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## Special Issue

Novel Studies in Crop Breeding for Promoting Agro-Biodiversity























Edited by

Prof. Dr. Roberto Pilu and Prof. Dr. Michela Landoni



Review

# Plant Genetic Resources for Food and Agriculture: The Role and Contribution of CREA (Italy) within the National Program RGV-FAO

Patrizia Vaccino <sup>1,\*</sup>, Maurizio Antonetti <sup>2</sup>, Carlotta Balconi <sup>3</sup>, Andrea Brandolini <sup>4</sup>, Silvia Cappellozza <sup>5</sup>, Angelo Raffaele Caputo <sup>6</sup>, Andrea Carboni <sup>7</sup>, Marco Caruso <sup>8</sup>, Andrea Copetta <sup>9</sup>, Giovanbattista de Dato <sup>10</sup>, Pasquale De Vita <sup>11</sup>, Giancarlo Fascella <sup>12</sup>, Luca Ferretti <sup>13</sup>, Nadia Ficcadenti <sup>14</sup>, Pietro Fusani <sup>15</sup>, Massimo Gardiman <sup>16</sup>, Daniela Giovannini <sup>17</sup>, Jessica Giovinazzi <sup>18</sup>, Angela Iori <sup>19</sup>, Rita Leogrande <sup>20</sup>, Vincenzo Montalbano <sup>21</sup>, Maria Antonietta Palombi <sup>22</sup>, Luciano Pecetti <sup>4</sup>, Enzo Perri <sup>23</sup>, Milena Petriccione <sup>24</sup>, Tea Sala <sup>25</sup>, Paolo Storchi <sup>26</sup>, Alessandro Tondelli <sup>27</sup>, Pasquale Tripodi <sup>28</sup>, Nino Virzi <sup>29</sup> and Ignazio Verde <sup>18,\*</sup>



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- <sup>1</sup> CREA-CI, Council for Agricultural Research and Economics—Research Centre for Cereal and Industrial Crops, 13100 Vercelli, Italy
- <sup>2</sup> CREA-OF, Research Centre for Vegetable and Ornamental Crops, 51017 Pescia, Italy; [maurizio.antonetti@crea.gov.it](mailto:maurizio.antonetti@crea.gov.it)
- <sup>3</sup> CREA-CI, Research Centre for Cereal and Industrial Crops, 24126 Bergamo, Italy; [carlotta.balconi@crea.gov.it](mailto:carlotta.balconi@crea.gov.it)
- <sup>4</sup> CREA-ZA, Research Centre for Animal Production and Aquaculture, 26900 Lodi, Italy; [andrea.brandolini@crea.gov.it](mailto:andrea.brandolini@crea.gov.it) (A.B.); [luciano.pecetti@crea.gov.it](mailto:luciano.pecetti@crea.gov.it) (L.P.)
- <sup>5</sup> CREA-AA, Research Centre for Agriculture and Environment, 35143 Padova, Italy; [silvia.cappellozza@crea.gov.it](mailto:silvia.cappellozza@crea.gov.it)
- <sup>6</sup> CREA-VE, Research Centre for Viticulture and Enology, 70010 Turi, Italy; [angeloraffaele.caputo@crea.gov.it](mailto:angeloraffaele.caputo@crea.gov.it)
- <sup>7</sup> CREA-CI, Research Centre for Cereal and Industrial Crops, 40128 Bologna, Italy; [andrea.carboni@crea.gov.it](mailto:andrea.carboni@crea.gov.it)
- <sup>8</sup> CREA-OFA, Research Centre for Olive, Fruit and Citrus Crops, 95024 Acireale, Italy; [marco.caruso@crea.gov.it](mailto:marco.caruso@crea.gov.it)
- <sup>9</sup> CREA-OF, Research Centre for Vegetable and Ornamental Crops, 18038 Sanremo, Italy; [andrea.copetta@crea.gov.it](mailto:andrea.copetta@crea.gov.it)
- <sup>10</sup> CREA-FL, Research Centre for Forestry and Wood, 52100 Arezzo, Italy; [giovanbattista.dedato@crea.gov.it](mailto:giovanbattista.dedato@crea.gov.it)
- <sup>11</sup> CREA-CI, Research Centre for Cereal and Industrial Crops, 71122 Foggia, Italy; [pasquale.devita@crea.gov.it](mailto:pasquale.devita@crea.gov.it)
- <sup>12</sup> CREA-DC, Research Centre for Plant Protection and Certification, 90128 Palermo, Italy; [giancarlo.fascella@crea.gov.it](mailto:giancarlo.fascella@crea.gov.it)
- <sup>13</sup> CREA-DC, Research Centre for Plant Protection and Certification, 00156 Roma, Italy; [luca.ferretti@crea.gov.it](mailto:luca.ferretti@crea.gov.it)
- <sup>14</sup> CREA-OF, Research Centre for Vegetable and Ornamental Crops, 63077 Monsampolo del Tronto, Italy; [nadia.ficcadenti@crea.gov.it](mailto:nadia.ficcadenti@crea.gov.it)
- <sup>15</sup> CREA-FL, Research Centre for Forestry and Wood, 38123 Trento, Italy; [pietro.fusani@crea.gov.it](mailto:pietro.fusani@crea.gov.it)
- <sup>16</sup> CREA-VE, Research Centre for Viticulture and Enology, 31015 Conegliano, Italy; [massimo.gardiman@crea.gov.it](mailto:massimo.gardiman@crea.gov.it)
- <sup>17</sup> CREA-OFA, Research Centre for Olive, Fruit and Citrus Crops, 47121 Forlì, Italy; [daniela.giovannini@crea.gov.it](mailto:daniela.giovannini@crea.gov.it)
- <sup>18</sup> CREA-OFA, Research Centre for Olive, Fruit and Citrus Crops, 00134 Roma, Italy; [jessica.giovinazzi@crea.gov.it](mailto:jessica.giovinazzi@crea.gov.it)
- <sup>19</sup> CREA-IT, Research Centre for Engineering and Agro-Food Processing, 00189 Roma, Italy; [angela.iori@crea.gov.it](mailto:angela.iori@crea.gov.it)
- <sup>20</sup> CREA-AA, Research Centre for Agriculture and Environment, 70125 Bari, Italy; [rita.leogrande@crea.gov.it](mailto:rita.leogrande@crea.gov.it)
- <sup>21</sup> Ministry of Agriculture, Food Sovereignty and Forestry, DISR III, 00187 Roma, Italy; [v.montalbano@masaf.gov.it](mailto:v.montalbano@masaf.gov.it)
- <sup>22</sup> CREA-VE, Research Centre for Viticulture and Enology, 00049 Velletri, Italy; [mariaantonietta.palombi@crea.gov.it](mailto:mariaantonietta.palombi@crea.gov.it)
- <sup>23</sup> CREA-OFA, Research Centre for Olive, Fruit and Citrus Crops, 87036 Rende, Italy; [enzo.perri@crea.gov.it](mailto:enzo.perri@crea.gov.it)
- <sup>24</sup> CREA-OFA, Research Centre for Olive, Fruit and Citrus Crops, 81100 Caserta, Italy; [milena.petriccione@crea.gov.it](mailto:milena.petriccione@crea.gov.it)
- <sup>25</sup> CREA-GB, Research Centre for Genomics and Bioinformatics, 26836 Montanaso Lombardo, Italy; [tea.sala@crea.gov.it](mailto:tea.sala@crea.gov.it)
- <sup>26</sup> CREA-VE, Research Centre for Viticulture and Enology, 52100 Arezzo, Italy; [paolo.storchi@crea.gov.it](mailto:paolo.storchi@crea.gov.it)
- <sup>27</sup> CREA-GB, Research Centre for Genomics and Bioinformatics, 29017 Fiorenzuola d'Arda, Italy; [alessandro.tondelli@crea.gov.it](mailto:alessandro.tondelli@crea.gov.it)

<sup>28</sup> CREA-OF, Research Centre for Vegetable and Ornamental Crops, 84098 Pontecagnano Faiano, Italy; pasquale.tripodi@crea.gov.it

<sup>29</sup> CREA-CI, Research Centre for Cereal and Industrial Crops, 95024 Acireale, Italy; nino.virzi@crea.gov.it

\* Correspondence: patrizia.vaccino@crea.gov.it (P.V.); ignazio.verde@crea.gov.it (I.V.); Tel.: +39-0161-391134 (P.V.); +39-0679348183 (I.V.)

**Abstract:** Conservation, characterization and exploitation of agrobiodiversity are key factors to guarantee food security and face future challenges such as climate changes. These issues are the subject of a series of international agreements, such as the Convention of Biological Diversity, with its Nagoya Protocol, and the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) adopted in 2001 and entered into force in 2004. Italy ratified the Treaty in 2004 and instituted a long-lasting program, RGV-FAO, to implement it. CREA is one of the three organizations involved in the RGV-FAO Program, together with the National Research Council (CNR) and Reti Semi Rurali. CREA maintains a total of 40,186 accessions including cereals, vegetables, fruits, forages, industrial crops, forest and woody crops, medicinal and aromatic plants, and their wild relatives. Accessions are conserved using different ex situ conservation systems (seeds, in vivo plants, vegetative organs and in vitro plantlets), and characterized using genetic, morpho-phenological and/or biochemical methods. Herein, we will present the CREA long-lasting program RGV-FAO with some examples of the use of plant genetic resources in breeding programs, including molecular approaches. Some critical issues related to access and benefit sharing in PGRFA, such as the Nagoya Protocol and the Digital Sequence Information, will be discussed, highlighting their potential impact on food security and on the advancement of knowledge.

**Keywords:** agrobiodiversity; plant germplasm; characterization; conservation; genotyping; phenotyping; genebank; Digital Sequence Information (DSI)

## 1. Introduction

Agricultural plant genetic resources are the most important assets to face human future challenges such as climate changes and are pivotal for ensuring global food security. The awareness of their importance is quite recent and can be traced back to the beginning of the past century. The Russian scientist Nikolai Vavilov was the first who pointed out their importance for food security and the risk of losing genetic diversity (genetic erosion). Agriculture itself is the major driver of the loss of genetic diversity for crops [1]. Massive losses of crop genetic diversity result from selection during domestication and subsequent dissemination and breeding efforts. The Food and Agriculture Organization of the United Nations (FAO) estimates that approximately 75% of crop genetic diversity has been lost in the last century [2]. Recent estimates of nucleotide diversity at whole genome scale in different species highlighted the same rate of loss (ranging from 69% to 83%) compared to natural populations [3–7].

The increased awareness of the importance of genetic resources gave a relevant input to the constitution of seed banks. The first and most important one was established by Vavilov himself in Saint Petersburg. It was the result of the expeditions that Vavilov and his collaborators carried out all around the world to collect specimens of diverse crops and their wild relatives [8].

The coordination of worldwide efforts to preserve and exchange genetic material is even more recent and can be traced back to the second half of the 20th century. In the 1980s, FAO started the process that ended in 2001 with the adoption of the International Treaty for Plant Genetic Resources for Food and Agriculture (ITPGRFA). A comprehensive description of the process leading to the ITPGRFA is reported in Coupe and Lewins (2007) [9]. The main steps are summarized in Table 1.

