation sites and site networks will ever be established (Maxted, 2019). Therefore, establishing links between *in situ* resources and use is fundamental to ensure additional germplasm access and the promotion of *in situ* conservation itself (Maxted and Brehm, 2023).

Maxted and Kell (2008); Maxted and Palmé (2016) and Maxted (2019) each reviewed potential models for how *in situ* conserved resources might be linked to user access, either accessed for use directly from the *in situ* population or indirectly via an *ex situ* conservation facility (Figure 3). Five potential options have thus far been proposed for promoting user access to *in situ* and on-farm conserved PGR and are elaborated in Table 2. Except for Option 3, users request an *in*

*situ* PGR population sample and  $\approx(20\text{--}40\text{--}50)$  viable seeds are dispatched to the end user, fulfilling the *in situ* to-use prerequisite outlined in the Principles of PGR Conservation and Use Congruence. The chosen option may vary based on GRC facilities, available resources, conservation practices, and constraints from PGR maintainers or national authorities. However, assuming resources are adequate and constraints do not limit distribution, Option 5 achieves the Principles of PGR Conservation and Use Congruence, making the *in situ* resource-to-user link via the GRC, while placing the minimum additional burden on the GRC staff and their resources.

However, such an approach has not been practically implemented in any country. The reason is not thought to be that Option 5 or the other options are not conceptually sound, but due to funding limitations, risk aversion, lack of formal incentives, or the necessary skills and tools to promote *in situ* utilization. It could also simply be that active CWR, WFP *in situ* or LR on-farm conservation itself is only now being tentatively initiated, *in situ* conserved resources are uncharacterized and evaluated, the potential of *in situ* or on-farm germplasm access is unflagged so potential users are unaware such resources are accessible or how to access them.





**Figure 3.** Five options to linkplant genetic resource (PGR) in situ conserved resources to user access: 1. Direct in situ supply, 2. Standard ex situ supply, 3. Blackbox in situ safety backup, 4. In situ demand and supply, and 5. In situ backup & supply. OEMC, other effective area-based conservation measures.

**Table 2.** Options proposed for promoting user access to *in situ* and on-farm conserved plant genetic resources (PGR). The addition of an asterisk to option number means the option meets the Principles of PGR Conservation and Use Congruence. ABS, Access and benefit sharing; CBD, Convention on Biological Diversity; CWR, crop wild relatives; GRC, Genetic Resource Centre; ITPGRFA, International Treaty on Plant Genetic Resources for Food and Agriculture; LR, landraces; WFP, wild food plants.

Option	Option description	Advantages	Disadvantages
1	<b>Direct <i>in situ</i> supply:</b> involves the user being made aware of the availability of particular <i>in situ</i> PGR populations and their characteristics, the user contacts the PGR <i>in situ</i> maintainer and the maintainer sends a sample directly to end user.	A simple procedure agreed and organized by the <i>in situ</i> or on-farm maintainer and the user, which would not necessarily imply GRC involvement. In some cases, users may be granted permission to autonomously collect by the appropriate national authority.	(a) In general, <i>in situ</i> population maintainers (protected area managers, farmers, land agents, gardeners, etc.) do not see germplasm supply as one of their core activities, have no experience with such activities and are unable to engage in direct user supply. Further, they rarely have legislative knowledge of CBD (2011) and ITPGRFA-related legislation or its national application and/or international ABS statutes (FAO (2001); Art. 12.3(h) and Art. 15.1(b)), therefore cannot enact the legislation. (b) Germplasm supply outside of the country of origin requires phytosanitary certification and testing to ensure seeds are free from specific pests/pathogens and the <i>in situ</i> population maintainers would not have the required processing skills. While it might be feasible to supply such knowledge to some maintainers, such as protected areas managers, extending it to all potential farmers, land agents and gardeners, is unrealistic. (c) Training <i>in situ</i> population maintainers in germplasm supplier skills would be almost meaningless as the chances of each individual supplier supplying conserved germplasm would be limited given their large number and the limited number of seed requests. (d) <i>In situ</i> population maintainers could only supply germplasm during the PGR fruiting season, so there would be significant delays between request and user supply.
2*	<b>Standard <i>ex situ</i> conservation:</b> describes the typical route by which germplasm enters the GRC: populations are sampled from the wild or on-farm location, transferred to the GRC, registered and documented, processed following the standard guidelines (FAO, 2014) and supplied to users.	A tried and tested route applied widely for <i>ex situ</i> conservation that effectively meets users’ needs, but here is applied to an <i>in situ</i> conserved population. It meets the Principles of PGR Conservation and Use Congruence.	(a) If each country maintains a substantial number of <i>in situ</i> conservation sites for CWR, WFP or LR population conservation and these all <i>ex situ</i> backup accessions in the GRC, the processing of additional <i>in situ</i> samples and making them available to users would require significant additional resources.

