agronomy

MDPI

Review

PGRFA Management of Outcrossing Plants Propagated by Seed: From On-Farm to Ex Situ Conservation and Some Italian Maize Case Studies

Michela Landoni [1,*], Anna Bertoncini [1], Martina Ghidoli [2], Graziano Rossi [1], Elena Cassani [2], Sabrina Locatelli [3], Carlotta Balconi [3] and Roberto Pilu [2]

[1] Department of Earth and Environmental Sciences, University of Pavia, Via S. Epifanio 14, 27100 Pavia, Italy; anna.bertoncini@unipv.it (A.B.); graziano.rossi@unipv.it (G.R.)
[2] Department of Agricultural and Environmental Sciences—Production, Landscape and Agroenergy, University of Milan, Via Celoria 2, 20133 Milan, Italy; martina.ghidoli@unimi.it (M.G.); elena.cassani@unimi.it (E.C.); salvatore.pilu@unimi.it (R.P.)
[3] Research Centre for Cereal and Industrial Crops, Council for Agricultural Research and Economics (CREA), Via Stezzano, 24, 24126 Bergamo, Italy; sabrina.locatelli@crea.gov.it (S.L.); carlotta.balconi@crea.gov.it (C.B.)
* Correspondence: michelaveronica.landoni@unipv.it

Abstract: In this review, the main issues related to the conservation and valorization of Plant Genetic Resources for Food and Agriculture (PGRFA) will be primarily addressed. The conservation of PGRFA concerning outcrossing plants poses a significant challenge. For this reason, this review will cover the key challenges related to all stages, starting from in situ sampling, collection in the germplasm bank, and conservative reproductive methods. Integrated approaches involving the combined use of classical and molecular techniques will be described for the characterization of accessions. Within this framework, some successful Italian case studies focused on maize will be reported as well.

Keywords: PGRFA; ex situ conservation; in situ conservation; germplasm bank; allogamous plants; controlled pollination; opv (open pollinated variety); molecular markers

Citation: Landoni, M.; Bertoncini, A.; Ghidoli, M.; Rossi, G.; Cassani, E.; Locatelli, S.; Balconi, C.; Pilu, R. PGRFA Management of Outcrossing Plants Propagated by Seed: From On-Farm to Ex Situ Conservation and Some Italian Maize Case Studies. *Agronomy* **2024**, *14*, 1030. https://doi.org/10.3390/agronomy14051030

Academic Editor: Niels P. Louwaars

Received: 23 March 2024
Revised: 1 May 2024
Accepted: 9 May 2024
Published: 12 May 2024

1. Introduction

Agrobiodiversity, a crucial subset of biodiversity, emerges from the interaction among the genetic resources of plants, animals, and microbes with environmental factors, management systems, and cultivation practices within diverse cultural contexts [1]. The plant agrobiodiversity which holds present or potential value for human nutrition is commonly known as Plant Genetic Resources for Food and Agriculture (PGRFA). The sustainable availability of a broad range of PGRFA, including landraces and crop wild relatives (CWR), is pivotal for our future food supplies, catering to the needs of farmers, researchers, and breeders. Given the accelerating impacts of climate change on food production and the transformation of socio-economic conditions, genetic resources have assumed even greater significance. Agrobiodiversity plays a crucial role in conferring resilience to agroecosystems, enabling them to buffer the negative effects induced by climate change [2,3]. However, the current situation is marred by an alarming rate of agrobiodiversity loss, in which landscapes, species, and diverse forms of within-species diversity are steadily disappearing [4]. To try to face this problem, a variety of strategies and corresponding actions must be implemented to prevent these losses. Currently, most PGRFA are preserved ex situ in gene banks, mainly as seed samples. However, to optimize the conservation of genetic diversity, there is a consensus that ex situ methods should be complemented with in situ conservation [5,6]. In situ conservation is regarded as a way to capture the evolutionary adaptation of resources that, when exposed to a changing environment, can serve as a valuable reservoir of adaptive traits [7,8]. However, despite the recognized importance of in situ

conservation, regarding outcrossing plants, the coexistence between landraces and modern neosynthetic varieties significantly limits the possibility of maintaining different cultivars in purity due to cross-pollination. Indeed, in developed nations, the majority of landraces were gathered ex situ between the 1950s and 1970s, coinciding with the establishment of modern genebanks in various countries, following the example set by the USA [9].

After the advent of modern agriculture based on hybrids and pure lines, there has been significant "genetic erosion", leading to the virtual disappearance of landraces in the territory to date. To cope with this problem, in the last few years, the definition of the term 'landrace' has been subject to various changes, with additional or broader definitions being provided to the term itself, focused on the materials that are the subject of in situ conservation [10]. Indeed, in a comprehensive definition, we should encompass 'true landraces', cross-composite populations, and varietal mixtures that have been cultivated in situ for several years [11]. However, some authors suggest a broader interpretation of landraces that includes either conventional or modern breeding methods within traditional or emerging agricultural settings in a specific ecogeographical region while also considering the local traditional culture [12].

In this review, we discuss the main criticisms regarding the ex situ conservation of allogamous plants propagated by seed (Figure 1) and the importance of the study of PGRFA by integrated methodology (i.e., molecular markers), which is valuable for the discovery of "lost genes" potentially useful for breeding programs.

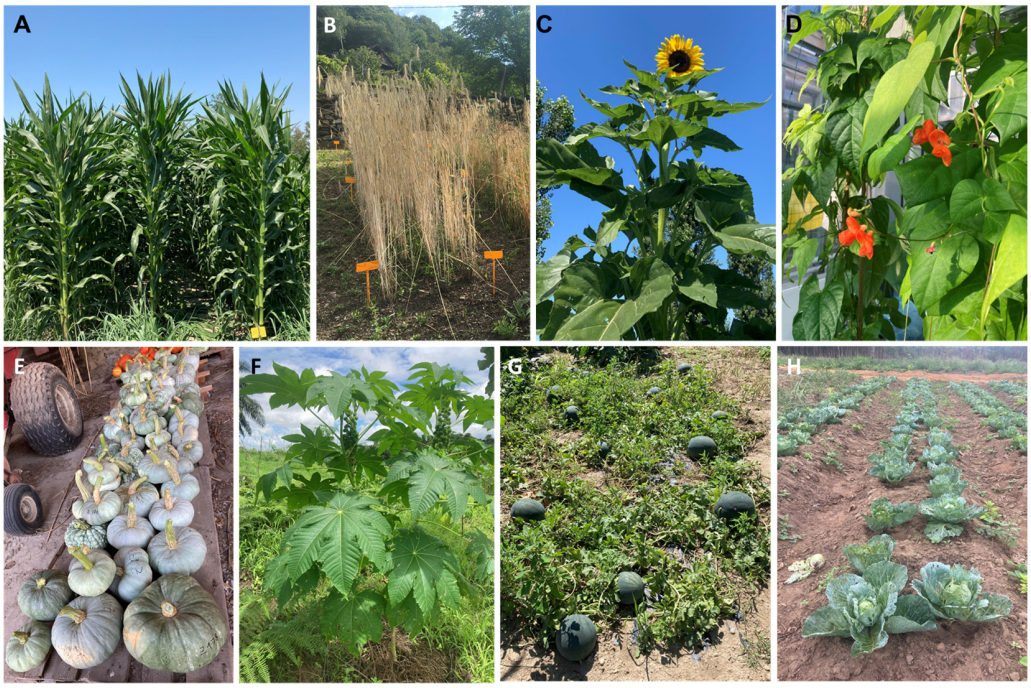

Figure 1. A survey of allogamous plants propagated by seed. (**A**) *Zea mays*; (**B**) *Secale cereale*; (**C**) *Helianthus annuus*; (**D**) *Phaseolus coccineus*; (**E**) *Cucurbita maxima*; (**F**) *Ricinus communis*; (**G**) *Citrullus lanatus*; and (**H**) *Brassica oleracea*.

2. On-Farm Conservation

Since the 1900s, a significant portion of plant genetic diversity has diminished due to global farmers forsaking their local varieties and landraces in favor of genetically uniform, high-yielding varieties [13].

To ensure efficient conservation of PGRFA, it is generally acknowledged that the two conservation approaches, the typically static ex situ conservation and the dynamic in situ conservation, allow for the continuous adaptation of the species to the environment and their natural evolution and should be considered complementary [14–16].