**Table 1.** Main steps leading up to the “Treaty”.

1983	Establishment of the Commission on Plant Genetic Resources (CPGR). Adoption of the voluntary International Undertaking (IU) on Plant Genetic Resources.
1989	FAO Resolution 4/89—Plant Breeders’ Rights (PBR) are not in contrast with IU.
1989	FAO Resolution 5/89—Recognition of the farmers’ rights: farmers are entitled as the main actors in preserving Plant Genetic Resources for Food and Agriculture (PGRFA).
1991	FAO Resolution 3/91—Nations have sovereign rights on their own genetic resources.
1993	Convention on Biological Diversity (CBD) enters into force.
1994	Start of the revision of IU, to harmonize it with CBD, by the First Extraordinary Session of CPGR.
1995	CPGR is extended to include all the Genetic Resources for Food and Agriculture—CGRFA.
1996	Leipzig International Technical Conference on PGR. The Leipzig Declaration and the Global Plan of Action are adopted by 150 countries.
2001	6th Contact Group Meeting (Spoleto, Italy) and 6th Extraordinary Session of CGRFA (Rome, Italy)—Revised IU is adopted. The International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) is adopted by FAO (November).
2004	Italy ratifies the ITPGRFA (April). The ITPGRFA comes into force (June).

In 1983, the FAO Commission on Plant Genetic Resources (CPGR) was established, and the International Undertaking (IU) was adopted. This was a voluntary non-binding agreement that guaranteed access to Plant Genetic Resources for Food and Agriculture (PGRFA) for research and breeding. The text was then the subject of some agreements, basically aimed at striking a balance between the needs of the producers and those of farmers: FAO Resolutions 4/89, 5/89 and 3/91. Harmonization of FAO IU with the Convention on Biological Diversity (CBD), entered into force in 1993, was accomplished with the First Extraordinary Session of CPGR in 1994. In 1995, CPGR was expanded to include all the genetic resources for food and agriculture and become the Commission on Genetic Resources for Food and Agriculture (CGRFA). A fundamental step toward the adoption of the Treaty was the Leipzig International Technical Conference on plant genetic resources [10], when 150 countries adopted the Leipzig Declaration and the Global Plan of Actions. In the declaration, the Parties agreed on a fundamental aspect of the future Treaty, namely maintaining the multilateral character of the system of Access and Benefit Sharing (ABS). Italy played a pioneering role during the negotiation process, hosting two negotiating meetings, one in Spoleto in April 2001 (6th Contact Group Meeting) and the other in Rome in October 2001 (6th Extraordinary Session of CGRFA). In November 2001, the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA), henceforth “the Treaty”, was adopted by FAO. The Treaty came, finally, into force in June 2004. The Treaty takes its starting point from the consideration that plant genetic resources are constantly eroding, a common concern for all countries. On the other hand, the conservation and enhancement of these resources play an essential role in world food security, allowing the availability of genetic resources for breeding by both classical and biotechnological methods.

The main objective of the Treaty is, therefore, to conserve and ensure the sustainable use of plant genetic resources; this objective is complemented by the comparably significant objective of structuring a system that allows for a fair and equitable sharing of the benefits arising from their use. The core of the Treaty is the Multilateral System (MLS) of Access and Benefit Sharing (ABS) that establishes the rules for exchanging genetic resources among contracting parties and for equitable and fair sharing of the benefits arising from their use. The MLS is innovative and easy compared with the complex and cumbersome bilateral system between contracting parties used by CBD but, unfortunately, it is limited to 64 crops (listed in Annex 1 of the Treaty). This list comes from a long negotiation, and the numerous

attempts to enlarge it to all the PGRFA have been unsuccessful. The negotiations, as well as the discussions about the access to the Genetic Sequence Data (GDS), also known as Digital Sequence Information (DSI), and the payment system for accessing PGRFA and their fair and equitable benefit sharing, are still ongoing, and will be discussed in 2025 at Governing Body 11 (GB11).

Italy ratified the Treaty with a specific law (L. 06, April 2004, n. 101); this law included a program for collection, conservation, characterization and documentation of PGRFA in Italy, known as RGV-FAO. The Program [11], which started in 2004, is funded by the Italian Ministry of Agriculture, Food Sovereignty and Forestry (MASAF) and aims at implementing the Treaty in Italy, in particular its art. 5 (conservation, characterization and documentation of genetic resources), art. 6 (sustainable use, valorization of genetic resources) and art. 9 (Farmers' Rights). The Program includes the Council for Agricultural Research and Economics (CREA), the National Research Council (CNR) and the Italian seed diversity network Rete Semi Rurali (RSR) and is coordinated by the Research Centre for Olive, Fruit and Citrus Crops (CREA-OFA, formerly Istituto Sperimentale per la Frutticoltura). Originally, the Program also included non-food crops such as woody, forest, medicinal and ornamental crops. Since 2020, in agreement with art. 1 and art. 12.3 of the Treaty, MASAF excluded from the program all the non-food/feed crops such as woody, medicinal and ornamental plants.

Herein, we will present the results achieved during 20 years of the RGV-FAO Program at CREA. Some examples of the use and valorization of the PGR will be discussed, e.g., their use in breeding programs and the resulting achievements, their use with molecular approaches such as Genome-Wide Association Studies (GWAS) for the identification of genes underpinning important agronomic traits, the path of domestication and diversification of important crops, and the establishment of important tools such as core collections and Single Nucleotide Polymorphism (SNP) arrays. The Italian and international framework will be discussed highlighting the complex aspects of the Nagoya Protocol (NP) of the Convention on Biological Diversity (CBD) that deals with all the genetic resources and its connection with the ITPGRFA, as well to other important issues such as the NGS/DSI.

CREA is the main agricultural research organization in Italy. It plays a central role in the collection, conservation, characterization and valorization of plant genetic resources. CREA actively participates to several Working Groups of the European Cooperative Programme for Plant Genetic Resources (ECPGR) [12]. Ten out of its 12 centers, with their capillary network of structures throughout the country, in the 20 years of RGV-FAO Program, collected and maintained in their repositories about forty thousand accessions of the major crops and their wild relatives (Table 2, Table 3 and Table S1).

**Table 2.** Crop accessions maintained at CREA.

Crop Group	N. of Accessions
Cereal crops	17,006
Fruit crops (including nuts, berries, citrus, olive and grape)	11,719
Vegetable crops	521
Forage crops	7643
Industrial crops (including grain legumes)	1950
Medicinal and aromatic plants	9
Ornamental crops	974
Forest and woody crops	733
TOTAL	40,555

Accessions are referenced with FAO multi-crop passport descriptors (MCPD) [13]. Most of the CREA accessions are included in the European catalogue EURISCO [14] that is updated on an annual basis. The phenotypic characterization of the materials has been achieved using the National Guidelines for the Conservation and Characterization of Plant Biodiversity of Agricultural Interest [15,16] developed in 2012 with the coordination of

MASAF, and the information is being stored in a structured database. In addition, all the collections are in the process of obtaining the Digital Identification Object (DOI). About 12 thousand of accessions are included in the ITPGRFA MLS and are currently exchanged with both public and private entities and about four thousand accessions are shared in AEGIS (A European Genebank Integrated System) [17]. During the RGV-FAO period, CREA has exchanged 4957 accessions (Table 4).

**Table 3.** Number of cultivated and wild relative species for each crop group.

Crop Group	Cultivated Species	Crop Wild Relatives (CWR)
Cereal crops	24	33
Fruit crops (including nuts, berries, citrus, olive and grape)	70	108
Vegetable crops	30	9
Forage crops	70	0
Industrial crops (including grain legumes)	19	6
Medicinal and aromatic plants	6	0
Ornamental crops	197	0
Forest and woody crops	2	0
<b>TOTAL</b>	<b>418</b>	<b>156</b>

**Table 4.** Number of accessions exchanged by CREA from 2004 to 2023.

Crop Group	N. of Accessions Transferred to			N. of Accessions Introduced from		
	Italy	Abroad	Total	Italy	Abroad	Total
Cereal crops	258	189	447	167	198	365
Fruit crops (including nuts, berries, citrus, olive and grape)	851	92	943	877	142	1019
Vegetable crops	87	0	87	80	152	232
Forage crops	30	50	80	71	98	169
Industrial crops (including grain legumes)	142	53	195	145	774	919
Medicinal and aromatic plants	63	10	73	13	9	22
Ornamental crops	19	1	20	11	0	11
Forest and woody crops	190	137	327	19	29	48
<b>TOTAL</b>	<b>1640</b>	<b>532</b>	<b>2172</b>	<b>1383</b>	<b>1402</b>	<b>2785</b>

CREA has been actively participating in many European projects aimed at conserving and exploiting plant genetic resources. Most of the projects are described in each crop section. Recently CREA is contributing to the setup of a European Research Infrastructure (RI) within the Horizon Europe Framework with the project “Promoting a Plant Genetic Resources Community for Europe (PRO-GRACE)” [18], whose aim is to bring together the European institutions working on the conservation and characterization of plant genetic resources and exploit their full potential. CREA genetic resources are currently being exploited within the Agritech National Center Spoke 1 [19] to cope with adaptation to climate changes.

## 2. Cereal Crops

Cereal crops, grown on more than 25 percent of the arable land (Table 5) play a central role in Italian agriculture and support agri-food, livestock and industrial activities. Nevertheless, the national surface area dedicated to their cultivation has been shrinking and today the production of major grains is largely insufficient to satisfy the internal demand. In addition, the Mediterranean basin is one of the most vulnerable regions to climate change and it is at great risk for food security in the coming years [20]. In this context, the contribution of public research for the development of advanced high-yielding and resilient varieties is fundamental to maintain the competitiveness of the Italian cereal sector.

Exploring the genetic diversity of cereals provides valuable insights into their evolutionary history and is the basis for genetic dissection of adaptive, yield and quality traits. For all these reasons, genetic resources from these species have been collected, and seeds are maintained ex situ in cold chambers (medium term storage) periodically regenerated in the field and characterized following UPOV guidelines [21] in different CREA centers.

**Table 5.** Surface and production of cereal crops in Italy in 2022 (source: FAOSTAT) [22].

Crops	Harvested Area (ha × 1000)	Grain Yield (t × 1000)
Durum wheat	1238	3690
Common wheat	539	2760
Maize	564	4682
Barley	268	1124
Rice	218	1237
Oat	104	242
Other cereals	25	74

### 2.1. Wheat and Triticale

Wheat is one of the most important crops worldwide, occupying 17 percent of the world total arable land and providing the staple food for 35 percent of the world population [23]. A predominant role is played in Italy, particularly in the southern regions, by durum wheat, which was grown on over 1.2 Mha in 2022 to feed the industrial pasta chain [24]. CREA conserves accessions of wild and domesticated wheats of different ploidy.