Continued on next page

Table 2 continued

Option	Option description	Advantages	Disadvantages
3	<b>Blackbox <i>in situ</i> safety back-up:</b> a sample is either collected by the <i>in situ</i> maintainer or collected by GRC staff and stored in the nominated <i>ex situ</i> facility and is only available to the donor for their use, or <i>in situ</i> population reinforcement or reintroduction.	A simple, inexpensive procedure agreed and organized by the <i>in situ</i> or on-farm maintainer and the GRC.	(a) This option does not meet one of the imperatives of the Principles of PGR Conservation and Use Congruence which mandates that conserved PGR should be available for utilization, therefore this cannot be considered effective as a primary PGR conservation measure. (b) If the <i>in situ</i> population is rare, highly threatened or has known unique, adaptive allelic diversity, then it should be conserved <i>in situ</i> and backed up <i>ex situ</i> <sup>4</sup> .
4*	<b><i>In situ</i> demand and supply:</b> proposed by <a href="#">van Hintum et al (2021)</a> to minimize the GRC additional workload. It involves users identifying the <i>in situ</i> population they wish to obtain, requesting a sample from the appropriate GRC, and a staff member travelling to the site, collecting and processing a sample and distributing it to the end user.	This option does minimize the additional GRC workload and ensures the <i>in situ</i> or on-farm maintained population is provided to the user.	(a) This option would involve additional work for the GRC staff in sampling and processing <i>in situ</i> samples, though GRC sampling costs could potentially be shared with the user. Costs could be reduced by providing guidance to <i>in situ</i> maintainer so that they collect and forward the sample either directly to the user or via the GRC. However, any additional costs of <i>in situ</i> supply might act as a disincentive to potential users, especially if no such cost is associated with <i>ex situ</i> GRC holdings. (b) User supply would involve one-off population sampling and would not be as cost-effective as expedient sampling while undertaking a routine GRC collection mission. (c) Seasonality would mean seed, cuttings or tissue samples would not be available year-round and this might significantly delay <i>in situ</i> sample supply to the user, which would add a further disincentive to potential users ( <a href="#">Maxted, 2019</a> ), while <i>ex situ</i> conserved GRC samples are available for distribution year-round. (d) For CWR and WFP taxa natural seed dispersal mechanisms make it difficult for collectors to gather the required target number of seed at the optimal time for conservation and supply during a brief one-off visit to a natural population. (e) Also, <i>in situ</i> populations are less likely to be characterized and evaluated for adaptive traits, although users could apply predictive characterization techniques to aid <i>in situ</i> population selection ( <a href="#">Thormann et al, 2014</a> ).

Continued on next page

<sup>4</sup> The assumption is that availability would be granted by the *in situ* maintainer in the future when target population levels have risen, and black box *in situ* back-up would not be a long-term preferred option.



Table 2 continued			
Option	Option description	Advantages	Disadvantages
5*	<b>In situ backup and supply:</b> <a href="#">Iriondo et al (2012)</a> proposed as a standard that <i>in situ</i> conserved populations should be backed up in nominated <i>ex situ</i> facilities. It involves users identifying the <i>in situ</i> population they wish to obtain, requesting a sample from the appropriate GRC, and a staff member supplying a sample from the <i>in situ</i> backup material to the end user.	Each <i>in situ</i> population should be backed up <i>ex situ</i> to facilitate reintroduction of the original material, if necessary. The sample could be collected by the <i>in situ</i> maintainer and sent by them to the nominated GRC. The backup sample could be maintained using a partial <i>ex situ</i> protocol and used for characterization and evaluation to promote user application.	(a) Backing up each <i>in situ</i> conserved population in the GRC would be costly, especially if all samples were collected and processed using standard <i>ex situ</i> models ( <a href="#">FAO, 2014</a> ). To minimize the GRC costs of <i>in situ</i> sample processing: (i) the sample and associated data could be collected by the <i>in situ</i> population manager and sent to the GRC, rather than collected by GRC staff; (ii) on arrival in the GRC, the <i>in situ</i> sample would be processed using <i>ex situ</i> protocols, except regeneration <sup>5</sup> and germination monitoring would be omitted ( <a href="#">Maxted, 2019</a> ), regeneration being replaced by regular <i>in situ</i> population resampling <sup>6</sup> , which would also reduce the requirement for periodic germination testing; (iii) periodically resampling will also ensure that the genetic diversity captured in the <i>ex situ</i> backup sample accurately reflects the ongoing evolutionary trajectory of the <i>in situ</i> population. (b) The <i>in situ</i> backup sample needs to be sufficiently large for the GRC to supply the end user.