In situ conservation on-farm is commonly intended as "the continuous cultivation and management of a diverse set of populations by farmers in the agro-ecosystems where a crop has evolved" [17,18]. Although various definitions have been given to the concept of landraces [19,20], the authors of this review agree with the definition by Villa et al. 2010, defining a landrace as "a dynamic population of a cultivated plant that has historical origin, distinct identity and lacks formal crop improvement, as well as often being genetically diverse, locally adapted and associated with traditional farming systems" [21].

The evolutionary potential of farm conservation has been reported in different studies not only for allogamous plants [22,23]. Comparing corn landraces conserved ex situ for a period of more than 50 years with the same varieties conserved on farm, it was shown that the on-farm conservation was associated with the presence of several loci under selection [24]. These data highlighted not only the evolutionary meaning of on-farm conservation but also the positive effect of traditional farming practices and cultural customs on genetic diversity [6].

2.1. Guidelines for On-Farm Conservation

In the last three decades, the interest in on-farm conservation of landraces in centers of crop diversity have been growing [17,25–27]. The main objective of on-farm conservation is to maintain crop evolution in farmers' fields and landscapes; on-farm conservation depends then on farmers' preferences, knowledge, management, practices, and social organization. Activities that support on-farm conservation include community seed banks, local germplasm collections, reintroduction of traditional and locally adapted varieties, diversity festivals, and community biodiversity registers.

The genetic characterization of PGRFA is a fundamental step for both in situ and ex situ conservation, enabling the univocal identification of each accession and reducing the conservation costs by recognizing redundancies (i.e., accessions with different names but which are genetically identical). A correct identification is also fundamental for the development of effective strategies for preserving and conserving germplasm. A recent document release by the Italian Society of Agricultural Genetics [28] highlights the need to define a minimal set of easily detectable markers for genotypic characterization, to be adopted by PGRFA conservators (see paragraph 5, evaluation of genetic diversity by molecular markers). In this way, all accessions characterized within different research programs worldwide could be phylogenetically related to each other and we can thus obtain a more comprehensive and continuously updated view with the latest published results.

Several initiatives at an international level have been focused on the topic of biodiversity conservation. Among the most important is the Convention on Biological Diversity [29] (CBD, signed in Rio de Janeiro on 5 June 1992. It included three main objectives as follows: the conservation of biological diversity, the sustainable use of the components of biological diversity, and the fair and equitable sharing of the benefits arising from the use of genetic resources. Following this initiative, in 2009, the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA), which shares the goals of biodiversity conservation with the CBD, was established [30].

From these agreements, guidelines for biodiversity conservation at the European and national levels have been derived. In particular, the European Catalogue of Conservation Varieties can be considered one of the most modern instruments for in situ conservation of landraces adopted by the EU [31], representing an effective method to preserve endangered genetic resources. It envisages the figure of the custodian farmer who is responsible for the production of seed (produced in purity) in the area where the landrace is traditionally cultivated.

In this perspective, the European Cooperative Program for Plant Genetic Resources (ECPGR) brings together European countries to promote long-term conservation and to increase the use of plant genetic resources in Europe for sustainable agriculture, food security, and food quality [11]. The ECPGR's main aims are devoted to consolidating the ex situ conservation of plant genetic resources by improving the efficiency of European

Genebank infrastructure; expanding in situ conservation of crop wild relatives, wild food plants and promoting conservation and management of on-farm diversity; strengthening information systems of plant genetic resources by improving the quality and quantity of data available in the European Search catalogue for Plant Genetic Resources [32,33].

Additionally, the ECPGR European Evaluation Network (EVA) for PGRFA is an international program of public–private partnerships aimed at focusing the interest on crop genetic resources maintained in European Genebanks and to increase their utilization as pre-breeding materials and subsequent valorization in breeding programs.

The EVA Maize Network [34] involves research institutions, genebanks, and private breeding companies from nine European countries, namely Croatia, France, Germany, Italy (CREA Bergamo as national representative), Portugal, Romania, Serbia, Spain, and Switzerland. Network activities are focused on the valorization of maize genetic resources across Europe such as landraces and traditional varieties, which are valuable sources of genetic diversity for facing climate change issues due to their local adaptation.

Concerning the Italian laws about the preservation of PGRFA, Italy ratified the CBD with law 124 of 14 February 1994 [35]. Furthermore, the law 194 of 2015, "Provisions for the Protection and Enhancement of Agricultural and Food Biodiversity" established, at a national level, the National Catalogue, the Network, and the Portal of PGRFA, including the methods/guidelines for their conservation [36].

The registration process required to inscribe each PGRFA in the National Register is based on a procedure that includes assessments by the respective Region and the MASAF (Ministry of Agricultural, Food, and Forestry Policies). The National Network is comprised of the custodians of PGRFA, who can be either ex situ conservation centers or farmers, who, as described above, are the main actors of in situ/on-farm conservation. The National Portal is tasked with consolidating information from individual databases [25].

Although in some cases genetic diversity is high even in self-pollinating plants [22], a specific challenge to face managing outcrossing species is the high genetic diversity within and among populations. This conflicts with the uniformity criterion required for registration. In the case of maize, however, this requirement could still be met despite outcrossing, due to a series of characteristics typical of the traditional varieties of this cereal. These varieties are generally cultivated in restricted geographical areas and have undergone generations of selection for specific use in human consumption (such as the traditional dish "polenta" in Northern Italy). This has led to varieties with a narrow genetic base that can adapt well to the uniformity criteria required for registration as conservation varieties [31].

2.2. The Role of Custodian Farmers

As previously reported, a fundamental role in the on-farm conservation is played by the custodian farmer. According to the ITPGRFA, this figure is described as "a farmer who conserves landraces' seeds over time, having great knowledge about these landraces in their environment (often inherited from their parents or grandparents), as well as the way of cultivating and their use as food. This farmer holds the responsibility for seeds, which constitute not only genetic but also cultural heritage" [37].

Furthermore, among the roles of custodian farmers, the following are highlighted: the safeguarding of rare and distinctive varieties, the participation in community seed banks and the use of seed-sharing practices, and the preservation of varieties during challenging growing seasons [38].

A specific challenge of on-farm conservation for outcrossing species is the reproduction of varieties. In fact, the on-farm conservation of different varieties of the same outcrossing species requires maintaining a safe distance between them. In the case of maize plants, a distance of at least 200 m is generally suggested [39], although under favorable climatic conditions, cross-pollination has been observed at higher distances, up to 800 m [40,41].

In cases where it is not possible to maintain these safety distances, controlled pollination methods need to be employed. Outside of structured environments such as universities

and research centers, this type of reproduction is not always feasible as it requires trained staff dedicated to this activity for the duration of the reproduction phase. This can be particularly challenging in small, family-run agricultural enterprises such as, for example, those typical of custodian farmers in the maize-growing areas in Northern Italy.

In this agricultural context, the only way to safeguard the identity of outcrossing varieties is to cultivate a single variety per farm, ensuring that the distance of 2–300 m is respected when the same species is cultivated in neighboring farms.

The custodian farmers are therefore asked to cultivate a traditional variety at the expense of cultivating the much more productive modern cultivar. This means that the conservation of crop genetic diversity entails costs, not only for the work dedicated to these conservation programs but also for the loss of income on their farms (i.e., the opportunity costs).

This problem is felt even more sharply, considering that the areas with the highest genetic diversity and therefore the greatest need for in situ conservation are often in semi-subsistence rural areas in poor countries or on small-scale farms in industrialized countries. Analyzing the literature data, different approaches have been used to develop an economic support strategy for agrobiodiversity conservation.

A model to reduce costs of in situ/on-farm conservation is based on the identification, inside a community, of specific landraces threatened by extinction and on the farmers for whom the cost of on-farm conservation was the lowest [42]. The integration of in situ conservation with ex situ conservation management has also been suggested as a method to generate compensation mechanisms for custodian farmers as private benefits [43]. Another study highlighted the need for on-farm conservation cost estimates to optimize costs so as to recover, at a national or international level, the necessary funds, and to plan their optimal use. Furthermore, the design of different types and levels of compensation systems, customized to the different profiles of custodian farmers, is suggested [44].