#### 2.1.1. Diploid Wheat

The Research Centre for Animal Production and Aquaculture (CREA-ZA) maintains more than 2000 diploid wheat accessions belonging to several species, i.e., 675 domesticated *Triticum monococcum* accessions, 867 wild *T. thaoudar* syn. *boeoticum* accessions, 366 *T. urartu* accessions, 97 *Aegilops* accessions from different species. Among the domesticated einkorns, a core collection of 158 accessions from different origin was characterized both morpho-physiologically and genetically through DARTseq markers [25,26]. The information collected is fueling a small breeding program aiming to reintroduce this neglected crop into cultivation. Five genotypes were recently released, i.e., ‘Monlis’ and ‘Norberto’ (good breadmaking quality), ‘Antenato’ (early-maturing), ‘Monili’ (good yield), ‘Ham-murabi’ (free-threshing and early-maturing) and are grown in Italy over a few hundred hectares. Also, einkorn-based food products (flour, pasta, biscuits) are now available on the market. The phenotypic and genotypic diversity of 299 *T. urartu* accessions has been described by Brunazzi et al. [27] and Talini et al. [28].

#### 2.1.2. Tetraploid Wheat

The tetraploid wheat species (*T. turgidum* ssp.,  $2n = 4x = 28$ ; AABB genome) encompass eight subspecies, including wild (*T. turgidum* ssp. *diccooides*) and domesticated ones (*T. turgidum* ssp. *dicoccum*, ssp. *paleocolchicum*, ssp. *carthlicum*, ssp. *polonicum*, ssp. *turgidum*, ssp. *turanicum* and ssp. *durum*) [29,30]. Among all these subspecies, only durum wheat is cultivated extensively, particularly in the Mediterranean region [24], to produce pasta and couscous, and to a lesser extent various bread specialties.

The Research Center for Cereal and Industrial Crops (CREA-CI) in Foggia hosts an important collection of about 1600 tetraploid wheat accessions, entirely genotyped with SNP markers. Part of this collection was exploited to analyze the evolutionary pressure exerted by the environment on the various tetraploid species, and more recently, the action of plant breeding on the polyphenol oxidases (*PPO*) genes [31]. This allowed to better clarify the origin of Persian wheat (*T. carthlicum*) from its wild emmer progenitor (*T. diccooides*). Furthermore, this study supported the idea that *PPO* genes were under strong selection pressure as they were linked to groups of adaptive and/or resistance genes influencing plant adaptation to environmental stresses. By studying the genetic diversity of a large

panel of Italian durum wheat accessions (i.e., landraces, old and modern cultivars), Taranto et al. [32] showed a strong effect of demographic history and breeding in chromosomal regions where the morpho-phenological genes, such as vernalization (*Vrn*), photoperiod sensitivity (*Ppd*) and dwarfing (*Rht*) genes, were located. All the studies conducted on tetraploid wheat suggested the need to recover the genetic diversity of wild or domesticated species and wheat landraces through intense pre-breeding activities.

At CREA-CI in Acireale, durum wheat accessions are maintained and characterized through morphological, phenological and bio-agronomic surveys. The collection includes several Sicilian landraces, whose description highlighted inter- and intra-population polymorphisms as well as protein compositions no longer detectable in modern wheats. Technological analyses were carried out on most of the accessions to evaluate their suitability for transformation, also in the frame of international and national projects, such as the Horizon 2020 DiverImpacts [33], Biodurum (Masaf), IN.TE.GRA. and INNO.MALTO (PSR Sicily). Breeding programs involving these accessions led to the release of the durum wheat varieties Sant'Agata and Ciclope.

At the Research Centre for Genomics and Bioinformatics (CREA-GB) in Fiorenzuola d'Arda (PC), a collection of 400 *T. dicoccoides* accessions has been assembled and genotyped to identify novel sources of disease resistance (e.g., stem rust at seedling level [34]) and to study traits related to spike fertility under different environmental conditions. The collection laid the foundation of a multiparental mapping population that will be characterized in the recently funded PRO-WILD project. In addition, 700 durum wheat landraces originated from worldwide geographic area traditionally dedicated to durum wheat cultivation (mainly around the Mediterranean basin and the Fertile Crescent), have been evaluated for resistance to wheat rusts and Septoria at adult stage in different Mediterranean field environments, and for traits related to spike fertility. The collection has been genotyped with SNP markers, and its genetic structure deeply characterized [35]. Most of these accessions are included in two large reference collections, the Global Durum Panel [36] and the tetraploid Global Collection [35]. The genome of a few subpopulation representatives of these wild emmer and durum landrace collections are being sequenced and assembled to be included in the tetraploid pangenome.

At the Research Centre for Engineering and Agro-Food Processing (CREA-IT) in Rome, 295 *T. dicoccum* accessions from different origin were collected and maintained as seeds both in cold chamber ( $T < 5\text{ }^{\circ}\text{C}$ ) and in sealed bags at  $-20\text{ }^{\circ}\text{C}$ . Over the years, the germplasm was regenerated in field and characterized for adaptability to autumn and spring sowings, disease resistance at adult-plant stage, protein content, SDS test, gluten and yellow index [37]. Some accessions were analyzed in the greenhouse to evaluate, at the seedling stage, the resistance to the main fungal pathogens (*Blumeria graminis tritici*, *Puccinia triticina*, *P. graminis tritici* and *Parastagonospora nodorum*). In addition, few accessions of the tetraploid *T. timopheevii* (Lonigo, Far 72 and Tr110) were proven to be potentially usable to produce foods with enhanced nutritional and health properties, thanks to their high protein content, low gluten index and high total antioxidant capacity. Technological and rheological assessments indicate they could serve as promising raw materials for flatbreads, biscuits and pasta formulations [38].

### 2.1.3. Hexaploid Wheat

The largest germplasm collection of *T. aestivum* is preserved at CREA-CI in Vercelli. With more than 5000 accessions, it represents a wide source of genetic variability for many agronomic and qualitative traits. An important set of accessions is represented by more than 100 cultivars released by Nazareno Strampelli, one of the most important plant geneticists of the 20th century. Such varieties, characterized by rust resistance, early flowering and maturity and short straw, allowed Italy to increase crop production during the so called "Battaglia del grano" (Battle for Grain; 1925–1940), launched to achieve self-sufficiency in wheat production. Some of Strampelli's wheats served as parents in the breeding programs of several countries (e.g., China, Argentina, Russia, Yugoslavia) after the Second World



War and had a key role also in the first phase of Norman Borlaug's Green Revolution. Even today, Strampelli germplasm represents a valuable asset for the constitution of new genotypes. A thorough activity of genetic characterization of these varieties was carried out by means of protein and DNA molecular markers [39]. Another panel of 160 accessions, representative of common wheat breeding in Italy during the 20th century, was deeply characterized at the phenotypic and genotypic level. This research allowed to describe the evolutionary trends of common wheat breeding throughout this period and to survey the shift of key traits [40], but also to identify significant associations (MTAs) for traits related to crop-weed interference, of outermost importance in organic and low-input agriculture [41]. Today CREA-CI participates in the H2020 project AGENT [42], which aims to unlock the full potential of the biological material stored in gene banks around the globe. Within the project, one thousand bread wheat accessions from CREA-CI are being studied at the genomic and phenotypic level and will contribute to the prediction of phenotypic values through genomic selection, and to the identification of genes responsible for important agronomic traits.

Other CREA centers focused their attention on different subspecies of hexaploid wheats that often possess interesting agronomic, qualitative and nutritional characteristics, potentially serving as valuable source of raw materials for functional foods and of valuable genes for pre-breeding activities. For example, CREA-GB has recently assembled a collection of about 200 accessions of uncultivated hexaploid wheats belonging to the *compactum*, *sphaerococcum*, *macha* and *spelta* subspecies including landraces and wild forms from all geographical areas of the world. These accessions, not yet included in EURISCO, were genotyped with SNP markers and phenotyped for different phenological and morphological traits. A different set of 212 *T. aestivum* ssp. *spelta* conserved at CREA-IT has been characterized for key bio-agronomic and qualitative traits, such as adaptability to autumn and spring sowings, resistance to fungal pathogens, protein content, SDS test, gluten and yellow index [43]. Researchers of CREA-IT have also evaluated the agronomical, biochemical, nutritional and technological performance of nine perennial wheat genotypes obtained from crosses between *T. aestivum* and *Thinopyrum* spp. (*Th. elongatum*, *Th. intermedium* and *Th. ponticum*) in comparison to common wheat cultivars. Results showed higher soil respiration [44] and higher content of carotenoids and 5-n-alkylresorcinols (AR) known to potentially mitigate cardiovascular diseases and cancer risks [45]. The same center hosts 117 differential wheat Near Isogenic Lines (NILs) carrying one or more resistance genes against major fungal diseases. These lines are useful in breeding efforts against pathogenic fungi that hinder both quantitative and qualitative production and aid in monitoring pathogen evolution [46].

#### 2.1.4. Triticale

Thanks to RGV-FAO, a triticale collection consisting of 166 accessions with unique characteristics for cultivation in Italy is preserved at CREA-GB, where a small breeding program for the species is currently running in collaboration with Italian seed companies. While molecular data are not yet available, the accessions have been continuously enriched of morpho-physiological descriptors, yield data and observations on disease resistance.

#### 2.2. Barley

Barley represents the model plant for genomic, genetic, molecular and physiological studies carried out at CREA-GB, where more than 3000 barley accessions from worldwide origin are hosted. Among them are germplasm collections extensively characterized both at the genotypic and the phenotypic level in the frame of EU-funded projects, such as ExBarDiv [47] and Whealbi [48]. More recently, a reference set of about 1000 landraces covering the different agro-climatic zones of the Mediterranean basin has been established within the PRIMA project GendiBar; it will serve for detecting traits and genes useful for barley adaptation to harsh environments. More than 1400 barley accessions are at present considered in the RGV-FAO Program, with special attention paid to a subset of

approximately 200 landraces and cultivars of Italian origin selected in collaboration with the CNR-IBBR Genebank of Bari for registration in the European AEGIS (A European Genebank Integrated System) catalog of the ECPGR. They are also under evaluation for disease resistance and other agronomical traits in the frame of the EVA Wheat and Barley network. At CREA-GB genetic stocks have been developed that include bi- and multi-parental mapping population, *Hordeum vulgare*/*H. spontaneum* introgression lines and developmental mutants for basic research. A large breeding program in collaboration with Italian seed companies has led to the registration of >40 cultivars that today cover > 25% of the certified barley seed in Italy. Pre-breeding activities are continuously fed with genotypes, traits and genes from the genetic resources described above.

### 2.3. Maize

Maize (*Zea mays* L.) is one of the major crops of the world for feed, food and industrial uses. It originated in Central America and was introduced into Europe and other continents after Columbus trips at the end of the 15th century. Due to its large adaptability, farmers have originated a wide variability of genetic resources with wide diversity of adaptation, characteristics and uses. Maize introduction into Italy was almost simultaneous to introduction into Spain and Portugal [49].