<sup>5</sup> Note for CWR samples, it may be difficult to collect recommended standard sample sizes quantities ([FAO, 2014](#)) and therefore, initial sample seed bulking may be required before formal seed storage, especially if the sample is to be used subsequently for characterization and evaluation, and user provision.

<sup>6</sup> Although germination testing as a relatively inexpensive task might be retained to confirm the initial quality of the sampled seeds and as an indicator to trigger *in situ* population resampling.

It is also true that there has been some initial resistance to changes in roles and responsibilities from both current *in situ* population maintainers and GRC staff; true *in situ* and *ex situ* GRC integration will add additional roles and responsibilities, especially when many staff are already over-committed and additional resources are limited.

Although *in situ* and *ex situ* GRC integration will add additional roles and responsibilities for both GRC staff and *in situ* population maintainer communities, it is likely to be mutually beneficial. For GRC staff it would extend the range of diversity they are able to provide to users, whereas for *in situ* maintainers, it presents a good example of applied additional ecosystem services from the PGR resources they manage, graphically demonstrating the fundamental value of area-based conservation and diversity-based farming systems to the public.

By providing access to *in situ* population samples, GRC extend their expertise in user seed supply — an area in which *in situ* population managers lack experience and have no institutional mandate. This aligns with the GRC's existing key role in effectively addressing user demand for genetic diversity. Furthermore, adoption of this option could be expanded if the additional commitment remained minimal for site managers and GRC staff, and if it were adequately resourced.

Such integration would also likely facilitate more coherent PGR policy development and implementation, rather than PGR policy being the responsibility of each discrete site managers and GRC communities, plus those from the third research community. It is appropriate that the GRC takes a lead role in PGR conservation and user provision because it: (1) has experience in PGR long-, medium- and short-term genetic conservation, collection management and meeting user requests for germplasm effectively, as well as promoting a supportive policy environment over the past 60 years globally; (2) possesses practical expertise in national and international germplasm transfer, as well as meeting associated phytosanitary and legislative requirements; (3) is already known as the germplasm source for diverse users and are accustomed to germplasm access procedures; and (4) has the potential to extend their role to supply samples from *in situ* conservation sites. It should be stressed that even if the GRC provides the overall PGR national lead they must ensure collective decision-making and implementation among the three communities involved, site managers and GRC communities, plus those from the third research community, site manager, PGR researcher and GRC communities, potentially plus more peripheral communities (e.g. biodiversity, informatics, systematics, etc.). How such managerial cooperation is achieved is likely to vary from country to country based on local contexts, species biology, resource constraints and broader socio-political factors, but it is likely to involve the establishment of a PGR conservation committee to

promote collection management, user access promotion research direction and policy development discussion, chaired by GRC staff.

It should be noted that the partnership between the *in situ* population maintainer and the *ex situ* component of the GRC is critical to facilitating *in situ* germplasm user access. To ensure this relationship is effective, it is preferable that each *in situ* population be partnered with a nominated *ex situ* GRC, this will be the national or a national GRC. However, in countries with a decentralized GRC network, matching specific crop group CWR, WFP or LR with their corresponding specialist GRC would be appropriate and beneficial.

The preceding discussion has focused on professional roles in PGR conservation and use, but locally, community biodiversity management is increasingly shown to be effective in facilitating local conservation management of PGR; a role that seems particularly pertinent in the *in situ* context linking local PGR conservation effort to local PGR use. It seems unlikely many local communities would be interested in CWR use because of the potential need for advanced techniques to overcome interspecific breeding barriers and problems associated with linkage drag of unwanted additional traits, though even here local communities have shown interest in CWR population surveying. However, WFP and LR could be conserved and used more directly via community seedbanks initiated by local communities. Local community seedbanks could also function as a conduit to the more formal GRC community (Bocci *et al*, 2025), aiding *in situ* characterization, adaptive trait recognition, *in situ* population sampling for *ex situ* duplication and backup, and even CWR prebred-varietal introductions, as well as provision of associated datasets. This could encourage greater recognition of the informal conservation sector, provision of resources and skills training, and inclusion of community seedbank holdings in national PGR inventories and EURISCO. Community seedbanks could take the role of LR population maintainers working in collaboration with GRC staff to maximize diversity maintenance. Improving integration between the PGR formal and informal systems will surely prove mutually beneficial and help secure existentially important food security resources.

