

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-RARe). 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, *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 prebreeding 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 3,000m² of greenhouses, 1,500m² 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 $1,500m^2$ of greenhouses, 2ha of field space, *in vitro*

culture facilities, cold storage rooms (950m³) 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 $250m^2$ of greenhouses, $500m^2$ of field space and cold storage rooms ($20m^2$).

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 (htt p://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). 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 BrA-CySol 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), Meloïdogyne 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).

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

Table 1. Composition of the collections.^a, Solanum taxonomy according to Hawkes (1990)



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 (ht tp://www.g2p-sol.eu); Prima BrasExplor (https://bra sexplor.hub.inrae.fr); Horizon Europe NemEmerge (https://nem-emerge.eu) and ProWild (https://www.p ro-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 genomewide 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 longstanding 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., Meloïdogyne 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: 'Garli-CCS' (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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