During the 1950s, when the cultivation of maize traditional varieties started to be replaced by hybrids, with the aim of avoiding a loss of precious germplasm, a survey of local Italian varieties was carried out by the Regional Inspectorates of Agriculture with the support by the Italian Ministry of Agriculture. In this context the Experimental Maize Station in Bergamo organized a systematic acquisition of Italian maize germplasm including over 600 samples of local populations cultivated by farmers in various regions [50]. Nowadays CREA-CI Bergamo preserves *ex situ* the largest maize collection in Italy and one of the richest in Europe: over 5300 accessions maintained as seeds in cold chambers and periodically field regenerated under controlled pollinations [51]. As listed in the European catalogue ECPGR [52] the collection includes the following: 3590 inbred lines, originating from United States (41%) and from Italy (40%); 1262 landraces, over half of which Italian and others from over 20 foreign countries; 33 selected synthetic populations mostly constituted in Italy.

The wide range of pedoclimatic conditions in Italy and the different cultivation practices originated a large number of local varieties, promoting selection of flint and semi-flint maize [53] with high milling yield, suitability in food flour production and pasting properties [54,55], wide genetic variability for main grain components (protein, lipid, starch and carotenoid contents [56,57], variability for susceptibility to *F. verticillioides* ear rot [58,59]. CREA Italian maize collection, recently genetically characterized, can be considered an important tool for mapping useful traits and associated loci/alleles to be utilized in maize breeding programs [60].

CREA-CI in Bergamo is involved (i) at national level in seed stock regeneration under controlled pollination, preservation and characterization of maize traditional varieties from Lombardy registered in the Varietal National Conservation Catalogue [61]; (ii) at international level as member and Italian Chair of the Maize Working Group of ECPGR and Italian Representative in EVA Maize Network [62]. Several European, National and Regional research projects aimed at the valorization of CREA maize collection are listed as follows: P.G.S.O. Km 0 Bolivia (AICS International Agency Cooperation and Development), GEMMA—Lombardy maize varieties valorization; VALOMAYS—Local varieties of maize: characterization for reintroduction in the Lombardy region; PRIMADROMAMED—Capitalization of Mediterranean maize germplasm for improving stress tolerance; MineLandDiv—Mining allelic Diversity of maize Landraces for tolerance to abiotic and biotic stresses, SusCrop—ERA-NET-Joint FACCE-JPI-AGROBIOVERSITY. CREA-CI Bergamo Genebank germplasm valorization is represented by pigmented maize materials developed in the frame of P.S.G.O. Km0 Bolivia project; Italian white local varieties were crossed to Bolivian (Morado) and Mexican (Azul) types. Derived purple and blue maize

varieties rich in antioxidant compounds could be a good tool to increase the functional food properties [55,63].

#### 2.4. Rice

Although in Italy, due to climatic conditions, rice cultivation is restricted to the temperate japonica type (*Oryza sativa* L. ssp. *japonica*), the long history of Italian rice breeding, which began at the beginning of the 20th century, has resulted in the release of a considerable number of varieties, many of which appreciated and cultivated in other countries of the Mediterranean basin, e.g., Spain, France, Greece and Portugal. This result is due, on the one hand, to the introduction and evaluation of foreign germplasm that allowed the identification of the best traits suitable for the Italian environment to be used in breeding programs and, on the other hand, to the valorization of heritage cultivars, no longer competitive for yield performance, but characterized by peculiar quality traits that make Italian rice particularly appreciated in the world. The rice collection present at the CREA-CI Vercelli Genebank consists of 700 *O. sativa* spp. *japonica* genotypes, both Italian and international, three accessions of the ancestor *O. rufipogon* Griff. and 5 accessions of *O. glaberrima* Steud., known as African rice, domesticated and grown in West Africa around 3000 years ago. During the regeneration cycles, materials are characterized at the morphological level with international descriptors according to the “Standard Evaluation System for Rice” [64] and the national guidelines [16]. The wide genetic variability of the collection is used for the investigation of the loci responsible for important agronomic traits, e.g., resistance to biotic and abiotic stress, and for quality traits.

Using a panel of 311 rice accessions, analyzed for two years in the open field and for one year in the growth chamber for resistance to blast (*Pyricularia orizae*), GWAS analysis allowed the identification of 11 rice lines with a high degree of resistance in both field and controlled environment conditions, which represent potential donors of new resistance genes [65]. A similar study conducted on a panel of 138 rice lines analyzed for resistance to the pathogen *Fusarium fujikuroi*, agent of bakanae, a seed-borne disease, led to the identification of two potential sources of resistance on rice chromosomes 1 and 4 [66].

Using a panel of 281 rice lines, grown for two years under two growing conditions, permanent flooding (PF) and alternate wetting and drying (AWD), and analyzed for 26 phenotypic traits related to phenology, plant and seed morphology, and physiology, Volante et al. [67] found great variability for adaptation to water stress conditions. The significant associations found for each of the two conditions through GWAS pave the way to the identification of genes to be used in breeding for drought tolerance.

As regards heritage cultivars, a comprehensive description of the historical background and of the varieties cultivated in Italy between the end of the 19th century and the middle of the 20th century was performed and the results collected in a book [68].

#### 2.5. Oat

The oat collection (*Avena* spp.) maintained at CREA-CI, Bergamo, consisting of almost 1000 accessions, was enriched since 2000 of new accessions, coming from different genebanks, which were evaluated for their nutritional quality [69–71]. A part of the collection was studied in the European project “Avena genetic resources for quality in human consumption” (AVEQ) and characterized for the content of bioactive molecules [72,73]. Some oat cultivars were used for the development of NIRS calibration curves [74,75]. The genetic relationships among *Avena* species with different ploidy levels were described by the study of a particular class of starch-bound proteins [76,77]. At CREA-GB, around 1000 European accessions (diploid, tetraploid and hexaploid), including old and modern varieties, local populations and wild oats, collected in the frame of AVEQ, are preserved. A set of ca 150 accessions has been genotyped using SNP markers to study the genetic basis of traits of interest, such as the resistance to biotic and abiotic stresses (e.g., freezing tolerance) [78], and the content of antioxidant compounds (e.g., avenanthramides and  $\beta$ -glucans). The oat genetic resources are exploited in a small breeding program running at

CREA-GB and in the development of mapping populations, such as a MAGIC population of 17 families from 11 different parents.

### 3. Fruit Crops (including Berries, Nuts and Morus), Olive, Citrus and Grape

Encompassing one third of the arable land, the fruit sector is a significant component of the Italian agro-industry (Table 6).

**Table 6.** Surface and production of fruit crops in Italy in 2022 (source: FAOSTAT) [22].

Crops	Harvested Area (ha × 1000)	Yield (t × 1000)
Fruit (including berries)	237.64	5342.22
Grape	709.89	8437.97
Olive	1076.52	2160.40
Citrus	145.92	3094.43
Nuts	147.82	199.46

CREA fruit collections are the primary centers for fruit germplasm conservation in Italy and are internationally significant. The traditional Italian germplasm accounts for a substantial share of the total germplasm conserved.

As most of fruit crops are woody perennials, vegetatively propagated and with a relatively long unproductive phase, fruit germplasm at CREA is mostly maintained as living collections, with accessions readily available for characterization and evaluation, exchange, fruit exhibitions and open-day events purposes. However, efforts were also made to generate and maintain virus-free propagation material, with emphasis on commercial varieties, and part of the fruit, grape, citrus and olive collections are maintained into insect-proof screen-houses and officially registered in the national list of the certified plant propagation material, available for national nursery people associations and growers. A sanitary selection based on visual inspection of symptoms and specific serological, molecular and biological diagnostic tests is at the base of the production of virus free-propagating material. Nuclear stocks of plants free from systemic pathogens (virus, viroids and phytoplasmas), maintained under protected conditions are generated following this procedure and used as starting propagation material in regulated certification schemes. Considering that no direct control strategies can be applied against systemic plant pathogens, the use of virus-free planting material for establishing new plantations is one of the few preventing measures that can be adopted to efficiently counteract the spread of these pathogens and to protect the plant germplasm from the detrimental effects they induce. The repositories also provide data and plant samples required by numerous research projects in various disciplines, including plant breeding, genetics, agronomy and biotechnology. The funding and scientific results of these projects depend on the existence of the maintained accessions.

#### 3.1. Fruit

The Fruit germplasm collection was first established in the second half of the 20th century, with a boost from 1980s, after three nation-wide censuses revealed the high risk of loss of the traditional and locally adapted landraces, increasingly replaced by modern cultivars [79]. Many of the fruit genetic resources inventoried were included in CREA collection, now representing the largest National collection of fruit germplasm (Table S1). Over 6200 accessions of the most important cultivated species, pome and stone fruit crops, strawberry and small fruits, mulberry and nuts are conserved in the collections of Rome, holding the National Repository Centre for Fruit crops species (Centro Nazionale Germoplasma Frutticolo, CNGF), Forlì, Caserta, Bari and Padova collections (Table S1). Accessions are maintained as living trees, in plots of 2 to 5 trees, grafted onto crop-specific rootstocks when required. An exception is strawberry, almost entirely preserved *in vitro*, in slow growth conditions (Baruzzi personal communication). Also, about 200 valuable fruit genetic materials are safety-duplicated in slow-growth conditions,

with a prospect of further enlargement [80] (Giovannini unpublished results; Caboni personal communication). Cryopreservation of dormant buds and/or shoot tips is under study as a back-up strategy to ensure long-term maintenance of strawberry and fruit woody plant germplasm [81]. Despite the many reports of successful cryopreservation in the literature, routine implementation of this technology in cryo-banks is often hindered by the criticality of technology transfer and protocol validation across laboratories [82].

Characterization is carried out by using standardized descriptors, mainly those from ECPGR [83–85], UPOV or the National Guidelines [16]. Phenology of blooming and harvest are the most frequently recorded traits, and historical series are used to assess the effect of climate change in almond [86], apple [87], sweet cherry [88] and peach [89,90]. Traits related to tolerance to abiotic [91] and biotic [92,93] factors are increasingly evaluated in many collections, as well as fruit quality, including secondary metabolites with health-preserving properties [94]. Recent studies on mulberry varieties maintained at CREA revealed that soroses are a precious source of secondary metabolites, e.g., stilbenoids, with anti-inflammatory, anti-tyrosinase and antioxidant actions [95,96].

Molecular characterization of part of peach [97], plum [98] and sweet cherry [99] CREA germplasm collections has been performed by using the SSR markers sets agreed by ECPGR and CPVO international networks [100–104]. The use of ECPGR cherry SSR sets allowed the alignment *ex post* of 2241 SSR sweet cherry profiles from six countries datasets, including 186 accessions of CREA cherry collection [105], and the development of a Joint International Database for cherry, with a significant step forward in the coordinated management of field collections of cherry in Europe [106]. A set of long core SSRs was also developed and proposed as universal set for *Prunus* [107] with the aim of overcoming some of the drawbacks of the most common di-nucleotides ones such as easy scoring and limitation of stuttering.