### Aiding user selection of *in situ* conserved populations via EURISCO

Significant progress has recently been achieved in advancing *in situ* PGR conservation documentation through the incorporation of information on active *in situ* population conservation into EURISCO (<https://www.ecpgr.org/working-groups/crop-wild-relatives/cwr-in-eurisco>). This was accomplished through a project funded by the German Federal Ministry of Food and Agriculture (referred to as EURISCO project below), commenced in November 2021 and focused on country-based case study incorporation of CWR *in situ* population data in EURISCO. Although this initiative was developed for CWR populations, a similar approach

could, in the future, be implemented for WFP and LR population data, marking a significant step forward in PGR science.

The extension of EURISCO is endowing the European region with a centralized, public and web-searchable inventory of priority *in situ* CWR populations' passport data, along with a fine-tuned data flow mechanism that uses an internationally agreed data exchange standard (Van Hintum and Iriondo, 2022). The new *in situ* module of the EURISCO catalogue was built in compliance with the 'FAIR principles': Findable, Accessible, Interoperable and Reusable data (Wilkinson et al, 2016). The online central catalogue of *in situ* CWR population data has been available since the beginning of 2024, and more European countries are being trained and encouraged to add their country data. This provides easy-to-access information to potential users seeking novel sources of diversity for breeding and pre-breeding programmes and other uses. The implementation of these international commitments prioritized by the CBD, Global Plan of Action (GPA) and ITPGRFA, as well as by the *European Plant Genetic Resources Strategy* (ECPGR, 2021), will prove beneficial to PGR conservationists and users alike, ultimately promoting food security and well-being.

A proposal, including principles and requirements for data inclusion, the definition of a data flow mechanism and the proposed data exchange standard (CWR passport descriptors), was developed and published on the ECPGR website (Van Hintum and Iriondo, 2022). It includes recommendations for identifying the most relevant CWR populations to be recorded in EURISCO. It also outlines a set of descriptors for *in situ* conserved populations, including their current location, precise coordinates, and where samples are being actively conserved to guarantee their long-term persistence. It addresses how samples from these populations can be accessed, potentially based on the terms and conditions of the ITPGRFA Multilateral System. Furthermore, it describes the structure of information shared between the CWR-National Inventory (CWR-NI) and EURISCO, the necessary steps to upload CWR-NI elements into EURISCO and the modifications to EURISCO to accommodate such type of data. Two annexes containing 'Descriptors recommended for the generation of a National Inventory of *in situ* Crop Wild Relatives' and 'Descriptors for uploading passport data of *in situ* CWR to EURISCO' complete the document. As of January 2025, eleven countries (Albania, Bulgaria, Cyprus, Germany, Italy, Lithuania, the Netherlands, Poland, Romania, Spain and the United Kingdom) have provided *in situ* CWR data to EURISCO, with data from a total of 5,764 populations.

Incorporating *in situ* data into EURISCO is a key step toward addressing some of the accessibility issues related to *in situ* material that have been discussed in this review. The EURISCO project has already played a key role in establishing *in situ* PGR conservation and documentation as being truly complementary to *ex situ*

efforts in Europe and in helping ensure that *in situ* conservation meets the Principles of PGR Conservation and Use Congruence. Without this initial step, the establishment of *in situ* genetic reserves would have progressed more slowly. Further initiatives are likely to be agreed between the PGR *in situ* site and population maintainers, researchers and GRC to ensure a future fully integrated and effective complementary *in situ*–*ex situ* conservation–use continuum. Some first thoughts include:

