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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; 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
Total number	**2,237**

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, populations, 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, trilaminate 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 bieticola*. 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. bieticola* 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.

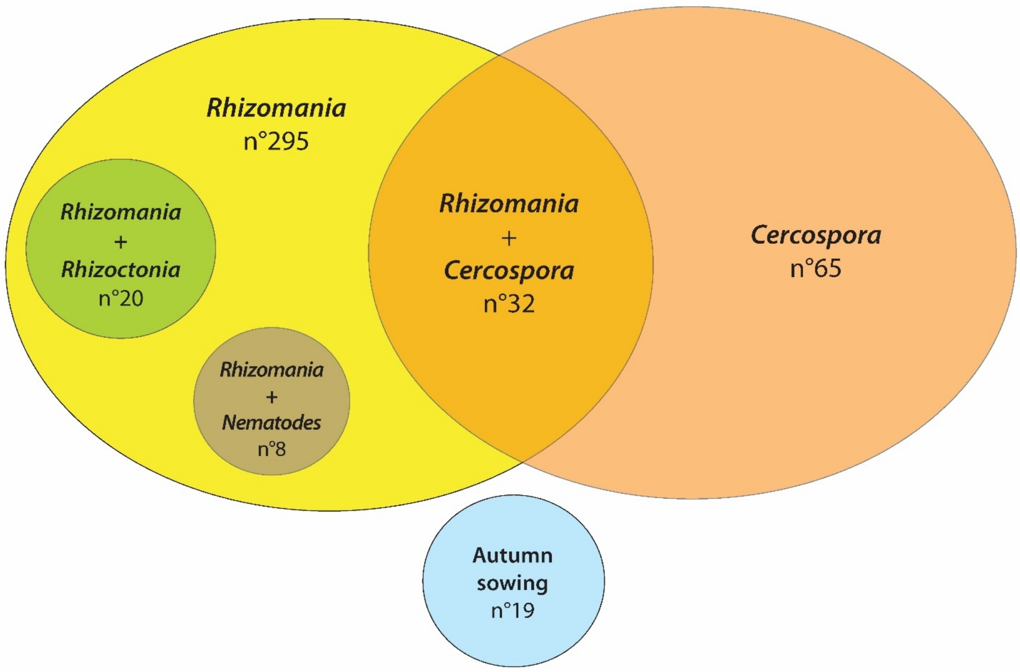

Figure 1. Resistance traits distribution in the *Beta* CREA-CI collection of Rovigo (*Beta vulgaris* subsp. *vulgaris* x *maritima*)

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. bieticola* 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-myosinase 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. *maritim*a 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 multi-functional plants, exhibiting different functions often exploited in the past. Some like *Barbarea verna* (Mill.) Asch., *Diplotaxis erucoides* (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., *Diplotaxis muralis* (L.) DC., *Diplotaxis 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

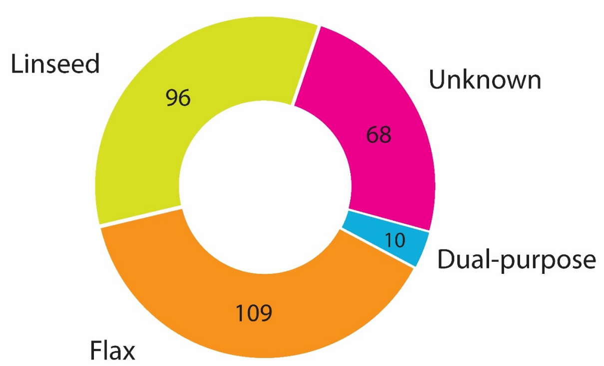

Figure 5. The CREA-CI collection includes the following accessions: 109 flax, 96 linseed, mostly ecotypes from Southern Italy, 68 unknown with uncertain suitability to the fibre or oil production and 10 designated as dual-purpose intended for both final outputs.