In the framework of FP7 Fruitbreedomics EU project, about 1500 accessions of the peach European collection that included about 400 CREA accessions were genotyped using the 9K IPSC SNP array [108]. Stratification of peach germplasm in 3 subpopulations (Oriental accessions, Occidental Ancient landraces and Occidental varieties from modern breeding programs) was detected in accordance with historical path of peach diversification from domestication in China to the westward dissemination; in addition, a GWAS analysis was performed allowing the detection of genomic regions involved in the control of seven monogenic traits [109] as well of Quantitative Trait Loci (QTL) [110]. Moreover, using the above information a Peach Reference Population (PeachRefPop) composed of 169 accessions was designed, reflecting the genetic and phenotypic diversity of the original collections [111]. This resource has been duplicated in several European environments and is currently used to detect important traits such as adaptation to climate changes and resistance to pests and disease as well as genotype by environment interaction ( $G \times E$ ). Within the Horizon ARACNE project (2023–2026), the CREA mulberry collection is being genetically compared to other European and Asian mulberry collections (up to 500 accessions analyzed via ddRADseq) to infer very precisely the genetic diversity and the population structure of the germplasm and reconstruct the possible route of mulberry introduction in Europe.

At regional scale, in the frame of DICOVALE and AGRIGENET projects the varieties with interesting bio-agronomic traits have been transferred on-farm [112]. The purpose of an on-farm conservation is to provide support in various forms to farmers to enable them to continue cultivating traditional varieties in their systems and practices, thereby conserving and perpetuating plant genetic diversity. In the frame of the projects GenFruLa and MADOGEN, funded by the Agency for the development and innovation in agriculture of the Lazio region (ARSIAL), aiming at safeguarding autochthonous crop species, 528 different apple trees, including reference plants from the CREA collection, were genotyped with the Apple 20K Illumina SNP Array [113] enabling true-to-type analysis of different samples grown in the nurseries of the Lazio region and highlighting synonymies, homonymies and mislabeling among the heritage apple germplasm of the territory. The

germplasm conserved represents a valuable source of diversity for breeding programs based on conventional and biotechnological methods. Specifically, breeding programs are ongoing for apple, sweet cherry, peach, pear, raspberry and strawberry [92,114,115].

### 3.2. Grapevine

The ampelographic collection has its origins in the establishment of the experimental station in Conegliano in the early 1900s [116,117] and is currently divided into sub-collections located at the experimental farms of the Research Centre for Viticulture and Enology (CREA-VE). The accessions are maintained in Susegana (Veneto) in Northeastern Italy, Arezzo (Tuscany) and Velletri (Latium) in Central Italy, Turi (Apulia) in Southern Italy. Overall, the collection includes cultivars of wine and table grapes, disease resistant hybrids, rootstocks and several *Vitis* species. A considerable portion of the accessions represent minor and less-known native varieties. The repository in Susegana also contains the National Catalogue of Grapevine Varieties.

The conservation of all accessions is carried out *ex situ* in dedicated vineyards with a minimum of five vines per accession. Few accessions are preserved *in vitro* in slow growth condition. With regard to cryopreservation, there are some Italian experiences, but the method has not yet been applied on a large scale [118,119]. Accessions are typically characterized using specific international ampelographic descriptors, provided by the Organisation Internationale de la Vigne et du Vin (OIV) and/or the National Guidelines [15,16]. The evaluation of agronomic and quality traits, which are often influenced by environmental factors, has been limited to a subset of grape varieties and vintages. However, the goal is to expand this recording to all conserved germplasm.

Microsatellite markers are commonly used to identify grapevine varieties because they enable easy comparison of data between different international collections and reference databases. Efforts have been made to improve and rationalize the ampelographic repository by completing the genetic characterization of all accessions held using at least 11 SSRs [120], nine of which are considered a minimal standard marker set used internationally. This process identified synonyms, homonyms and mislabeling. Additionally, a subset of the collection (over 600 of the most representative accessions) was genotyped using 18K SNP markers [121].

The grapevine germplasm in the collection has been and continues to be of interest to several significant national (Basivin, Bionet, Puglia\_INNO.VIT, Ri.Vi.Parco) and international (Grapegen06, Vitismart, OENOMED) research projects, and for the activities of ECPGR *Vitis* working group. The use of genetic fingerprinting provides information on the origin and phylogeny of different varieties [121–127].

The database obtained from recording phenological events, dating back to the 1970s, has been used in many studies to examine phenological models and grapevine adaptation to climate change [128–132].

Vines for commercial wine production must be registered in the National Register [133] and classified regionally for the relevant administrative areas. This process involves the technical-judicial recognition of rediscovered native grape varieties, after evaluating their morphological, physiological and agronomic characteristics. As an important part of the RGV-FAO Program, numerous minor native varieties were recovered, studied and subsequently included in the National Register (Table S2).

### 3.3. Citrus

The establishment of the citrus germplasm collection started approximately at the beginning of the 20th century and was mostly constituted by local cultivars. The number of accessions started to increase in the 1950s as a result of the development of the citrus breeding program in Acireale (Sicily), the generation of virus-free nucellar selections of the most important cultivars [134] and the introduction of foreign material [135]. Most of the local accessions and foreign cultivars of sweet orange, mandarins, pummelos and grapefruit, as well as most of the rootstocks and breeding materials are field-planted with

two or three replicates, while some relatives are propagated by seeds. The lemon collection is conserved in pots under screenhouses to protect the plants from “Mal secco” disease, caused by *Plenodomus tracheiphilus*.

The citrus collection is routinely used in the framework of the breeding program [136,137], which has released many scion and rootstock cultivars [138,139] and is also available for research purposes to national and foreign institutions. Part of the collection has been subjected to phenotypic evaluation for specific traits, including fruit quality [140] or resistance to biotic stresses [141]. In citrus, fingerprinting using traditional markers such as SSRs has been ineffective in discriminating accessions and cultivars within each species, because intraspecific variability of economically important citrus species, such as sweet orange, lemon and grapefruit is almost completely determined by somatic mutations. Recently, 22 sweet orange accessions were subjected to whole genome resequencing, and a robust and reliable set of SNPs, structural variants and indels, specific to each clonal selection or common to varietal groups, was identified [142]. The SNPs were used to develop two 384 SNPs—GoldenGate Assays that allowed the genotyping of 169 CREA sweet orange accessions (unpublished data). These molecular tools will facilitate the conservation of clonal accessions and reduce redundancy in the collection.

In the framework of different international projects, propagation material has been recently distributed as apomictic seeds or shoot tip grafted material and used for the implementation of multi-site collections of lemons and rootstocks [143], for the identification of sources of resistance to bacterial canker in citrus and related species [144] and to understand the genetic basis of Huanglongbing resistance in *Citrus glauca* and *Murraya* spp. [145,146].

#### 3.4. Olive

The main olive collection was established in 1997 and is located at the Regional Agency for Agricultural Development in Calabria (ARSAC) in Mirto Crosia (CS) and managed by the Research Centre for Olive, Fruit and Citrus Crops (CREA-OFA) in Rende (CS) thanks to a multi-year agreement. Recently, new accessions of cultivated and wild olive trees have been collected at CREA in Rende, Calabria. In November 2023, the CREA-OFA collections of Rende and Mirto Crosia were recognized as International Olive Germplasm Banks by the International Olive Council [147]. Another collection is also available at Tormancina (Rome) experimental farm belonging to CREA-DC, which plays the official role of Conservation Centre for Pre-multiplication (CCP) and Centre for Pre-multiplication (CP), acknowledged by the NPPO (National Plant Protection Organizations). It also includes certified varieties and ecotypes (39 in total), mainly representing the varietal panorama of Central Italy.

CREA-OFA olive collection has been extensively characterized at a molecular [148,149] morphological and agronomic level. Several varietal catalogues have been produced [149] which describe overall 317 Italian varieties. Currently, 216 Italian and foreign varieties have been authenticated. The molecular analysis conducted with a set of 8 to 12 microsatellite markers (SSR) highlighted the presence of several cases of synonymy between the Italian varieties. For olive, CREA has developed an internal varietal identification method based on the use of 12 multiplex SSR markers which is being validated at an inter-laboratory level and will be proposed for official accreditation.

The olive collection is evaluated for various agronomic aspects and for the quality of the olive oil in the context of national and international projects and is used for a breeding program for yield and yield stability, oil quality and vegetative architecture. The collection is currently subjected to phenotyping for quality traits (olive oil quality) and for susceptibility to abiotic stress (water and cold stress) and biotic stresses (olive fly and pathogens) in the framework of national and international projects (SALVAOLIVI, GEN4OLIVE, GENOLICS and SAMOTHRACE). To date, thanks to the multi-year phenotypic evaluation of fruit and plant traits in field conditions, CREA-OFA contributed to register 23 varieties in the National Register of Fruit Varieties.

#### 4. Vegetable Crops

Italy is among the main producers of vegetables in Europe thanks to the presence of a high number of crops on the national territory (Table 7).

**Table 7.** Surface and production of vegetable crops in Italy in 2022 (source: FAOSTAT) [22].

Crops	Harvested Area (ha × 1000)	Yield (t × 1000)
Artichokes	38.17	378.11
Asparagus	7.46	51.55
Beans	76.51	285.08
Cabbages	21.71	403.89
Melons	22.89	590.23
Carrots and turnips	8.34	353.5
Cauliflowers and broccoli	14.73	352.07
Peppers	9.39	232.68
Cucumbers	1.98	61.83
Eggplants	9.6	307.43
Garlic and other alliaceous vegetables	4.07	41.82
Lettuce and chicory	26.21	638.18
Lupins	0.71	0.95
Onions	12.85	402.19
Other legumes	18.99	29.48
Peas	32.46	120.74
Potatoes	47.03	1332.98
Pumpkins	19.05	558.94
Spinach	5.57	96.87
Tomatoes	97.61	6136.38
Watermelons	12.39	656.7

In the frame of the RGV-FAO Program the Research Centre for Vegetable and Ornamental Crops (CREA-OF) holds accessions of approximately 30 plant species belonging to Solanaceae, Brassicaceae, Cucurbitaceae, Fabaceae and Asteraceae families. Germplasm is conserved ex situ as seeds in climate chambers that guarantee temperatures below 5° C and maximum humidity of 30%. Once extracted from fruits, seeds are disinfected and dried, then placed in vacuum bags. These conditions ensure medium-long term conservation and vitality of seeds for an average period of 10 years. Small aliquots of seeds are also stored at −20 °C to ensure long-term viability. Regeneration and multiplication activities are performed in protected environments (e.g., glasshouse, insect proof greenhouse) to avoid the risk of pollen contamination and to guarantee adequate standards for purity of the seed.