- While a periodic update of *in situ* data to EURISCO, such as every five years, may be suitable long-term, more frequent updates might be necessary during the initial establishment of *in situ* genetic reserves.
- Recently, EURISCO has begun to support the linkage of characterization and evaluation data with the germplasm passport data held in EURISCO as a means of aiding user selection of germplasm and promoting further utilization of conserved resources. There is significant opportunity for further extending utilization by building Tools to Aid Germplasm Selection (TAGS) and links to additional data sets. One obvious TAGS would be a predicted characterization tool, where the crop and the desired trait required are selected and the tool suggests a subset of CWR and LR accessions that might have the trait for the crop. Another tool is a LR repatriation tool that allows the user to choose LR from certain localities to aid repatriation of LR lost from those locations.
- Just as CWR and WFP diversity is actively conserved in other non-PGR contexts, e.g. as a rare or threatened taxa by biodiversity specialists or as wild species by botanic gardens, so biodiversity specialists and botanic gardens are interested in CWR and WFP diversity, and organic crop producers and diversity-based farmer specialists, for example, are interested in PGR germplasm for their own non-PGR based utilization. To this end, EURISCO could be better designed to meet additional user communities.
- It is widely agreed that national *in situ* and on-farm conservation should be managed in a network structure rather than each site being managed independently. The likely benefits include systematic conservation coordination and reporting, stronger partnerships and mutual support, integration of global, regional, national and local actions, truly *in situ*–*ex situ* conservation integration with improved data interoperability and coordinated policy development, facilitation of ABS for protected areas and farmers/farming communities, and tools and methodologies to aid safeguarding *in situ* PGR populations. With so many potential benefits and many different potential governance structures possible, it would be wise to start planning now to maximize national PGR *in situ*



networking that links *in situ*, *ex situ*, user access and impact.

- It would also be useful to define what data will be included and excluded from EURISCO. What data might be better maintained at individual CWR, WFP or LR population site level and or at national network level, and where there is no benefit in collating at the regional level. One example that could be considered such data is currently provided by genebank holding curatorial data (e.g. size of seed collection, germination percentages, location of sample in genebank). Similar curatorial data exists for *in situ* populations (e.g. monitoring data for *in situ* populations over time, levels and timings of management interventions, or age of LR maintainer cultivating a LR). Some such data might appropriately be recorded in National Inventories and some at site level, but boundaries need to be established to maximize overall efficiency.

### Future challenges and opportunities for *in situ*–*ex situ* integration

With agrobiodiversity conservation budgets limited and becoming tighter, it is imperative to maximize the efficiency of conservation expenditure. Horizon scanning, a participatory approach to the establishment of future priorities, is getting increasingly recognized as a useful tool to help prioritize and plan conservation action, inform resource allocation and provide an evidence base for conservation implementation (for its PGR application see Maxted *et al.* (2012a)). This exercise is carried out here in the context of *in situ*–*ex situ* integration for CWR and LR conservation over the next ten years and the results are summarized in Supplemental Tables 2 and 3, respectively. Those involved in the 2025 assessment were partners in the EU-funded project PRO-GRACE<sup>7</sup> (23 experts from 11 countries + ECPGR Secretariat), members of the ECPGR On-farm Conservation and Management (85 experts from 43 countries) and CWR Working Groups (87 experts from 38 countries). These experts were also asked to identify emerging PGR-related issues with implications for *ex situ* and *in situ* conservation that they felt were of European importance to CWR, WFP and LR diversity in Europe, and required resolution by 2035.

The experts identified a set of 23 issues related to CWR and WFP, and 24 issues related to LR. It is anticipated that the issues detailed in Supplemental Tables 2 and 3 will be used in three primary ways. Firstly, that policymakers will critically examine the issues identified, assessing their potential impact on policy development and considering appropriate implementation timelines. Secondly, it is expected that this exercise will help the integrated *ex situ* and *in situ* PGR community better target their activities for

the immediate and longer-term future, considering the relative success of the previous PGR Horizon scanning initiative. It is hoped that researchers, funders, and those working on PGR policy and regulation will use the outcome of this exercise when considering the future direction of strategic CWR, WFP and LR research. Finally, this exercise may encourage further consideration and debate about the issues that are on the horizon and the ways in which scientists and decision-makers can best communicate about them.

### Discussion

This paper discusses the largely unexplored challenges and opportunities associated with integrating *ex situ* and *in situ* plant genetic resources (PGR) communities. Historically, these communities have worked semi-independently, but there are now significant mutual benefits for humanity in their integration, transforming and enhancing the paradigm of PGR conservation and use. Traditionally, formal PGR conservation has relied almost exclusively on *ex situ* storage of seed samples in genebanks, providing users with easy access to meet evolving needs. Conversely, *in situ* and on-farm applications for PGR conservation have been extensively discussed (Jain, 1975; Maxted *et al.*, 1997b, 2002, 2020; Safriel *et al.*, 1997; Brush, 2000; Eyzaguirre and Linares, 2004; Heywood and Dulloo, 2005; Jarvis *et al.*, 2007, 2016; Iriondo *et al.*, 2008, 2021; Veteläinen *et al.*, 2009; Hunter and Heywood, 2011; FAO, 2013; Hunter *et al.*, 2017), particularly post-CBD established prioritized *in situ* techniques (CBD, 1992), but rarely practically applied except for farmer-based maintenance of LR. The strength of integrating both conservation strategies, *ex situ* and *in situ*, lies in maximizing the long-term and sustainable maintenance of a more comprehensive representation of PGR diversity.