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

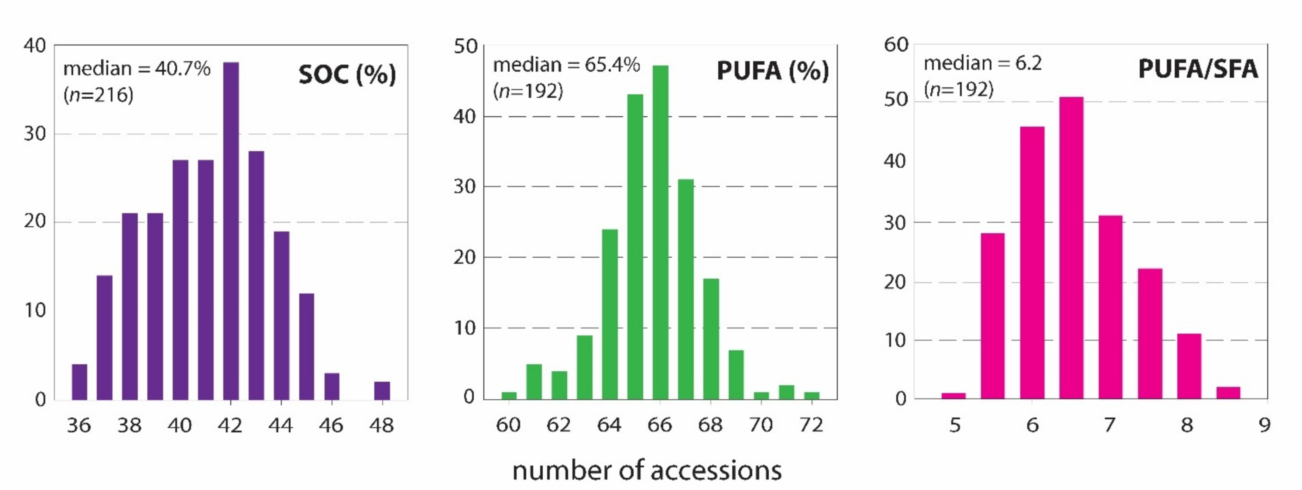

Figure 6. Distribution of accessions tested during 1989-2022 period in relation to seed oil content (SOC % w/w), polyunsaturated fraction (PUFA % w/w), and ratio of polyunsaturated to saturated fraction (PUFA/SFA, Elaborated from Fila *et al* (2024)).

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 trilaminate 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, homogeneous 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).