Major efforts for genotypic and phenotypic characterization have been made by CREA-OF, in pepper (*Capsicum* spp.), tomato (*Solanum lycopersicum* L.), melon (*Cucumis melo* L.) and cultivated and wild rocket salad species (*Eruca sativa* L. Cav., *Diplotaxis tenuifolia* L. DC.). Thousands of SNP markers have been developed via Next Generation Sequencing (NGS) approaches involving Genotyping-By-Sequencing (GBS) and restriction-site associated sequencing (RADseq) methods. In pepper, a survey with over 26 thousand GBS-SNP markers on 10,000 accessions held in international gene banks and representing almost the global diversity allowed to establish strategies to improve the management of ex situ collections [150]. This study also provided new insight on the evolutionary routes of pepper, shedding light on the links from its center of origin to Europe and Asia, important centers of secondary diversification. In this frame, the Italian germplasm provided by CREA-OF represented the diversity enclosed in Mediterranean environments and contributed to build a wide representative germplasm core collection. In tomato, 33 thousand RAD-SNPs markers have been used to explore the diversity of long shelf-life germplasm providing new clues on the existence of genetic variants in genes involved into stress tolerance and fruit maturation mechanisms [151]. Phenotyping activities have been performed in replicated trials in both



open field and protected environments and included agronomic and morphologic traits with the aid of international descriptors established for the different species [13]. Selection during domestication and subsequent dissemination and breeding caused massive loss of crop genetic diversity. Digital tools to dissect the size and the shape of different vegetative and reproductive part of plants [152], as well as metabolic platforms for the investigation of main primary and secondary metabolites have been also used [153]. In both pepper and tomato, the integration of genomic and phenomic data allowed the identification of genomic regions controlling plant architecture and phenology, quality, fruit size and shape traits via GWAS [154,155]. Among these, over 125 novel candidate genes were discovered in both crops. Furthermore, local varieties and derived breeding materials have been used to dissect the influence of genotype and environment (including crop management) on the variation of bioactive compounds [156–158], and in combination with the reduction of nitrogen inputs [157,158] and water supply [159].

The melon germplasm recovered and characterized across the past 20 years by CREA OF includes both autochthonous varieties representing the diversity existing in the Italian peninsula [160] and advanced materials selected within genetic improvement programs for resistances [161]. These activities led to the development of diplohaploid lines highly resistant to different races of *Fusarium oxysporum* f.sp. *melonis* (FOM). These latter have been used as a basis for genetic marker discovery for early detection of FOM as well as for the investigation of the transcriptional network involved in the response of plants to infection [162,163].

In rocket salad, the activities have been mostly addressed to the deep characterization of germplasm providing new evidence on the metabolic profile diversity that distinguish the cultivated from the wild gene pool [164]. Additionally, a core collection of 155 cultivated rocket salad accessions have been established and deeply assessed for 54 morpho-agronomic and quality-related traits [165]. The collection enriches the germplasm available at CREA for future GWAS studies and breeding activities.

Moreover, CREA-OF has been hosting fields featuring local biodiversity of spring artichokes populations from Abruzzo and Marche regions. These landraces have been thoroughly analyzed for their morphological and qualitative traits as well as their peculiar end-use attitudes [166].

CREA-GB in Montanaso Lombardo (LO) is also involved in the RGV-FAO Program to maintain the purity and germination ability of more than 300 accessions of Amaryllidaceae, Asparagaceae, Asteraceae, Brassicaceae, Cucurbitaceae, Fabaceae and Solanaceae; here, the regeneration of plant material takes place both in the field and in the greenhouse, and the seeds are stored at  $-20^{\circ}\text{C}$  in vacuum bags. During the 20 years of the RGV-FAO Program, phenotypic and agronomic analyses carried out at CREA-GB have led to the valorization, especially at local level, of onion, melon, pumpkin and radicchio varieties, and studies have been carried out to analyze traditional agronomic varieties and chili peppers [167,168].

Several national and international projects allowed further valorization of genetic resources. Among these, two consortia were built in the frame of the Horizon Europe work programs. The Horizon 2020 project G2P-SOL [169], by bringing partners from four continents allowed to define a deep link between *Solanaceae* accessions held in genebanks defining a common strategy to enhance their exploitation for breeding purposes. Both CREA-OF and CREA-GB participated in this project with the aim to link phenotypes to genotypes toward the identification of candidate genes of agronomic and qualitative interest in pepper and eggplant core collections. The Horizon 2020 project BRESOV [170] aimed to increase the tolerances of tomato and brassica species to biotic and abiotic stresses toward the improvement of competitiveness in harsh environments and low input conditions.

## 5. Grain Legume Crops

Grain legumes are a rich source of proteins for animal feeding and, ever more, for human food, being an important share of the arable land surface (Table 8). Owing to their biological nitrogen fixation, they also contribute to reducing the negative effects caused

by intensification of the farming systems. The activity on genetic resources at CREA-ZA in Lodi is greatly focused, therefore, on these species. The collection of grain legumes encompasses over 2300 accessions, the majority of which belong to the species pea (*Pisum sativum* L.), white lupin (*Lupinus albus* L.), soybean (*Glycine max* (L.) Merr.) and cowpea (*Vigna unguiculata* (L.) Walp.). For pea and lupin, a world germplasm collection is included together with a large set of breeding lines and national cultivars and ecotypes. All the accessions are kept as dehydrated seeds (7–8% humidity), preserved for long-term storage in sealed bags at  $-15\text{ }^{\circ}\text{C}$ . These conditions should ensure a good conservation of the germination ability in the time, reducing, thereby, the need of germplasm regeneration. Dehydration is performed in a custom-made drying cabinet with forced air cycling in silica gel. Most accessions have undergone morpho-physiological characterization and bio-agronomic evaluation for economically relevant traits. The evaluation activity represented the foundation for germplasm valorization through breeding.

**Table 8.** Surface and production of main grain legume crops (mostly for feed) and forage crops in Italy in 2022 (source: ISTAT) [171].

Crops	Harvested Area (ha × 1000)	Yield (t × 1000)
Grain legume crops (grain yield)		
Soybean	342	918
Faba bean	50	91
Field pea	10	30
Forage crops (dry-matter yield)		
Alfalfa	684	15,550
Clovers	31	426
Other temporary forage species	65	865

The pea world collection was evaluated for key agronomic traits such as phenology, plant height, grain yield, protein content and drought tolerance. Traditional germplasm tended to display higher grain yield compared with improved variety material. Several ecotype/old cultivar accessions outperformed the best-ranking varieties for grain yield or protein content [172]. The collection also underwent molecular characterization through GBS. Genetic variation based on geographic patterns was revealed by the analysis of population structure, and a model for the spread of pea cultivation from its center of domestication to other regions of the world was defined. Genome-wide analysis identified putative selective sweeps associated with domestication and breeding, which included genes involved in shoot branching, cotyledon color and resistance to lodging [173]. The development of GBS approaches, once tuned for specific crop genomes [174], and the subsequent availability of several thousand polymorphic SNP markers may open avenues for genome-enabled selection of complex traits such as forage and grain yields or stress tolerances. Large germplasm collections can be exploited as potentially useful breeding populations and are, therefore, made available to research projects for this purpose. CREA-ZA collection of over 300 breeding lines of pea was used in the European projects Core Organic COBRA and ArimNet Reforma for the definition of genomic selection models for grain yield in Italian environments [175] and under severe drought stress in the Mediterranean basin [176]. Genomic prediction of agronomic and morphological traits was also performed in the pea world collection [177].

A multi-site evaluation based on grain yield performance was carried out on the white lupin world collection, which enabled the classification of regional germplasm pools according to their specific adaptation to different environments [178]. Landraces from the same collection were also compared versus elite varieties in a multi-environment assessment within the European project FP7 Legato. Adaptive responses highlighted the outstanding agronomic value for specific agro-climatic conditions of a few landrace germplasm pools in comparison with improved varieties and emphasized their interest for breeding [179]. A molecular characterization of the collection through GBS to perform GWAS revealed

candidate genes involved in anthracnose (*Colletotrichum lupini*) resistance [180]. Results on grain yield across contrasting environments supported the exploitation of genomic selection for this trait and provided economic justification for the genotyping of the collection [181]. The exploitation of the collections has produced several varieties, such as the peas 'Pantera Rosa', adapted to Italian conditions also under organic farming, and 'Pifor', selected for biomass production in intercropping with cereals, the white lupin 'Arsenio', adapted to southern European environments, and the faba bean 'Tano' with no tannins.

## 6. Forage Crops

Intensification and crop simplification of Italian farming systems in the last decades caused a severe erosion of forage crop genetic resources, especially in areas with most intensive agriculture. Nonetheless, alfalfa, clovers, sulla and other minor forage species still represent a major asset for the livestock industry in the country (Table 8). CREA ZA in Lodi carried out an activity of collection, rejuvenation, safeguarding and characterization of forage species, avoiding the irreversible loss of a large number of farm landraces, which represented the traditional basis of local forage productions in alfalfa (*Medicago sativa* L.), white clover (*Trifolium repens* L.) and red clover (*T. pratense* L.). Attention was paid to wild populations of some species whose main centers of origin or primary diversification were in Italy, such as the Ladino white clover type in the northern plains and subterranean clover (*T. subterraneum* L.) in Sardinia and Sicily. Wild populations were also collected for several forage and turf grass species, whose risk of genetic erosion increased with the abandonment of permanent meadows and pastures. The collection of forage species is held in Lodi as dehydrated seeds at  $-15\text{ }^{\circ}\text{C}$ , as previously described for grain legume crops.

Genetic structure, diversity and agronomic value of alfalfa landraces were thoroughly assessed phenotypically [182]. Landrace diversity based on SNP data was associated with both adaptation pattern across different environments and drought stress at collection sites [183]. Local germplasm showed a marked genotype  $\times$  environment interaction. Landrace evaluation across a range of conditions highlighted the importance of breeding for specific adaptation to achieve yield progress over commercial cultivars and resulted in the selection of new varieties [184]. Different breeding schemes were compared on local germplasm for the development of synthetic [185]. The great diversity available in the alfalfa collection, also including exotic germplasm from central Europe, North America, North Africa and Australia, was exploited to tackle specific breeding targets, such as grazing tolerance [186] or salt tolerance, or to develop the innovative variety type of semi-hybrids [187]. All these facets of the evaluation work on alfalfa genetic resources resulted in the selection of new varieties or the generation of superior germplasm for further variety release. Large collections of breeding materials specifically devised for sub-continental or Mediterranean conditions were obtained from locally adapted germplasm and made available to the European project ArimNet Reforma for the first ever genomic selection approach in alfalfa for forage yield under moderately-favorable and drought-stressed conditions and for forage quality traits [187–189].

The genetic variation among and within populations was studied in the collected Italian white clover genetic resources. The overall variation of the Ladino local germplasm proved higher than that of any foreign germplasm attributed to the same type. Molecular characterization by SSR markers provided evidence for the origin of Ladino white clover from indigenous germplasm from northern Italy, although morpho-physiological diversity was substantially unrelated to molecular diversity [190]. Evolutionary adaptation to different agronomic practices was a major determinant of the overall variation found in landraces. A relatively low number of collected ecotypes or landraces could provide a wide range of genetic variation. A thorough evaluation for agronomic value and morpho-physiological diversity of non-Ladino Italian populations was also performed [191]. Effective selection procedures enabled breeding and releasing varieties able to persist under severe competition exerted by grass companions, thereby enhancing the agronomic usefulness of this legume forage crop [192].