3. Ex Situ Conservation, from Sampling to Germplasm Bank Processing and Storing

Ex situ (i.e., in genebanks) and in situ (i.e., in farmers' fields) conservation represent complementary efforts to safeguard agrobiodiversity. Ex situ conservation involves the collection, preservation, and management of genetic resources outside their natural environment through various methods, including conservation in germplasm banks, such as seed banks. Genebanks established worldwide store representative germplasm samples of crop species, their wild relatives, and wild plant species, providing researchers access to this germplasm for evaluation and integration into modern breeding programs and for specific conservation purposes (i.e., core-collections) [24].

3.1. Guidelines for Ex Situ Conservation

Currently, 4.6 million accessions of the world's PGR are conserved in over 1750 seed banks dispersed worldwide, with wheat leading the way in terms of the number of conserved accessions, followed by rice, barley, and maize [45–47].

Each germplasm bank is expected to follow specific international standards [46,48,49], which are essential guidelines for the storage conditions provided by the ageing rate characteristic of seed of individual species studied [50].

Seed species can be categorized into three main groups, which also reflect the possibility and technique of conservation, namely recalcitrant (desiccation intolerant), intermediate (partly desiccation tolerant and sensitive to low temperature), or orthodox (desiccation tolerant) [46]. Examples of PGRFA with orthodox seeds are all the major cereals, forage grasses, onion, carrot, beet, papaya, pepper, chickpea, cucumber, squash, soybean, cotton, sunflower, lentil, tomato, beans, eggplant, spinach, and brassicas. Intermediate seeds seem to be a characteristic of a population and its ecology rather than a species [51], while cocoa, coconut, mango, cinnamon, nutmeg, avocado, tea, breadfruit, and jackfruit are examples of PGRFA with recalcitrant seeds [10].

The process of conservation for orthodox seeds starts from the seed collection and the post-harvest handling of the collected seeds, which must be properly identified during all the steps [49].

Regarding the quantity of seeds to be collected, ideally the sample should represent all the genetic diversity present in the accession selected for ex situ conservation. This would mean having as many seeds in the sample as necessary to have at least one copy of each allele for each locus. If the distribution of the different alleles within and between populations is random, the best strategy would be to collect as many seeds from as many plants as possible [10]. But since this is an ideal situation, several strategies are proposed to find the most appropriate sample size. The parameters that are considered are based on the taxonomy, morphology, phenology, and physiology, the population structure (i.e., single population/multiple genetically interconnected populations), the type of reproduction (vegetative/sexual, allogamous/autogamous plants), and the purpose of the conservation (one copy of each allele/multiple copies of each allele) [52,53].

To preserve 95% of the alleles present, in an FAO report, it is suggested to collect samples from 30 plants if allogamous and from 50 plants if autogamous, and to set a number of seeds to be sampled in order to reduce the number of regenerations necessary following seed distributions [49]. The frequency of seed regeneration in a germplasm bank is a balance between the need to maintain a sufficient number of seeds with high viability and the need to avoid the loss of genetic diversity that is at risk with each regeneration. In general, regeneration is necessary when the number of seeds decreases due to seed distribution or due to a reduction in their germinability. While it is not possible to put a limit on this last event since the loss of vitality is due to the characteristics of the seed, it is possible to reduce the number of regenerations due to the distribution of the seeds by planning a sampling of a sufficiently high number of seeds [49].

An indication of the sample size to be collected for effective ex situ conservation is suggested by Biodiversity International organization. A sample of 2000–5000 seeds is indicated for allogamous species and of 1000–2000 seeds for autogamous species, while in the case of vegetative reproduction, a minimum of two propagules should be sampled [54]. Similar sample sizes are recommended by Hawkes and colleagues, who suggested collecting 30–50 seed from 30 individuals in case of allogamous plants and from 50 individuals in case of autogamous plants [55].

The rate at which seeds age during the post-harvest phase is determined by the ambient relative humidity (RH) and temperature. A safe moisture level for field collection is around 50% equilibrium relative humidity (eRH) [53], then the seed moisture content is reduced to the recommended levels for storage, using techniques that are not detrimental to seed viability [56]. It is known that when seed moisture content is between 5% and 14%, each 1% decrease in seed moisture doubles the shelf life of the seeds [57,58].

Seeds for storage should be clean and free from weed seeds, pests, and diseases. Seed cleaning, in fact, aims to process field-harvested material into a collection of clean, viable plant propagules without incurring damage or loss [53]. To reduce the risk of losing genetic diversity during seed cleaning, some rules have been suggested as follows: (i) use equipment that allows for the processing of the majority of seeds of the population under study to avoid eliminating viable seeds of non-standard size; (ii) if smaller but viable seeds have been unintentionally discarded, recover them; (iii) clean seeds from different lots separately using specific cleaning protocols for the different species and harvest year [59].

After cleaning and drying, the seeds are ready to be stored in the germplasm bank. For medium term storage, the seeds are generally stored at 5–10 °C, while for long-term conservation, a temperature of −18 °C is preferred [48,49]. Viability, i.e., the capability of the seed to germinate under the suitable conditions, and longevity, i.e., seed viability after dry seed storage, should also be monitored [60,61].

Germination tests are performed according to specific protocols [53] to determine what proportion of seeds in an accession will germinate under favorable conditions and produce normal seedlings, capable of development into reproductively mature plants. This

is necessary in order to test the vitality of the seeds before storage and, periodically, during ex situ conservation.

3.2. Examples of Italian Germplasm Banks

Germplasm seeds banks can be organized in different ways, with some dedicated to a single species and others encompassing multiple species within their collections. While single species banks offer focused conservation and research opportunities, multi-species banks contribute to broader ecosystem preservation and resource efficiency. The choice between these approaches depends on factors such as the conservation goals, available resources, and the characteristics of the species being targeted.

As an example of a germplasm bank dedicated to the conservation of various species, we can describe the University of Pavia Germplasm Bank in Italy.

This seed bank was established in 2005 and has since been improved and expanded. It serves as a focal point for a variety of projects aimed at the sustainable use of phytogenetic resources, as well as diverse target species involved in specific research and conservation initiatives. Currently, it serves as storage for more than 4000 accessions, including alpine species, crop wild relatives (CWR), wild and cultivated plants regarded as endangered [62]. To further safeguard the seed samples, most of accessions of the Pavia germplasm bank are duplicated in other seed banks, such as the Millennium Seed Bank (Royal Botanic Gardens, Kew, UK).

This seed bank is a member of the European Native Seed Conservation Network (ENSCONET Consortium) and acts as Italy's delegate in the global project for the ex situ conservation of CWRs, which is supported by the Global Crop Diversity Trust and the Government of Norway [63]. Furthermore, since 2017, it has been an associate member of AEGIS (A European Genebank Integrated System), which is an initiative by ECPGR aimed at conserving and facilitating access to unique germplasm in Europe through the establishment of the European Collection [64].

The CREA Bergamo germplasm bank (Italy) is an example of germplasm bank dedicated to a single species.

Maize (*Zea mays* L.) is the most important grain and forage crop in Italy with a production of over 5.3 million tonnes of grain per year [65]. Ex situ conservation of maize germplasm is a crucial aspect to ensure the genetic diversity of this important food and feed crop. Maize germplasm, which encompasses a wide range of genetic varieties, is valuable for agricultural research and the development of new varieties capable of addressing challenges such as climate change, diseases, and pests. Genebanks serve as valuable genetic reserves, available for scientific research and the introduction of more resistant and adaptable maize varieties [66–69].

In 1954, researchers at the Experimental Station for Maize Cropping in Bergamo initiated the systematic acquisition of Italian maize germplasm through a national sampling program of "Indentata" and "Indurata" types, supported by the Italian Ministry of Agriculture. Over 600 samples of local populations were collected from various regions all over Italy and transferred to Bergamo for studies devoted to their classification [70]. Currently, the Bergamo Genebank preserves the largest collection of maize in Italy with over 5300 accessions preserved ex situ in cells at 7 °C and periodically regenerated in the field under controlled pollination. Seed lots of landraces preserved at the CREA Genebank are regenerated through successive generations of multiplication using between 100 and 200 full-sib ears for each genotype; the frequency or multiplication is generally every ten years, depending also on monitoring the viability of the seeds stored. To avoid genetic drift, inbreeding, and subsequent loss of allele and to maintain an equal and large effective population size, the number of plants sown for multiplication should be two to three times the number of hand-pollinated ears collected throughout the regeneration cycles, as each plant should be used only as male or as female [71].