Historically, commercial plant breeding has been hesitant to utilize non-domesticated or highly heterogeneous CWR and LR germplasm, likely due to a lack of economic incentive for broader diversity, limited availability of non-domesticated CWR or diverse LR germplasm, challenges in identifying germplasm with known adaptive and desirable traits, and the economic, time and complexity costs associated with pre-breeding and elimination of unwanted traits inadvertently introduce via linkage drag. However, the status quo is shifting: climate change and ecosystem instability necessitate a greater breadth of PGR diversity to sustain agricultural production, while precision techniques facilitate the identification of valuable adaptive traits and enhance the precision of trait introgression (Prohens *et al.*, 2017). This knowledge highlights that *ex situ* approaches alone cannot satisfy users' demands for a comprehensive range of diversity, prompting a renewed focus on *in situ* conservation.

Despite recent progress in experimental *in situ* applications, experience over the past 30 years indicates that implementing *in situ* methods independently of *ex situ* approaches is both ineffective and counterproductive.

<sup>7</sup> <https://www.grace-ri.eu/pro-grace>, Grant n. 101094738

*In situ* conservation should be complemented by *ex situ* strategies to: (1) provide long-term backup for security and potential population reinforcement or reinstatement; (2) assist in characterization and evaluation; and (3) ensure ease of access for end users. Likewise, *ex situ* conservation should be complemented by *in situ* approaches to: (1) maximize the preservation of taxonomic and genetic diversity; (2) allow for the evolution of adaptive traits in changing environments; and (3) address the evolving demands of end users. Thus, both *ex situ* and *in situ* conservation methods are interdependent and should function in a mutually supportive manner. However, unlike *ex situ* conservation, which can be largely managed within controlled environments, *in situ* conservation necessitates the active participation of diverse actors with various skill sets (ecology, wild plant biology, field geno- and phenotyping, remote monitoring, climate change management, invasive species and pest management) to enact conservation actions, adding layers of complexity and associated challenges. Integration of these diverse actors in a distributed Research Infrastructure on Plant Genetic Resources is likely to unite these additional actors.

Moreover, a critical question arises: who will take primary responsibility for coordinating *in situ* conservation efforts? The experiences of the ECPGR CWR and On-farm Conservation and Management Working Groups have demonstrated that neither protected areas nor farming communities can effectively coordinate *in situ* PGR diversity conservation activities, and many are reluctant to engage in formal *in situ* PGR conservation. Protected area managers focus on biodiversity rather than crop diversity conservation, while farming communities are primarily engaged in commercial agricultural production rather than systematic diversity conservation. Therefore, there is a pressing need for additional training for GRC staff and/or extending collaboration with actors possessing the necessary skills and experience in ecology, pest management and field conservation to complement the existing GRC staff's expertise in genotypic, phenotypic and agronomic evaluation, sampling, viability and phytosanitary testing, documentation, data upload to EURISCO, and distribution to users, including knowledge of national and international legislative implementation. This collaboration is fundamental to enhancing the conservation of *in situ* diversity, its description and its availability to end users.

Additionally, the existential problem of user supply is often underestimated by the *in situ* PGR community. For PGR conservation to be effective, meaningful, and serve a utilitarian purpose beyond its intrinsic value in nature preservation, a link must exist between conservation and utilization. However, neither protected areas nor farming communities possess experience in germplasm supply within the context of access and benefit-sharing legislation. Consequently, it can be argued that without the involvement of the *ex situ* community in these roles, *in situ* implementation risks becoming limited to 'academia,' 'hobbyists' or short-

term project support without long-term sustainability. Therefore, it is evident that the application of *ex situ* and *in situ* strategies is mutually dependent, and their complementary integration should be led by GRC. Leadership from GRC would entail adjusting their perspective to encompass both *ex situ* and *in situ* aspects, along with appropriately increased resources to fund the necessary structural and skill provisions for practical implementation. Conversely, if the *in situ* or on-farm community was able to take such a leadership role, would the genebanks welcome the competition?