The valorization of forage crop germplasm resulted in the release of a range of new varieties, including ‘Costanza’, ‘Alfitalia’, ‘Verbena’ and ‘Camporegio’ in alfalfa, ‘Giga’ and ‘Trefor’ in white clover, ‘Antas’, ‘Campeda’ and ‘Losa’ in subterranean clover.

## 7. Industrial Crops

The history of the recovery, conservation, characterization and valorization of industrial crop genetic resources at CREA-CI in Bologna and Rovigo dates back to the beginning of the last century.

The main crops covered by these studies are hemp (*Cannabis sativa* L.), potato (*Solanum tuberosum* L.), sugar beet (*Beta vulgaris* L.), sunflower (*Helianthus annuus* L.), different species of the Brassicaceae family, flax (*Linum usitatissimum* L.), castor (*Ricinus communis* L.), different grain legumes species with a main focus on common bean (*Phaseolus vulgaris* L.) (Table 9). Over the years, the work of different scientists led to the establishment of a genebank extremely heterogeneous in its composition, with about 2000 accessions and at least hundreds breeding lines (Table S1). The collection reflects the multidisciplinary approach to the study of these crops and, depending on the species, consists of wild crop relatives collected in Italy or in Europe, traditional ecotypes and landraces, breeding lines or populations, as well as ancient or modern varieties, many of which were constituted by the breeders of this Center.

**Table 9.** Surface and production of main Industrial crops in Italy in 2023 (source: ISTAT) [171].

Crops	Harvested Area (ha × 1000)	Grain Yield (t × 1000)
Common bean (dry + snapbean + romano type)	20	146
Lentil	5	4
Chickpea	14	23
Potato	45	1265
Sugarbeet	25 *	1110 *
Flax	1	1
Hemp	1	2
Rapeseed	30	82
Sunflower	122	305
Soybean	310	135
Other oil species	1	10

\* Sugarbeet data for 2022.

The germplasm is maintained in compliance with international standards; most of the accessions are kept as seeds in triple-layer vacuum bags and generally preserved in two distinct places, at  $-20$ – $-25$  °C and in cold chamber at  $5$  °C, while the potato collection is maintained as microtubers in growth chambers.

In recent years, the collection has been heavily studied for different purposes. Genetic and genomic studies allowed the characterization of the collections [193–198] at the same time investigating their genetic structure and diversity [197–199], as well as insights into quantitative [195,200–203] and qualitative agronomic traits [204–209].

Chemical analyses allowed the characterization of primary and secondary metabolites that are typical of each of these plants with a focus on health, nutraceutical and bioactive functions [194,196,204,207–221].

A significant example of the interaction between traditional breeding approaches and biotechnological ones is represented by hemp, studied, characterized and selected at CREA-CI in Bologna since the postwar period. The preliminary analyses of genetic structure and diversity of *Cannabis sativa* L. germplasm can be traced back to the early 1990s [222]; since then, molecular markers for faster hemp breeding were developed [208,223–226] and the first explanation of the genetic inheritance of cannabinoids [227], i.e., the main secondary metabolites, peculiar and distinctive of this species, was proposed. In the following years a cascade of innovative results has been generated, underpinning the synthesis and accumulation of key metabolites [194,208,210,214,221,228], and their genetic

expression [194,208,210]. The studies allowed the successful establishment of forensic and pharmaceutical applications, as well as agronomical, qualitative and industrial characterization of this crop [199,207,219,229–232].

With regard to breeding activities, germplasm characterization has been financed over the past 20 years not only by public funds through national and European projects, but also by private companies, leading, for example, to the registration of five potato, 21 bean and two pea varieties, eight varieties (sunflower, flax, hemp) for oil and fiber and two Brassicaceae varieties for bio-fumigation.

## 8. Medicinal and Aromatic Plants

The RGV-FAO Program also deals with the conservation and study of aromatic and medicinal plants for food use. CREA-OF of Sanremo has established, within the RGV-FAO Program, collections of rosemary plants (*Salvia rosmarinus*, ex *Rosmarinus officinalis*—around 750 accessions), sage (around 50 of *Salvia officinalis* as well as similar species for food use—*S. fruticosa*, *S. blancoana*, *S. pomifera*, *S. sclarea*) *Helichrysum italicum* (200 accessions) and accessions belonging to the Apiaceae family. Throughout the entire program, accessions were maintained and renewed, and new accessions were introduced into the collections. Specific descriptors were applied for the morphological characterization and more than 550 descriptive cards of rosemary, *S. officinalis* and *H. italicum* accessions were edited. Rosemary and helichrysum accessions were selected and analyzed for volatile compounds, composition of leaf essential oils, antioxidant capacity and their antimicrobial properties for food use. Rosemary trials in pots have highlighted that light conditions and water availability in open fields can influence the aromatic profile and optimize the yield of essential oil [233]. Moreover, the cultivation of helichrysum and rosemary requires low nutritional and water input and can be considered at low environmental impact because they are adapting to arid climates, well sunny areas and significant temperature ranges. The genotypic analysis based on accessions of rosemary from different areas of the Mediterranean regions showed the biodiversity of the species and the subdivision of the populations into four macro-areas (Liguria-France; Corsica-Sardinia-Tuscany; Iberian Peninsula; Southern Italy) [234].

The Research Centre for Forestry and Wood (CREA-FL) of Trento participated in the RGV-FAO Program since its beginning in 2004, with a research line focused on Medicinal and Aromatic Plants (MAPs), by the conservation and characterization of a collection of about 100 species previously collected within different research projects. In the following years the activity was mainly focused on mountain and alpine plants, such as *Arnica montana* L., *Gentiana lutea* L., *Rhodiola rosea* L., by the domestication of natural populations of the species, with the aim to promote their cultivation and replacing the harvest in the wild with cultivation [235–242]. Some edible species, such as *Cicerbita alpina* (L.) Wallr. and *Aruncus dioicus* (Walter) Fernald, traditionally harvested in the wild in the North-East of Italy, were included. The conservation activity of MAPs gave the Research Centre the opportunity to participate in several research projects, such as “PARMA—edible, Aromatic and Medicinal Plants of the Alps—a resource to value” funded by the Autonomous Province of Trento, and “HERBs and Mountain Plants as an Alternative Medication for Anthelmintic Treatments in Livestock Species (HERBAL)” funded by Euregio. Since 2020 the activities carried out in Trento within the RGV-FAO Program have been exclusively focused on plant species with an aromatic and edible use. At the moment, different accessions of the species *Achillea moschata* Wulfen, *Armoracia rusticana* G.Gaertn., B.Mey. and Scherb., *C. alpina* (L.) Wallr., *G. lutea* L. and *Petroselinum crispum* (Mill) Nyman are conserved in vivo, ex situ, both at the Research Centre headquarters in Trento and, concerning the alpine species, in a field trial located in Alpine environment, at 1540 m a.s.l., and their characterization is in progress [213,243,244].

## 9. Ornamental Crops

Many ornamental species can be used for food purposes; for this reason, orchids, passion flowers, rosehips and more recently edible flowers have been included among

the species conserved and studied within the RGV-FAO Program. Regarding orchids, the activities are focused on species of food interest, belonging to genera traditionally used in some Eastern Mediterranean areas for the preparation of “salep” [245]. The main activities on wild orchids consist of data collection of phenological and territorial distribution of Italian populations, capsules collection in full compliance with current laws for environmental protection, asymbiotic in vitro sowing, micropropagation and ex situ conservation of wild orchid selected accessions [246]. At present, the germplasm collection, mainly maintained in vitro, includes 55 accessions referring to 28 species and 23 interspecific hybrids belonging to 9 genera: *Anacamptis*, *Cephalanthera*, *Dactylorhiza*, *Gymnadenia*, *Himantoglossum*, *Ophrys*, *Orchis*, *Platanthera*, *Serapias*. Moreover, a new interspecific hybrid (*Himantoglossum adriaticum* × *robertianum*) was created and genetically characterized [247]. Similarly to orchids, roses were initially collected and cultivated in the Program for ornamental scopes only as garden plants and valuable rootstocks for many species and cultivars. The main representative species of the collection are *Rosa blanda*, *R. canina*, *R. corymbifera*, *R. micrantha*, *R. moschata*, *R. rubiginosa*, *R. rugosa*, *R. sempervirens* and *R. spinosissima*. The collection is based on soil and soilless-cultivated plants grown with low-input techniques as well as on micropropagated plantlets. Since 2019, roses were grown for food uses as their pseudo-fruits (hips) contain vitamins, polyphenols, carotenoids, anthocyanins, flavonoids with high antioxidant effects and nutraceutical value [248,249]. The rosehips collection was created by collecting seeds and cuttings from species, cultivars and ecotypes naturally grown in Sicily and in other Italian regions to evaluate their potentialities for producing functional foods (jams, teas and wines) as it occurs in other parts of the world [250,251]. Edible flowers were used over the centuries as ingredient in traditional dishes. Among the genera with edible flowers there are *Anthirrinum*, *Begonia*, *Calendula*, *Dianthus*, *Pelargonium*, *Tagetes*, *Tropaeolum*, *Tulbaghia* and *Viola*. The collection of edible flowers is mainly made up of potted plants grown organically. *Agastache*, *Polianthes tuberosa*, *Mertensia maritima* are maintained in vitro [252–254]. Edible flowers are often characterized by different flavors (sweet, bitter, sour and spicy) and have been characterized nutritionally [255]. They are rich in essential amino acids, minerals, polyunsaturated fatty acids and above all in substances with antioxidant, antimicrobial and anti-inflammatory properties (e.g., polyphenols and vitamins) [220]). These nutritional characteristics make edible flowers ideal for vegan and vegetarian diets.

## 10. Forest and Woody Crops

CREA-FL in Arezzo addressed the conservation of the germplasm of forest tree species, mainly in vivo. Forest trees can be utilized for wood and food production, and their role in agro-forest ecosystems to increase biodiversity, resilience and livelihood is fundamental. In the framework of the RGV-FAO Program, from 2020, the conservation efforts were mainly focused on two food species: the chestnut tree (*Castanea sativa*) and the stone pine (*Pinus pinea*).

The chestnut tree has had an essential role in the food subsistence of Italian Apennine populations for centuries, also providing goods and services of vital importance for the economy (fruit, wood, pasture, non-woody secondary products, litter, soil, tannins). The damaging effects of climate change and phytopathologies have led to the almost total disappearance of chestnut groves that survive in a few suitable areas. The collection of chestnut trees is made up of Italian and foreign provenances and hybrids, with 368 accessions. The aim is to preserve this critical genetic heritage, recovering and renewing the selected genotypes by silvicultural and nursery activities, including thinning and grafting.

The stone pine has been used since ancient times to produce the seeds (pine kernels) used as food. Italy is one of the largest producers and consumers of pine kernels, with world-class exports mainly to Nordic countries. The market value of pine kernels has increased considerably in recent years following the drop in production caused by biotic adversities (*Leptoglossus occidentalis*, *Diplodiasa pinea*). The national production chain also suffers from a lack of innovation, being essentially based on the harvesting of pine cones from mature plants (25–35 m high) in coastal pine forests, especially in Tuscany, suffering

from competition from countries such as Tunisia, Spain, Chile, Argentina, China and the USA. The selection, genetic characterization and establishment of clonal archives of *Pinus pinea* with genotypes that are more tolerant to *Leptoglossus occidentalis* attacks were started as the first steps of a selection program. Tuscan plantations are registered as seed woods in the national register of basic forest materials (European Directive 1999/105/EC and Legislative Decree 386/03).