Germplasm accession regeneration is performed at the CREA Bergamo Farm "La Salvagna" (GPS 45°41′42″ N 9°40′12″ E) in a dedicated field sector (nursery); the experimental

unit is a two-row plot, 5.1 m long, thinned to 20 plants per row, with rows spaced 0.75 m apart. Fertiliser (kg ha^{-1}: N = 280, P_2O_5 = 115, K_2O = 120) and irrigation are applied during the growing season to limit drought stress.

The method of artificial pollination is used for multiplication of CREA Genebank accessions; ears are hand pollinated; to prevent contamination by pollen migration from outside the regeneration plot, the silks are covered with paper envelopes and tassels with pollen bags, followed by swift and accurate pollination.

At maturity, the materials derived from controlled pollination are manually harvested, ears are dried at 40 °C for 7 days up to 14% relative humidity; biometric traits are recorded [72]. Then, if the recorded traits fit with the registered parameters for the specific accession, the ears are shelled, and kernels stored a 4 °C in the genebank facility.

Nowadays, the collection includes 3590 inbred lines, both public and private, with the majority originating from the United States (41%) and Italy (40%); 1262 local populations, over half of which are Italian (682 as reported in AEGIS catalogue, ECPGR: European Accessions [73]), while the others come from 23 different countries; 33 selected synthetic populations representative of an original larger number (476 including subsequent selection cycles) mostly constituted Italy (61%), but also coming from other countries. Additionally, a collection of inbred lines carrying mutations at different plant development stages and in metabolic pathways of the kernel is stored at CREA Genebank.

Recently, a panel of 360 accessions of the Genebank at CREA Bergamo, which includes the inbreds derived from traditional Italian maize open-pollinated varieties (OPV) and advanced breeding lines (Elite Inbreds), was analyzed to identify SNP markers. The results obtained demonstrate that the genetically characterized CREA Italian maize collection, as studied in this research, can be considered an important tool for mapping, and characterizing useful traits and associated loci/alleles to be utilized in maize improvement programs [74].

4. Evaluation of Inbreeding Depression and Genetic Diversity by Classical Methods

The likelihood of genetic drift during seed collection renewal is a challenge that all germplasm banks face. The process of genetic drift gradually elevates homozygosity among diploid organisms, leading to a rise in the inbreeding coefficient (F coefficient) and increases the probability of losing alleles (Figure 2).

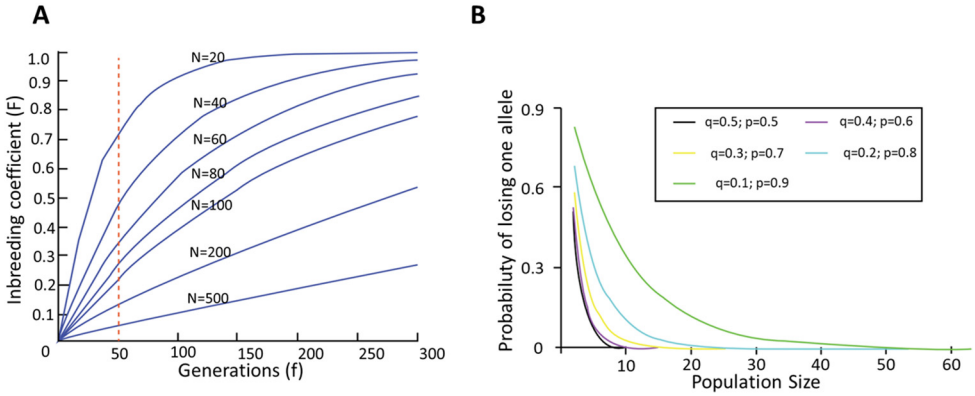

Figure 2. Effect of population size on the inbreeding coefficient (F) and the resulting probability of losing alleles. (**A**) Relationship between inbreeding level (ranging from F = 0, 100% heterozygous to F = 1, 100% homozygous) and the population size across generations. (**B**) Probability of losing alleles (drift away from population) in any single generation in relation to allele frequencies and population size. Modified from McDonald's in 2004 [75].

Inbreeding depression occurs when closely related individuals mate, leading to a reduction in the overall fitness and vigor of the offspring. Inbreeding depression can lead to decreased yield, susceptibility to diseases, and compromised adaptation to environmental

changes. Sustainable agricultural practices should prioritize maintaining genetic variability to ensure long-term resilience in plant populations. In allogamous plants, inbreeding depression is closely linked to the coefficient of inbreeding (F), that represents the probability that two copies of a gene in an individual are identical by descent from a common ancestor. The higher the coefficient of inbreeding, the greater the probability that an individual inherits identical genetic copies from its parents. We can use two different simple methods to estimate inbreeding depression. The first is based on the study of genetic variance, in fact it is a measure of genetic diversity within a population. When there is inbreeding, genetic variance decreases because some alleles are inherited from common ancestors. The relationship between genetic variance and the coefficient of inbreeding can be expressed by the following equation: F= ½ [(Var. Gen. 0 − Var. Gen. 1)/Var. Gen. 0] where Var. Gen. 0 is the initial genetic variance and Var. Gen. 1 is the following generation. Hence by measuring genetic variance in a population and knowing the initial genetic variance, it is possible to calculate the estimated coefficient of inbreeding using this formula [76]. However, it is important to note that this is only an estimate and may vary based on different factors, including population structure and the actual rate of inbreeding. Another method to estimate inbreeding depression in grain yield is the measure of the difference between the means of the S0 (original population) and S1 generations (obtained by bulking selfed plants). Hence, estimating the yield of OPVs in comparison with selfed progenies, we can infer depression. In fact, the rate of inbreeding depression is influenced by allele frequency, directional dominance, and the number of segregating loci. For example, the paper of Lamkey and Smith in 1987 [77] reported a study regarding the rate of inbreeding depression for yield over different eras (from 1930s to 1980s) of maize breeding and compared these with two pre-1930 era varieties, represented by the OPVs 'Reids Yellow Dent' and 'Lancaster Sure Crop'. The era populations were produced by one generation of intermating of single-cross hybrids. The results obtained are shown in Table 1.

Table 1. Estimation of inbreeding depression of maize populations used for hybrid production using three different measures. (i) calculated as yield difference between S0 and S1 population; (ii) expressed as percentage of inbreeding depression (calculated as (S0 − S1)/S0 × 100); (iii) calculated using the formula (S0 − S1)/0.5/100, where 0.5 is the theoretical value of the inbreeding coefficient (F = 50.0% for S1 population). Modified from Lamkey and Smith (1987) [77].

Population	Yield (Mg ha^{-1})		Inbreeding Depression		
	S0	S1	Difference (i)	Percent (ii)	Rate (iii)
Reid opv	2.91	2.25	0.66	22.7	0.013
Lancaster opv	3.14	2.27	0.87	27.7	0.017
Era 1 (1930′)	3.05	2.23	0.82	26.9	0.016
Era 2 (1940′)	3.91	2.92	0.99	25.3	0.020
Era 3 (1950′)	4.42	3.08	1.34	30.3	0.027
Era 4 (1960′)	4.77	3.38	1.39	29.1	0.028
Era 5 (1970′)	5.63	4.25	1.38	24.5	0.028
Era 6 (1980′)	6.07	4.41	1.66	27.3	0.033

5. Evaluation of Genetic Diversity by Molecular Markers

The use of molecular markers for the conservation of genetic resources, particularly ex situ, can assist in the management of the germplasm bank in following ways:

(i) genetic identity verification: molecular markers help ensure the accuracy of germplasm identification and prevent mislabeling or mix-ups within germplasm collections. For example, agrobiodiversity can either be conserved [78] or lost [79,80] by the pressures of selection for adaptation that has favored the emergence of new alleles introduced through crosses with modern varieties [24,80].