There is also an economic argument for GRC to adopt a more proactive role in *in situ* conservation. As outlined, one justification for PGR conservation is to enhance user access and benefits, which may encompass various industries, with the most prominent being those related to economic and food security, medicinal products and material uses. The most recent estimate of the use value for CWR closely related to 29 globally important crops is US\$42 billion, with a potential future value of \$120 billion. The annual gross added value was \$581 billion in 2010, indicating that CWR are already valued at about 7% of the annual production value of these 29 crops (PWC, 2013). This valuation is conservative, as it does not account for the potential expansion of CWR use in breeding these or other crops, nor the value of utilizing LR diversity. Therefore, the overall annual gross added value of using PGR diversity in crop improvement could approach a trillion US dollars. This significant valuation raises the question: does not the potential revenue stream justify the modest investment required now in PGR conservation to secure future substantial benefits? The rationale for integrating *ex situ* and *in situ* conservation lies in the fact that *ex situ* collections typically capture only a snapshot of the genetic diversity present in natural populations at the time of collection. It also should be acknowledged that over time, genetic drift or selection during storage and regeneration can lead to the loss of some of this genetic variation. In contrast, *in situ* conservation allows the remaining spectrum of genetic diversity to persist and evolve naturally in response to environmental changes. Without leveraging both approaches, a significant portion of the genetic diversity available in natural populations remains untapped, limiting its potential contribution to crop improvement and other industries.

While the practical establishment of CWR genetic reserves or LR on-farm diversity maintenance sites has progressed more slowly than anticipated, this may be partly attributed to the PGR community's longstanding focus on the established 'in-nature and on-farm sampling to genebank to user' paradigm (Guarino et al, 1995, 2012; Hawkes et al, 2000; Smith et al, 2003; FAO, 2014). This paradigm has proven resilient and successful over the last century, consistently meeting the needs of breeders and consumers. However, the very success of this established paradigm poses a significant challenge to the adoption of *in situ* conservation approaches. To gain wider acceptance, these approaches

must articulate an equally robust and straightforward model that demonstrates long-term effectiveness – the PGR germplasm user is indifferent to the conservation source if it meets their trait needs. Promoting *in situ* application includes clearly communicating the value of the proposed *in situ* to *ex situ* to use paradigm and its mutual advantage in diversity breadth. Although the clarification of the Principles of PGR Conservation and Use Congruence and the derived proposals presented provide an initial foundation for a proposed *in situ* to *ex situ* to use paradigm led by the national GRC, further development will be necessary based on a growing evidence base.

Another related topic that has progressed more slowly than anticipated is the systematic *ex situ* and *in situ* conservation of WFP. These include fruits, leafy vegetables, woody foliage, bulbs and tubers, cereals and grains, nuts and kernels, saps and gums, mushrooms and seaweeds (Wunder, 2014). WFP have historically served as a coping strategy for many rural households, particularly during the 'hungry season' before the next season's crops ripen and as part of subsistence farming systems (Hunter *et al*, 2015; Kennedy *et al*, 2017). FAO (2019a) estimates that around one billion people globally incorporate wild foods into their diets regularly, and forests alone provide livelihoods and food for approximately 300 million people through non-timber forest products. However, WFP are rarely included in PGR conservation initiatives and are unlikely to be targeted for biodiversity conservation only if they are threatened or rare. FAO (2019a) calls for (1) active *ex situ* and *in situ* conservation and sustainable use, (2) breeding of improved varieties, and (3) raising awareness of the importance of WFP, particularly local and traditional foods that are vital for nutritionally balanced, healthy diets and food security. WFP, like CWR, are simply wild species with specific food value, although the former is associated with direct consumption rather than trait provision. Therefore, WFP planning and conservation implementation are unlikely to differ significantly from CWR-based actions, making it timely to test this assumption. Implementing WFP conservation falls within the remit of national GRC activities and should be integrated with other PGR activities. Most importantly, WFP can provide material for future domestication efforts, thereby expanding the foundation of our food production systems.