In the past, other species included in the program were wild cherry (*Prunus avium*), Douglas fir (*Pseudotsuga mentiesii*), silver birch (*Betula pendula*), *Abies nebrodensis*, with some sites included in the National Register of Basic Forest Materials (Dlgs 386/2003).

Overall, these collections were included in regional/national (Do.Na.To.) and European projects (H2020 B4EST, LIFE Resilfor, COST Action FP1202 Marginal and Peripheral populations, RI Treebrex, FP7 Trees4Future), besides being part of IUFRO collections.

## 11. Italian and International Issues

The main issue of the Italian strategy for the conservation, characterization and valorization of PGRFA is the extreme fragmentation of the management, in which three Ministries are involved. The Ministry of Agriculture, Food Sovereignty and Forest (MASAF) oversees the management of PGRFA, is responsible for reporting internationally on the status of implementation of the Treaty and leads the international policy within the FAO organizations. The Ministry of University and Research (MUR) has competence with plant collections maintained by the National Research Council and Universities. The Ministry of the Environment and Energy Security (MITE) plays an important role in the management of biodiversity as a whole (plant, animal, microorganisms), representing Italy in important international organs such as CBD and its Nagoya Protocol. In addition, other subjects with responsibility for implementing and enforcing the Treaty and for the management of the genetic resources are Italian Regions and Autonomous Provinces (Trento and Bolzano), supervised by MASAF. The Regions and Autonomous Provinces must notify MASAF and MITE, by June 30 of each year of the measures they have taken or intend to take to implement the provisions contained in Articles 5, 6, 9, 11 and 12 of the Treaty.

The negative aspect of this extreme fragmentation is the complexity in the coordination that gives rise to inefficiencies and waste of financial and human resources. It is not uncommon, for example, that the different actors (CREA, CNR, Regions) characterize their collections with different set of molecular markers, thus preventing the comparison of the results and the evaluation of the redundancies, synonymies, homonymies and labeling errors. A more comprehensive coordination with a central role on this matter would be helpful and welcome to rationalize the system.

In 2008, MASAF, with the collaboration of the Regions and Autonomous Provinces, implemented the National Plan on Biodiversity of Agricultural Interest (PNBA). The overall objective of the PNBA is to coordinate the initiatives with national and international Bodies that deal with biodiversity in agriculture, and to give the Regions and Autonomous Provinces concrete answers to the problems arising over time. The final aim was to establish a national system of protection of agrobiodiversity, capable of effectively bringing back to the territory much of the agrobiodiversity that has disappeared or is at risk of extinction, for the benefit of environmental protection, sustainable agriculture and rural development. As a result, in 2012, the "National Guidelines for the in-situ, on-farm and ex situ conservation of plant, animal and microbial biodiversity of agricultural interest" [15,16] were published.

In 2015 Italy adopted an additional tool for the protection and enhancement of Food and Agriculture Biodiversity, Law No. 194. The law lays down the principles for the establishment of a National System for the Protection and Enhancement of Food and Agriculture Biodiversity, aimed at the protection of local genetic resources of food and agricultural interest from the risk of extinction and genetic erosion, pursued also through the protection of the rural territory, helping to limit the phenomena of depopulation and to preserve the territory from phenomena of genetic pollution and loss of genetic heritage. The National System for the Protection and Enhancement of Food and Agriculture

Biodiversity consists of the following: (i) the National Registry of Food and Agricultural Biodiversity, established at MASAF, where vegetable, animal or microbial genetic resources at risk of extinction or genetic erosion are listed; (ii) the National Network of Food and Agricultural Biodiversity, coordinated by MASAF, consisting of local, regional and national institutions for in situ or ex situ germplasm conservation; (iii) the National Portal of Food and Agricultural Biodiversity, a website where to find information and monitor the state of conservation on the genetic resources characterized and present on the national territory; (iv) the Standing Committee, with the task of ensuring national coordination of actions, composed of one representative of MASAF, one of MITE, one of the Ministry of Health, six from Italian Regions and three representatives of the custodian farmers and ranchers. An active part of the system is played by the custodian farmers and breeders, engaged in the conservation—within their farms, i.e., in situ—of local genetic resources at risk of extinction or genetic erosion, according to the modalities defined by the Regions and the Autonomous Provinces.

Another significant issue is represented by the Nagoya Protocol (NP) on Access and Benefit Sharing (ABS) to Genetic Resources [256], a supplementary agreement to the Convention on Biological Diversity (CBD), entered into force in 2014, and to date ratified by 141 countries. It is focused on one of the three objectives of the CBD: the fair and equitable sharing of benefits arising from the utilization of genetic resources. The core of NP is the bilateral mechanism of ABS with the Mutual Agreed Terms (MAT) and Prior Informed Consent (PIC) to be signed by the actors involved. The Protocol gives contracting countries the power to determine the way of access and benefit-sharing of their own PGRFA not contained in Annex I of the Treaty. In such a manner, many important crops (e.g., tomato, pepper, peach, soybean, grape) might be subject to cumbersome and complex bilateral agreements, instead of the easier multilateral SMTA. Italy has not yet ratified the Protocol, therefore uncertainty still remains on this matter. In our opinion, the application of the bilateral system of Nagoya Protocol to PGRFA would hamper innovation in agriculture and crop improvement. As an example, access to a variety obtained using several parental lines from several different countries would be quite impossible to achieve, since bilateral agreements should be signed with all countries from which all the parental lines come.

Another issue that would have important consequences for the characterization, valorization and sustainable use of PGRFA is the Digital Sequence Information (DSI) derived from the genetic resources. The topic came up during CBD 13 (2016) when some developing countries raised the issue that ABS rules should also apply to the information derived from the genetic resources, e.g., their genetic sequences. At present, DSI is a placeholder since no agreement has been reached on the name nor what DSI includes (DNA, RNA, proteins, metabolites, phenotypic traits). If accepted as it was proposed, the issue would upset the international practice on the use of sequences associated with genetic resources: the Open Access. All the sequences obtained in the last 50 years are stored in about 1700 public databases with open access. To date, the US National Center for Biotechnology Information (NCBI) alone stores about 2 tera of DNA nucleotides (about 250 million sequences) that double approximately every 18 months. Other specialized databases exist, dealing with specific taxa, e.g., Genome Database of Rosaceae (GDR), or with other biomolecules such as protein (UNIPROT). The change of rules for accessing all the information associated with genetic resources (DSI) would potentially hamper the advancement of science and innovation hindering global food security [257–259]. On the other hand, developing countries that often hold important genetic resources often lack the technology to generate, access and use DSI, so they cannot have direct benefit from them. This issue does not have a simple solution: we need to recognize the benefits (monetary and non-monetary) to developing countries, but we need to preserve open access as well. An important step was achieved in December 2022 at the Kunming-Montreal Global Biodiversity Framework agreement at CBD COP15 with the Decision 15/9 on DSI [260]. The most important point agreed is the recognition that a Multilateral mechanism would



be the most suitable one to meet open access allowing the sharing of benefits with all the actors.

DSI had important effects on ITPGRFA negotiations. At GB 8 (2019, Rome), DSI was one of the main issues that blocked the negotiations on the enhancement of ITPGRFA, mainly the enlargement of Annex 1 including all PGRFA and the adoption of mandatory payments for accessing to PGRFA. Negotiations restarted at GB 9 (2022, India) with the reappointment of the ad hoc Open-ended Working Group to Enhance the Functioning of the Multilateral System (OWG-EFMLS), and the decision to adopt a new denomination adding Genetic Sequence Data (GSD) to DSI (DSI/GSD). The working group at GB 10 presented an important proposal to enhance ITPGRFA MLS with three hotspots: (1) expansion of Annex 1, including all PGRFA; (2) introducing mandatory payments and propose structure and rates; (3) DSI/GSD and possible solution to monetary and non-monetary benefit-sharing from their use. The next GB 11 in late 2025 has great expectations to find a positive solution for these important issues.

## 12. Conclusions

Conservation, characterization and exploitation of PGR are at the forefront to tackle future global challenges such as climate changes and new emerging pests and diseases. ITPGRFA gave us the framework to deal with these challenges. Nevertheless, many important issues remain to be addressed. Preserving and characterizing genetic resources are highly demanding tasks in terms of labor, equipment and funding. Moreover, PGR management needs a systematic approach to maximize their value and use, with high quality standards that, unfortunately, not all the genebanks can achieve, agreed ontologies and FAIR (Findable/Accessible/Interoperable/Reusable) principles for open data sharing, documentation and archiving. The many efforts being made in this regard at the international level will hopefully have a significant impact on the world's food and nutrient security.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy14061263/s1>, Table S1: Number of accessions for each species\_Crop categories. Table S2: Minor grapevine native varieties included in the National Register.

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## Abbreviations

ABS	Access and Benefit Sharing;
AEGIS	A European Genebank Integrated System;
CBD	Convention of Biological Diversity;
CGRFA	Commission on Genetic Resources for Food and Agriculture;
CNR	National Research Council;
COP	Conference of the Parties;
CPGR	Commission on Plant Genetic Resources;
CPVO	Community Plant Variety Office;
CREA	Council for Agricultural Research and Economics;
DSI	Digital Sequence Information;
ECPGR	European Cooperative Programme for Plant Genetic Resources;
EURISCO	European Cooperative Programme for Plant Genetic Resources;
EVA	European Evaluation Network;
FAIR	Findable/Accessible/Interoperable/Reusable;
GB	Governing Body;
GBS	Genotyping-By-Sequencing;
GDS	Genetic Sequence Data;
GWAS	Genome Wide Association Studies;
ISTAT	Italian National Institute of Statistics;
ITPGRFA	International Treaty on Plant Genetic Resources for Food and Agriculture;
IU	International Undertaking;
MAPs	Medicinal and Aromatic Plants;
MASAF	Ministry of Agriculture, Food Sovereignty and Forestry;
MAT	Mutual Agreed Terms;
MCPD	Multi-Crop Passport Descriptors;
MITE	Ministry of the Environment and Energy Security;
MLS	Multilateral System;
MTA	Material Transfer Agreement;
MUR	Ministry of University and Research;
NGS	Next Generation Sequencing;
NP	Nagoya Protocol;
NPPO	National Plant Protection Organizations;
OWG-EFMLS	Open-ended Working Group to Enhance the Functioning of the Multilateral System;
PBR	Plant Breeders' Rights;
PIC	Prior Informed Consent;
PNBA	National Plan on Biodiversity of Agricultural Interest;
QTL	Quantitative Trait Locus;
RADseq	Restriction-Site Associated Sequencing;
SNP	Single Nucleotide Polymorphism;
UPOV	Union for the Protection of New Varieties of Plants.

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