(ii) genetic diversity management: molecular markers enable the assessment of genetic diversity within ex situ collections, allowing curators to prioritize the conservation of genetically distinct or rare accessions.
(iii) assessment of genetic erosion: molecular markers facilitate the monitoring of genetic erosion due to factors like population bottlenecks, inbreeding, or selection pressures [24,41,80].
(iv) conservation prioritization: molecular markers assist in prioritizing accessions for conservation based on their genetic uniqueness, adaptive potential, or significance for breeding programs.
(v) germplasm utilization and breeding: molecular markers aid in the efficient utilization of ex situ germplasm resources for breeding purposes [81]. They facilitate the identification of desirable traits, genetic mapping of important genes, and the selection of suitable parental lines for breeding programs, thereby accelerating the development of improved cultivars with desired traits.

Choice of Molecular Markers

The choice of molecular markers to assist the work of germplasm banks depends on several factors, including the genetic diversity of the species, the specific objectives of the germplasm bank, and the available resources. The commonly used molecular markers that can be valuable for germplasm bank management are Single Nucleotide Polymorphisms (SNPs) and Microsatellites (Simple Sequence Repeats, SSRs). SNPs are the most abundant type of genetic variation in genomes and are widely used due to their abundance, stability, and suitability for high-throughput genotyping. SSRs are highly polymorphic markers characterized by short tandem repeat motifs and are widely used for genetic diversity studies, population genetics, and linkage mapping.

However, a common problem regarding the utilization of molecular markers to characterize populations is the ascertainment bias, which is a common issue in genetic studies. It occurs when the markers used for genotyping are not representative of the entire genetic variation present in the population under study [82].

This can lead to skewed results and affect the interpretation of genetic data. The problem of ascertainment bias can vary depending on the marker system used. For example, in the case of (SNPs) can occur when the SNPs selected for genotyping are discovered in a biased sample of individuals, such as individuals from a specific population or with specific phenotypic characteristics. This can lead to an overrepresentation of common variants and an underrepresentation of rare variants in the dataset [82].

To mitigate ascertainment bias, researchers should aim to use marker panels that are representative of the genetic variation present in the population of interest. For example, Diversity Array Technologies (DArT) panels can be utilized, representing samples from different populations of *Medicago* ssp. [83]. Additionally, incorporating multiple marker systems and employing statistical methods designed to account for ascertainment bias can help mitigate its effects and improve the accuracy and robustness of genetic analyses [84]. However, in general, based on the direct experience of the authors of this review, the most significant issue in the management of allogamous plants, both in situ and ex situ, is the risk of genetic drift or inbreeding during regenerations.

Estimating the F-coefficient using molecular markers typically involves conducting a genetic analysis to assess the genetic variation within and among populations or groups. The F-statistic is commonly used in population genetics to quantify the degree of genetic differentiation. The most important parameters that will be taken into consideration are as follows: proportion of polymorphic loci (P); average heterozygosity (H); allelic diversity (A); and molecular analysis of variance (AMOVA). For example, we can use the GenAlex program [85] to calculate these parameters. In order to study the genetic structure of the population, we can use different molecular markers such as SSR (Simple sequence repeats) or SNP (Single-Nucleotide Polymorphism) identified by GBS analysis (Genotyping by sequencing). The utilization of Genotyping-by-Sequencing (GBS) analysis has become

increasingly important in the management of PGRFA. GBS analysis allows for the high-throughput genotyping of large numbers of genetic markers distributed throughout the genome. This enables the assessment of genetic diversity within accession collections, providing valuable insights into the genetic structure, relatedness, and population dynamics of plant germplasm. Overall, the cost-effectiveness of GBS analysis for PGRFA management depends on various factors, including the scale of genotyping, sequencing costs, sample multiplexing strategies, and data analysis approaches. However, nowadays, the biggest issue in using NGS techniques concerns the cost of managing and processing the vast amount of information which, in ordinary germplasm bank management, is often disproportionate to the available resources and the objectives of a germplasm bank. In fact, CoreSNP recently emerged as an efficient pipeline for selecting core marker profiles from genome-wide SNP datasets in crops [84].

Hence, given the chronic shortage of funds dedicated to PGRFA, microsatellites, CoreSNP, and DArT panels seem to be the best choice with which to compare populations in relation to the objectives of germplasm banks, which aim to conserve biodiversity in the best possible way within the available resources of institutions [83,84,86]. Indeed, until now, germplasm banks have not extensively used molecular markers to characterize genetic resources, except for a few targeted projects funded through specific competitive calls. The use of a molecular tool based on SSRs could provide crucial support to avoid redundancies in accessions and to select the best accessions for collection. In fact, the polymorphism information content (PIC) is a measure that permits an estimate to be made of the level of genetic diversity uncovered by each different SSR [87]. However, for allogamous plants, several studies suggested that the use of a few SSRs (from 5 to 10) with a high PIC value should be sufficient to resolve ambiguities among accessions and determine the main genetic structure clustering [28,88–90].

6. Study, Promotion and Valorization of PGRFA: Some Case Studies

The conservation and study of agrobiodiversity are important not only for cultural reasons and as a heritage to future generations but also as a reservoir of new genetic resources that could prove to be useful in modern genetic improvement. Institutions and private companies establish several genebanks worldwide collecting germplasm, including landraces, and wild relatives that have been little explored due various factors such as lack of money, space, and time, and last but not least, information about desirable traits useful in modern breeding.

The high costs for ex situ conservation, mainly due to the costs of genebank management, can be reduced through an exhaustive characterization aimed at a correct/univocal identification of the different accessions. This procedure will avoid the waste of resources caused by the conservation of identical accessions registered with different names. A general scheme of PGRFA management is shown in Figure 3.

The multiplication of collected materials will allow both the study of genetic materials and redistribution to farmers interested in enhancing small-scale value chains in developed countries or as staple food in developing countries.

In this context, some Italian traditional varieties of maize collected in different regions in the 1950s, when the cultivation of landraces began to be replaced by hybrids, and preserved ex situ at the CREA Bergamo Genebank, were characterized and included in the Varietal National Conservation Catalogue [91]. Five maize open-pollinated varieties (Spinato di Gandino", "Rostrato Rosso di Rovetta", "Scagliolo di Carenno", "Nero Spinoso" and "Mais delle Fiorine" [92]), which were originally cultivated in different areas of Lombardy, were registered from 2014 to 2022, and their reintroduction in the original cultivation areas was successfully achieved; their on-farm conservation was carried out by farmers devoted to managing the cultivation following a specific policy and legal framework. CREA Bergamo, as they are responsible for seed stock regeneration under controlled pollination, actively contributes to the management and monitoring of on-farm conservation of the

above-mentioned maize local varieties, planning and setting up controls following standard protocols [11].

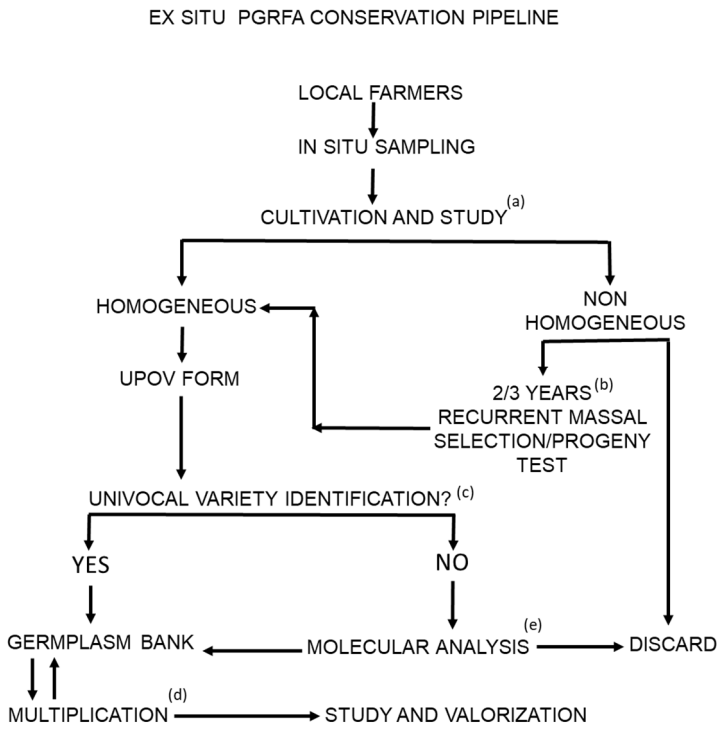

Figure 3. Workflow for ex situ conservation management. [(a)] The cultivation of sampled accessions should involve an estimation of the level of inbreeding as previously reported in this review. [(b)] If the accessions are not homogeneous, they may be discarded (e.g., crossed with varieties of neo-synthesis) or subjected to selection with respect to the considered ideotype. [(c)] Homogeneous varieties will be described using UPOV forms as follows: "Yes" indicates that the accession is recognized as a new variety and will be collected in the germplasm bank; "No" indicates that the accession has already been described and can be further characterized through molecular analysis [(e)] to identify differences if present, or it may be rejected because it is already present in the germplasm bank. [(d)] study, multiplication, and delivery to local farmers.