Here much has been made of expanding *in situ/ex situ* integration, but there is also significant leverage in *in situ*/on-farm working more closely with biodiversity communities. CWR and LR could be used as 'cultural ambassadors' to help promote PA-based conservation or traditional cultivation practices. The collaboration offers opportunities to marry biodiversity conservation management with food security or traditional foods associated with healthier lifestyles. While such collaboration would also help conserve the critical PGR resource more extensively and effectively – demonstrating the mutual relevance of each community contribution – PA don't

only maintain birds, mammals and reptiles, they conserve the founding resource for our food. Traditional farming is not just picturesque, it sustains cultural benefits such as recreation, education, spiritual and creative enrichment, and improved mental health and wellbeing. Whilst PA management may recognize the importance of these ecosystem services, their consideration and usefulness in site management decision-making is worth closer understanding.

There exists an opportunity and a central role for the proposed GRACE research infrastructure (see <https://www.grace-ri.eu/pro-grace>), which builds on 55 years of ECPGR collaborative networking aimed at ensuring long-term conservation and facilitating utilization of PGR to implement the necessary transition from genebanks to GRC and enact more effective *in situ* PGR conservation. This role may prove existentially important for humanity in the future. Without appropriate financing, skills and capacity provision, and cooperation with the broader biodiversity community, establishing and maintaining *in situ* and on-farm networks would be unsustainable in the medium to long term, even under GRC direction. The core mission of the PGR community remains unchanged, as summarized in the Principles of PGR Conservation and Use Congruence, and it is essential to reassess and reconfigure this mission to ensure it is fit for purpose today and in the future.

## Conclusions

The dual challenges of human population growth and climate change's negative impact on crop production have resulted in increased demand from germplasm users and consumers for greater breadth of diversity. *Ex situ* genebanking alone is unable to secure such breadth of diversity, as are *in situ* or on-farm conservation activities; the urgency of the situation is such that the much-discussed but rarely applied implementation of complementary PGR conservation offers the only practical and expedient solution. The Principles of PGR Conservation and Use Congruence describe the fundamental principles of PGR conservation (long-term, sustainable conservation, application of complementary conservation techniques, and documentation and availability of the conserved resource for utilization) and provide a framework for indicating success. Evidence and experience have shown that neither *ex situ*, *in situ* nor on-farm conservation functions adequately in isolation, but further that systematic *in situ* and on-farm genetic conservation is not a priority for practitioners of either biodiversity-focused conservationists or production-based farmers. The comprehensive integration of *ex situ*, *in situ* and on-farm conservation communities and their activities, with the local communities where the bulk of the genetic resources exist, led by national GRC and CGIAR institutes, is now critical for global, regional, national and local food security; failure to address this issue could have devastating consequences for humankind in the 21<sup>st</sup> century. Specific recommendations are outlined for collaborative resource management, user access to *in*



*situ* and on-farm conserved PGR, improving user selection of *in situ* conserved populations and what the future challenges and opportunities there might be for future *in situ*–*ex situ* integration. Other recommendations will undoubtedly come from further steps toward PGR community integration. Although realistically this initiative is doomed to failure unless national GRC step up to take the lead, skill gaps are filled, and they are adequately resourced.

### Supplemental data

**Supplemental Table 1.** Genetic conservation strategies and techniques (Maxted et al, 2020).

**Supplemental Table 2.** Horizon scanning issues associated with CWR *in situ*–*ex situ* conservation in 2025 that require resolution by 2035.

**Supplemental Table 3.** Horizon scanning issues associated with LR *in situ*–*ex situ* conservation in 2025 that require resolution by 2035.

### Acronyms used

- ABS – Access and Benefit Sharing
- AEGIS – A European Genebank Integrated System
- C & E – Characterization and evaluation
- CBD – Convention on Biological Diversity
- CWR – Crop wild relatives
- CWR-NI – CWR-National Inventory
- ECPGR – European Cooperative Programme for Plant Genetic Resources
- EURISCO – European Search Catalogue for Plant Genetic Resources
- FAO – Food and Agriculture Organization of the United Nations
- GPA – Global Plan of Action
- GR – Genetic reserve
- GRC – Genetic resource centre
- IRCC – Internationally Recognized Certificate of Compliance
- ITPGRFA – International Treaty on Plant Genetic Resources for Food and Agriculture
- LR – Crop landrace
- NGO – Non-governmental organization
- OECM – Other effective area-based conservation measures
- PA – Protected area
- PGR – Plant genetic resources
- SMTA – Standard material transfer agreement
- TAGS – Tools to Aid Germplasm Selection
- WFP – Wild food plant

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### Conflict of interest statement

The authors declare no conflict of interest.

### Author contributions

NM drafted the first iteration of the text and coordinated production of the final text, all authors contributed to the conception, discussion, and text of the paper.

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