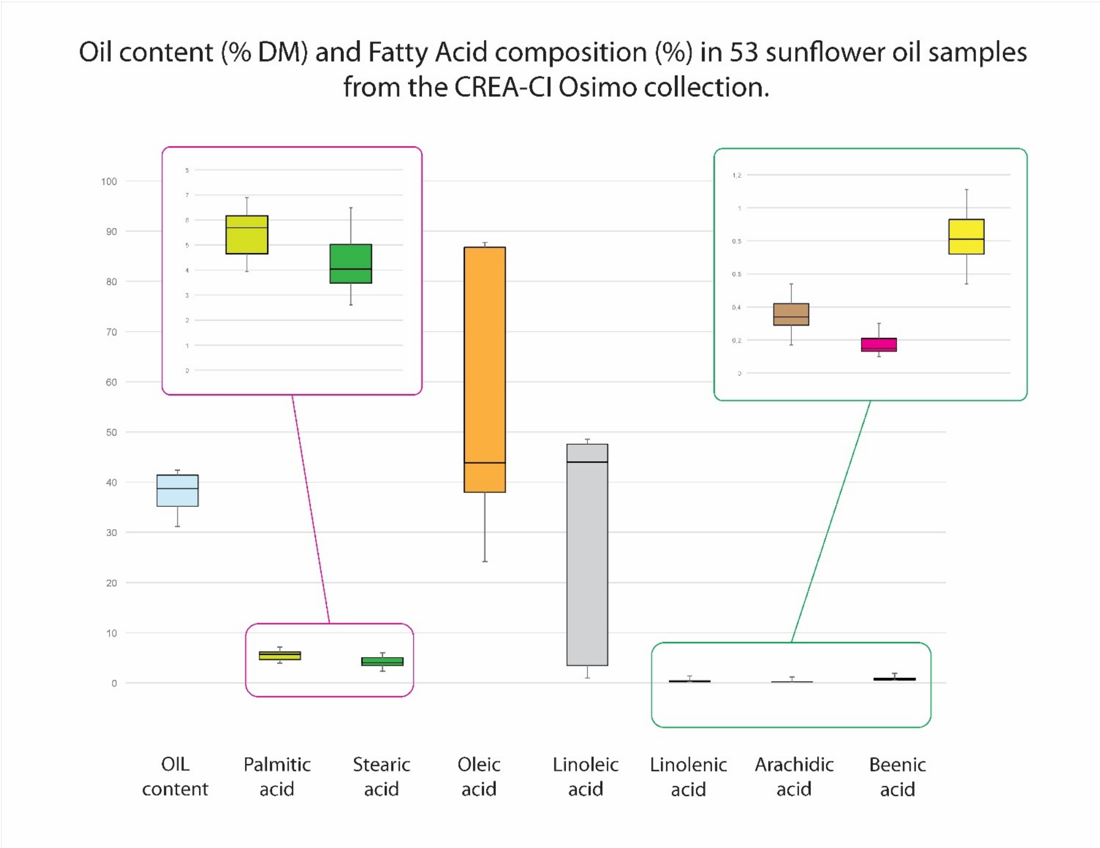

Figure 9. Oil content (% Dry Matter) and fatty acid composition (%) in 53 accessions of the Osimo CREA-CI collection.

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	Dioicious	Fibre/Biomass
CS	Dioicious	Fibre/Biomass
Fibranova	Dioicious	Fibre/Biomass
Fibrante (ex Red Petiole)	Dioicious	Fibre/Biomass
Asso	Dioicious	Fibre/Biomass
Codimono	Monoecious	Seeds/Biomass
Carmaleonte	Monoecious	Seeds/Biomass
Eletta Campana	Dioicious	Fibre/Biomass
Felsinea	Dioicious	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 sanisation 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). 'Ricciona' (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 (Pacifico *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 α-chaconine (Pacifico 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% (Pacifico *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 (Pacifico, 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 (Pacifico *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 genepools, 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 genepool (MG), Andean genepool (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

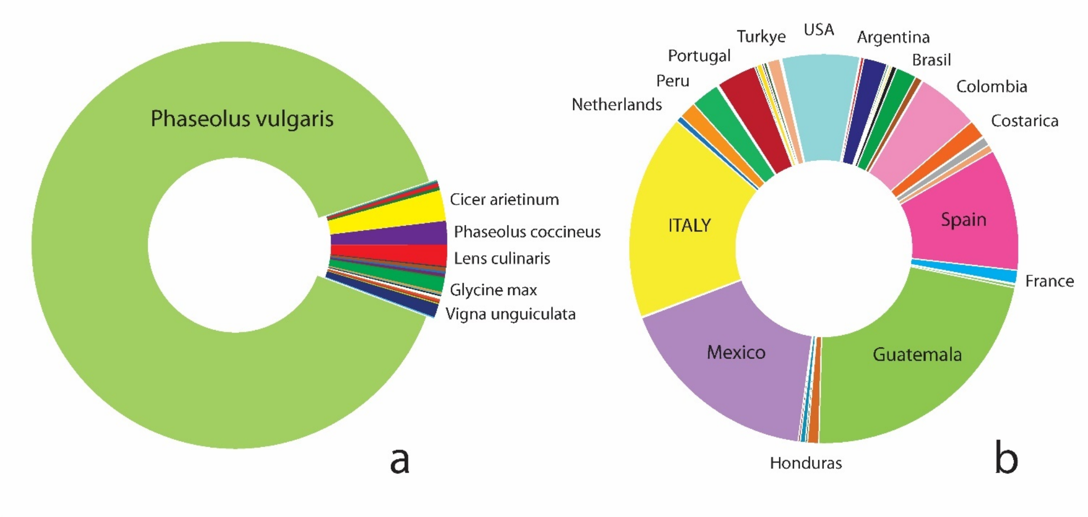

Figure 14. a, Species and b, Countries of origin of the accessions maintained in the grain legume collection.