The growing fascination with traditional varieties finds support in numerous studies that highlight the abundance of phytonutrients in these varieties, contributing to the health and well-being of both livestock and humans. Among the key phytonutrients, flavonoids, and particularly anthocyanins, are acknowledged as potent antioxidants capable of deterring chronic diseases in humans [93]. Furthermore, several studies conducted on landraces have revealed intriguing characteristics (such as lower susceptibility to fungal and pest attacks) that could be useful in reducing the use of agrochemicals in fields [94]. The accumulation of phlobaphenes (and flavonoids in general) grants the landrace "Nero Spinoso" beneficial properties, distinguishing it as a functional food when compared to colorless varieties [81]. Phlobaphenes are insoluble phenolic compounds, accumulated in specific plant tissues (e.g., pericarp layer, husk, cob glumes) that confer a reddish-brown color. They are secondary metabolites derived from 3-deoxy flavonoids in the phenylpropanoid pathway. The regulation of phlobaphene accumulation in the maize pericarp layer is governed by the transcription factor known as pericarp color 1 (*p1*). Different alleles of *p1* result in varying colors of the pericarp and cob glumes [95]. For instance, the *p1-rr* allele leads to coloring of both pericarp and cob glumes (allele present in the "Nero Spinoso" variety), whereas the *p1-rw* allele colors only the pericarp (allele present in the "Rostrato Rosso di Rovetta" variety [81]). Furthermore, several studies suggest a

potential association between phlobaphene pigments and reduced levels of mycotoxin contamination, particularly a decrease in fumonisin B1, in maize kernels [96].

Hence, *p1* genes could contribute to keeping low levels of mycotoxins present in the kernels, emerging as an effective "lost gene" in modern genetic improvement.

The concept of "lost genes" in modern genetic improvement refers to those genes present in wild species during evolution and domestication and corresponding landraces selected by humans, which have been lost during the years of genetic improvement in the last century.

For this reason, in recent years, the figure of the Pre-Breeder has been gaining prominence in various seed companies; their job is to study and "screen" genetic resources in order to propose to the actual breeder traits/genes that can be introduced into the main genetic pools. For example, the study of ancient varieties of maize rich in anthocyanins, originating from Mesoamerica, has enabled the recovery of regulatory genes in the anthocyanin biosynthetic pathway (e.g., Red 1 (*r1*) and Purple plant (*pl1*) genes) to be used to obtain new pigmented varieties adapted to our latitudes (Figure 4) [97].

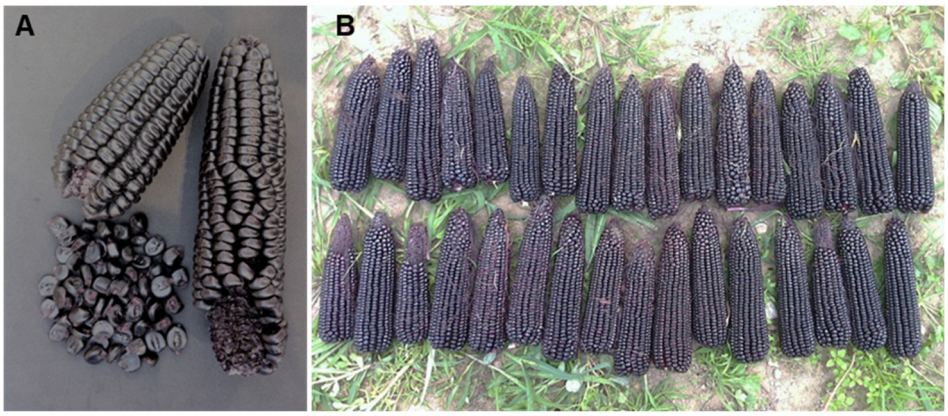

Figure 4. Hybrid corn rich in anthocyanins: 'Morado' variety native to Peru, used as a donor of the pl (purple plant1) gene regulating the anthocyanin biosynthetic pathway (**A**). Experimental hybrids derived, rich in anthocyanins and suitable for our latitudes (**B**).

Pigmented maize materials were also developed in the framework of an International Cooperation Project (P.S.G.O. Km0 Bolivia (2018–2021) funded by AICS-Agenzia Italiana per la Cooperazione allo sviluppo, AID 011.457); for this purpose, two Italian white local varieties from Bergamo CREA Genebank, were crossed to the germplasm of Bolivian type 'Morado' and Mexican type 'Azul' [98]. Purple and blue maize rich in antioxidant compounds could be a good tool to increase the functional food properties and improve beneficial effects on human health [99]. Additionally, pigmented maize kernels could express plant defense functions against biotic (fungal pathogens and pests) and abiotic stress (drought). In this perspective, maize pigmented genotypes, for their content in specific phytochemical bioactive compounds, could be also suitable for introduction into advanced breeding programs aimed to enhance and valorize biodiversity potential on resistance to mycotoxigenic pathogens and on grain quality and safety improvement.

7. Conclusions

The conservation of PGRFA is vital to ensuring food security, biodiversity preservation, and resilience in the face of changing climatic conditions and emerging agricultural challenges.

In this paper, we reviewed the two strategies for PGRFA conservation, in situ and ex situ, with a specific focus on allogamous plants. Literature data, besides underlining the complementarity of the two conservation approaches, highlighted the importance of genetic analysis as a fundamental prerequisite for proper conservation. Furthermore, the case studies analyzed have highlighted that the characterization of landraces has led to the discovery of interesting traits, useful both for future breeding programs and to valorize and

promote their present cultivation and use. The particular link to the territory, traditions, and culture, furthermore, ensures that with the conservation of these genetic resources, the entire cultural heritage associated with them can be preserved for future generations.

Author Contributions: Conceptualization, R.P. and M.L.; Writing—Original Draft Preparation, R.P., M.L., S.L., C.B., G.R., A.B., M.G. and E.C.; Writing—Review and Editing, R.P., M.L., S.L., C.B., G.R. and A.B.; Supervision, R.P. and M.L.; Project Administration, R.P. and M.L.; Funding Acquisition, R.P. and M.L. All authors have read and agreed to the published version of the manuscript.

Funding: This publication is part of the project NODES, which has received funding from the MUR—M4C2 1.5 of PNRR funded by the European Union—NextGenerationEU (Grant agreement no. ECS00000036) (to M.L.) and Agritech National Research Centre, which received funding from the European Union—NextGenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR)–MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4–D.D. 1032 17/06/2022, CN00000022) (to R.P.). This manuscript reflects only the authors' views and opinions; neither the European Union nor the European Commission can be considered responsible for them.

Data Availability Statement: Not applicable.

Acknowledgments: We wish to thank Lesley Currah for her editing and suggestions.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. FAO. Agricultural Biodiversity, Multifunctional Character of Agriculture and Land Conference, Background Paper 1. September 1999. Available online: https://www.fao.org/3/x2775e/x2775e03.htm (accessed on 5 March 2024).
2. Cardinale, B.J.; Duffy, J.E.; Gonzalez, A.; Hooper, D.U.; Perrings, C.; Venail, P.; Narwani, A.; MacE, G.M.; Tilman, D.; Wardle, D.A.; et al. Biodiversity loss and its impact on humanity. *Nature* **2012**, *486*, 59–67. [CrossRef] [PubMed]
3. Newton, A.C.; Begg, G.S.; Swanston, J.S. Deployment of diversity for enhanced crop function. *Ann. Appl. Biol.* **2009**, *154*, 309–322. [CrossRef]
4. FAO. The state of the world's biodiversity for food and agriculture. In *FAO Commission on Genetic Resources for Food and Agriculture Assessments*; B'elanger, J., Pilling, D., Eds.; FAO: Rome, Italy, 2019. Available online: https://www.fao.org/documents/card/en?details=ca3129en (accessed on 5 March 2024).
5. Maxted, N.; Hawkes, J.G.; Ford-Lloyd, B.V.; Williams, J.T. A practical model for in situ genetic conservation. In *Plant Genetic Conservation: The In Situ Approach*; Maxted, N., Ford-Lloyd, B.V., Hawkes, J.G., Eds.; Chapman & Hall: London, UK, 1997; pp. 339–367.
6. Wang, Y.; Wang, Y.; Sun, X.; Caiji, Z.; Yang, J.; Cui, D.; Cao, G.; Ma, X.; Han, B.; Xue, D.; et al. Influence of ethnic traditional cultures on genetic diversity of rice landraces under on-farm conservation in southwest China. *J. Ethnobiol. Ethnomed.* **2016**, *12*, 51. [CrossRef]
7. Tiranti, B.; Negri, V. Selective microenvironmental effects play a role in shaping genetic diversity and structure in a *Phaseolus vulgaris* L. landrace: Implications for on-farm conservation. *Mol. Ecol.* **2007**, *16*, 4942–4955. [CrossRef] [PubMed]
8. Vigouroux, Y.; Barnaud, A.; Scarcelli, N.; Thuillet, A.C. Biodiversity, evolution and adaptation of cultivated crops. *C. R. Biol.* **2011**, *334*, 450–457. [CrossRef]
9. Curry, H.A. The history of seed banking and the hazards of backup. *Soc. Stud. Sci.* **2022**, *52*, 664–688. [CrossRef] [PubMed]
10. Maxted, N.; Hunter, D.; Ortiz Ríos, R. *Plant Genetic Conservation*; Cambridge University Press: New York, NY, USA, 2020.
11. ECPGR. *ECPGR Concept for On-Farm Conservation and Management of Plant Genetic Resources for Food and Agriculture*; ECPGR: Rome, Italy, 2017. Available online: www.ecpgr.cgiar.org/fileadmin/bioversity/publications/pdfs/ECPGR_Concept_for_on_farm_final__05_05_2017_bis.pdf (accessed on 5 March 2024).
12. Casañas, F.; Simó, J.; Casals, J.; Prohens, J. Toward an evolved concept of landrace. *Front. Plant Sci.* **2017**, *8*, 145. [CrossRef] [PubMed]
13. FAO. What Is Happening to Agrobiodiversity? Available online: https://www.fao.org/3/y5609e/y5609e02.htm (accessed on 5 March 2024).
14. Dulloo, M.E.; Hunter, D.; Borelli, T. Ex situ and in situ conservation of agricultural biodiversity: Major advances and research needs. *Not. Bot. Horti Agrobo* **2010**, *38*, 123–135.
15. Volis, S. Complementarities of two existing intermediate conservation approaches. *Plant Divers.* **2017**, *39*, 379–382. [CrossRef]
16. Zegeye, H. In situ and ex situ conservation: Complementary approaches for maintaining biodiversity. *Int. J. Res. Env. Std.* **2016**, *4*, 1–12.
17. Bellon, M.R.; Pham, J.L.; Jackson, M.T. Genetic conservation: A role for rice farmers. In *Plant Conservation: The In Situ Approach*; Maxted, N., Ford-Lloyd, B.V., Hawkes, J.G., Eds.; Chapman and Hall: London, UK, 1997; pp. 263–289.

18. Bellon, M.R.; van Etten, J. Climate change and on-farm conservation of crop landraces in centres of diversity. In *Plant Genetic Resources and Climate Change*; Jackson, M., Ford-Lloyd, B., Parry, M., Eds.; CABI: Oxford, OK, 2013; pp. 137–150.
19. Zeven, A.C. Landraces: A review of definitions and classifications. *Euphytica* **1998**, *104*, 127–139. [CrossRef]
20. Negri, V.; Maxted, N.; Veteläinen, M. European landrace conservation: An introduction. In *European Landraces: On farm Conservation, Management and Use: Biodiversity Technical Bulletin No 15*; Veteläinen, M., Negri, V., Maxted, N., Eds.; European Cooperative Programme for Plant Genetic Resources: Rome, Italy, 2009.
21. Villa, T.C.; Maxted, N.; Scholten, M.A.; Ford-Lloyd, B.V. Defining and identifying crop landraces. *Plant Genet. Res.* **2005**, *3*, 373–384. [CrossRef]
22. Tin, H.Q.; Berg, T.; Bjørnstad, Å. Diversity and adaptation in rice varieties under static (ex situ) and dynamic (in situ) management. A case study in the Mekong Delta, Vietnam. *Euphytica* **2001**, *122*, 491–502. [CrossRef]
23. Gómez, O.J.; Blair, M.W.; Frankow-Lindberg, B.E.; Gullberg, U. Comparative study of common bean (*Phaseolus vulgaris* L.) landraces conserved ex situ in genebanks and in situ by farmers. *Genet. Resour. Crop Evol.* **2005**, *52*, 371–380.
24. McLean-Rodriguez, F.D.; Costich, D.E.; Camacho-Villa, T.C.; Pe, M.E.; Dell'Acqua, M. Genetic diversity and selection signatures in maize landraces compared across 50 years of in situ and ex situ conservation. *Heredity* **2021**, *126*, 913–928. [CrossRef] [PubMed]
25. Bretting, P.K.; Duvick, D.N. Dynamic conservation of plant genetic resources. *Adv. Agron.* **1997**, *61*, 1–51.
26. Brush, S.B. *Farmers' Bounty. Locating Crop Diversity in the Contemporary World*; Yale University Press: New Haven, CT, USA, 2004.
27. Gepts, P. Plant genetic resources conservation and utilization: The accomplishments and future of a societal insurance policy. *Crop. Sci.* **2006**, *46*, 2278–2292. [CrossRef]
28. Valorizzazione e Salvaguardia delle Risorse Genetiche Vegetali D'interesse Agrario e Alimentare (RGVAA). Available online: http://www.geneticagraria.it/attachment/SocietaScuolaRicerca/Documento_SIGA_Agrobiodiversita_2023.pdf (accessed on 5 March 2024).
29. Convention on Biodiversity (CBD). Available online: https://www.cbd.int/ (accessed on 8 March 2024).
30. FAO. *International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA)*; FAO: Rome, Italy, 2009. Available online: https://www.fao.org/3/i0510e/i0510e.pdf (accessed on 8 March 2024).
31. Spataro, G.; Negri, V. The European seed legislation on conservation varieties: Focus, implementation, present and future impact on landrace on farm conservation. *Genet. Resour. Crop Evol.* **2013**, *60*, 2421–2430. [CrossRef]
32. EURISCO. Available online: https://eurisco.ipk-gatersleben.de/apex/eurisco_ws/r/eurisco/home (accessed on 8 March 2024).
33. Kotni, P.; Van Hintum, T.; Maggioni, L.; Oppermann, M.; Weise, S. EURISCO update 2023: The European Search Catalogue for Plant Genetic Resources, a pillar for documentation of genebank material. *Nucleic Acids Res.* **2023**, *51*, 1465–1469. [CrossRef]
34. EVA Maize Network. Available online: https://www.ecpgr.cgiar.org/eva/eva-networks/maize (accessed on 8 March 2024).
35. Italian Law for the Ratification and Execution of the Convention on Biodiversity, with Annexes (Rio de Janeiro, 5 June 1992). Available online: https://www.mase.gov.it/normative/l-14-febbraio-1994-n-124-ratifica-ed-esecuzione-della-convenzione-sulla-biodiversita-conannessi (accessed on 8 March 2024).
36. Italian Law n° 194, Provisions for the Protection and Enhancement of Agricultural and Food Biodiversity. 2015. Available online: https://www.gazzettaufficiale.it/eli/id/2015/12/11/15G00210/sg%20 (accessed on 23 March 2024).
37. FAO/ITPGRFA. Views, Experiences and Best Practices as an Example of Possible Options for the National Implementation of Article 9 of the International Treaty. Available online: www.fao.org/3/ca7834en/ca7834en.pdf (accessed on 8 March 2024).
38. Byrnes, A.; Sthapit, B. *A Guide to the Identification and Importance of Custodian Farmers*; Bioversity International: Rome, Italy, 2016; 2p. Available online: https://cgspace.cgiar.org/server/api/core/bitstreams/707ead85-7b15-406f-b017-8479c0d7486a/content (accessed on 5 March 2024).
39. Barcaccia, G.; Falcinelli, M. *Miglioramento Genetico In Genetica e Genomica*, 2nd ed.; Liguori ed Srl: Napoli, Italia, 2005; Volume II.
40. Bannert, M.; Stamp, P. Cross-pollination of maize at long distance. *Eur. J. Agron.* **2007**, *27*, 44–51. [CrossRef]
41. Bøhn, T.; Aheto, D.W.; Mwangala, F.S.; Fischer, K.; Bones, I.L.; Simoloka, C.; Mbeule, I.; Schmidt, G.; Breckling, B. Pollen-mediated gene flow and seed exchange in small-scale Zambian maize farming, implications for biosafety assessment. *Sci. Rep.* **2016**, *6*, 34483. [CrossRef] [PubMed]
42. Poudel, D. On Farm Conservation of Crop Genetic Resource: Declining De Facto Diversity and Optimal Funding Strategy. *Nat. Resour. J.* **2015**, *6*, 196–207.
43. Amancah, E.; Mercado, W.; Gómez-Pando, L.; Escalante, R.; Sotomayor, D.A. Integrating in situ conservation of plant genetic resources with ex situ conservation management: Involving custodian farmers, benefits and their willingness to accept compensation. *Sci. Agropecu.* **2023**, *14*, 447–464. [CrossRef]
44. Wale, E. Costing on-farm conservation of crop diversity: The case of sorghum and wheat in Ethiopia and implications for policy. *Afr. J. Agric. Res.* **2011**, *6*, 401–406.
45. FAO. *Second Report on the World's Plant Genetic Resources for Food and Agriculture*; FAO: Rome, Italy, 2010; p. 299. Available online: https://www.fao.org/3/i1500e/i1500e00.htm (accessed on 5 March 2024).
46. Hay, F.R.; Probert, R.J. Advances in seed conservation of wild plant species: A review of recent research. *Conserv. Physiol.* **2013**, *1*, cot030. [CrossRef] [PubMed]
47. Wambugu, P.W.; Ndjiondjop, M.N.; Henry, R.J. Role of genomics in promoting the utilization of plant genetic resources in genebanks. *Brief. Funct. Genom.* **2018**, *17*, 198–206. [CrossRef]