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

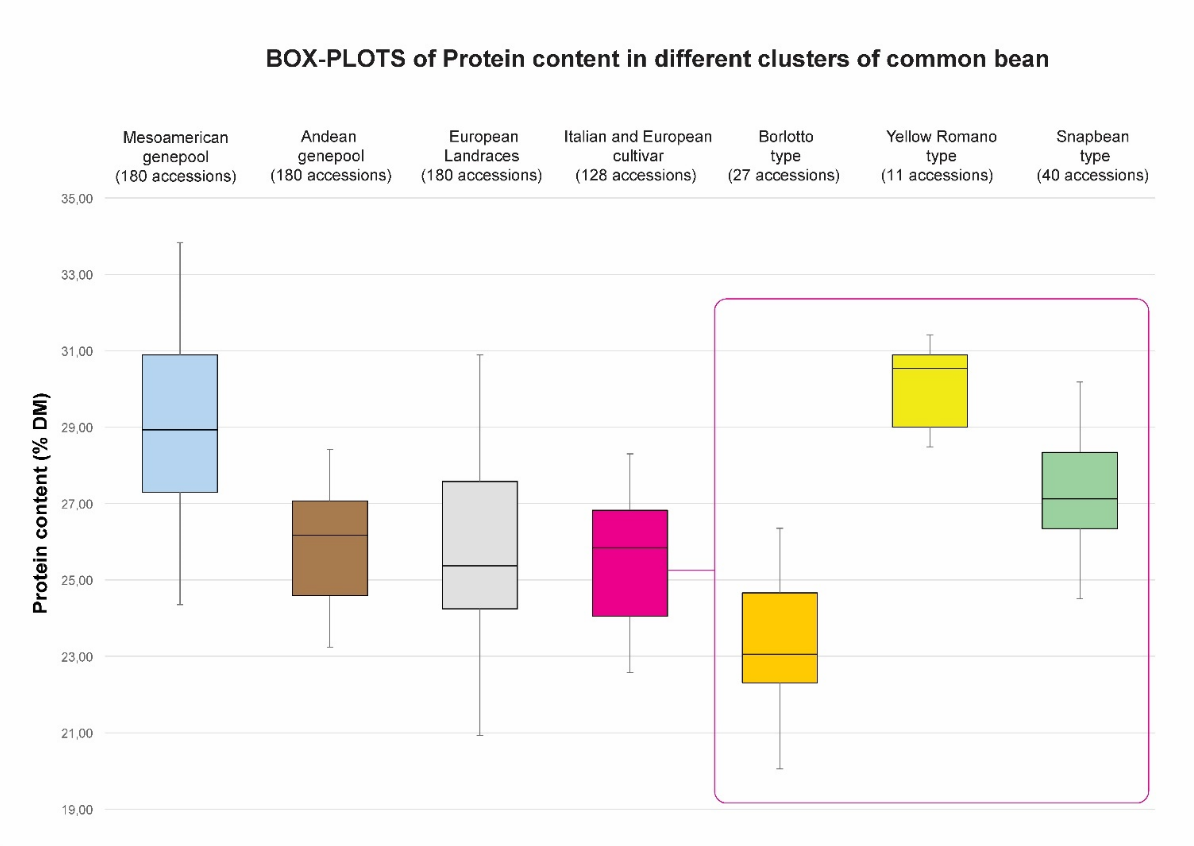

Figure 16. Box-plots of protein content (%DM, dry matter percentage) in different clusters of the CREA-CI common bean collection.

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.

Acknowledgements

It would take an enormous amount of space to name all the people who contributed to CREA-CI in Bologna and Rovigo over the last 100 years, and we would run the danger of overlooking some of them. We are profoundly grateful to each and every one of them.

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If the collection is so alive, it is also because of them. Thank you!

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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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