48. FAO/IPGRI. *Genebank Standards*; Food and Agriculture Organization of the United Nations: Rome, Italy; International Plant Genetic Resources Institute: Rome, Italy, 1994. Available online: https://cropgenebank.sgrp.cgiar.org/images/file/learning_space/genebank_standards.pdf (accessed on 5 March 2024).
49. FAO. *Genebank Standards for Plant Genetic Resources for Food and Agriculture, Rev. Ed.*; FAO: Rome, Italy, 2014. Available online: www.fao.org/3/i3704e/i3704e.pdf (accessed on 8 March 2024).
50. Walters, C.; Wheeler, L.M.; Grotenhuis, J.M. Longevity of seeds stored in a genebank: Species characteristics. *Seed Sci. Res.* **2005**, *15*, 1–20. [CrossRef]
51. Center for Plant Conservation. Best Plant Conservation Practices. Available online: https://saveplants.org/best-practices/difference-between-orthodox-intermediate-and-recalcitrantseed/#:~:text=Recalcitrant%20seeds%20cannot%20be%20stored,between%2045%20and%2065%25%20R (accessed on 23 April 2024).
52. Hoban, S. New guidance for ex situ gene conservation: Sampling realistic population systems and accounting for collection attrition. *Biol. Conserv.* **2019**, *235*, 199–208. [CrossRef]
53. Kew Seed Bank Resources. Available online: https://brahmsonline.kew.org/msbp/Training/Resources (accessed on 5 March 2024).
54. Ex Situ Conservation of Plant Genetic Resources. Biodiversity International. Available online: https://cgspace.cgiar.org/server/api/core/bitstreams/f66c1a4b-b390-42ab-9478-a6ba439cb35d/content (accessed on 8 March 2024).
55. Hawkes, J.G.; Maxted, N.; Ford-Lloyd, B.V. *The Ex Situ Conservation of Plant Genetic Resources*; Kluwer Academic Publishers: Dordrecht, The Netherlands, 2000.
56. Rao, N.K.; Hanson, J.; Dulloo, M.E.; Ghosh, K.; Nowell, D.; Larinde, M. *Manual of Seed Handling in Genebanks*; Handbook for Genebanks No. 8.; Biodiversity International: Rome, Italy, 2006.
57. Harrington, J.F. Seed storage and longevity. In *Seed Biology, Insects, and Seed Collection, Storage, Testing and Certification*; Kozlowski, T.T., Ed.; Academic Press: New York, NY, USA, 1972; pp. 145–245.
58. Ranganathan, U.; Groot, S.P.C. Seed Longevity and Deterioration. In *Seed Science and Technology*; Dadlani, M., Yadava, D.K., Eds.; Springer: Singapore, 2023.
59. Basey, A.C.; Fant, J.B.; Kramer, A.T. Producing native plant materials for restoration: 10 rules to collect and maintain genetic diversity. *Native Plants J.* **2015**, *16*, 37–52. [CrossRef]
60. Ellis, R.H.; Roberts, E.H. Improved Equations for the Prediction of Seed Longevity. *Ann. Bot.* **1980**, *45*, 13–30. [CrossRef]
61. Engels, J.M.M.; Ebert, A.W. A Critical Review of the Current Global Ex Situ Conservation System for Plant Agrobiodiversity. II. Strengths and Weaknesses of the Current System and Recommendations for Its Improvement. *Plants* **2021**, *10*, 1904. [CrossRef]
62. Laboratorio di Ecologia Vegetale (University of Pavia). Available online: http://www.labecove.it/index.php?option=com_content&view=article&id=55&Itemid=225&lang=en (accessed on 5 March 2024).
63. ENSCONET (European Native Seed Conservation Network). Seed Collecting Manual for Wild Species. 2009. Available online: http://ensconet.maich.gr/ (accessed on 5 March 2024).
64. A European Genebank Integrated System (AEGIS). Available online: https://www.cgiar.org/aegis (accessed on 8 March 2024).
65. ISTAT. 2023. Available online: https://dati.istat.it/Index.aspx?DataSetCode=DCSP_COLTIVAZIONI (accessed on 21 February 2024).
66. Butrón, A.; Revilla, P.; Sandoya, G.; Ordás, A.; Malvar, R.A. Resistance to reduce corn borer damage in maize for bread, in Spain. *J. Crop Prot.* **2009**, *28*, 134–138. [CrossRef]
67. Santiago, R.; Cao, A.; Malvar, R.A.; Butrón, A. Assessment of corn resistance to fumonisin accumulation in a broad collection of inbred lines. *Field Crop Res.* **2013**, *149*, 193–202. [CrossRef]
68. Balconi, C.; Berardo, N.; Locatelli, S.; Lanzanova, C.; Torri, A.; Redaelli, R. Evaluation of ear rot (Fusarium verticilliodes) resistance and fumonisinaccumulation in Italian maize inbred lines. *Phytopathol. Med.* **2014**, *53*, 1426.
69. Torri, A.; Lanzanova, C.; Locatelli, S.; Valoti, P.; Balconi, C. Screening of local Italian maize varieties for resistance to Fusarium verticillioides. *Maydica* **2015**, *60*, 1–8.
70. Brandolini, A.; Brandolini, A. Maize introduction, evolution and diffusion in Italy. *Maydica* **2009**, *54*, 233–242.
71. Wang, J.; Crossa, J.; van Ginkel, M.; Taba, S. Statistical genetics and simulation models in genetic resource conservation and regeneration. *Crop Sci.* **2004**, *44*, 2246–2253. [CrossRef]
72. IBPGR. *Descriptors for Maize*; International Maize and Wheat Improvement Center: Mexico City, Mexico; International Board for Plant Genetic Resources: Rome, Italy, 1991. Available online: https://www.google.com/url?sa=i&url=https://www.genebanks.org/resources/publications/descriptors-for-maize/&psig=AOvVaw1swVN6EOl6YBRbs2LSP5XD&ust=1714492561029000&source=images&cd=vfe&opi=89978449&ved=0CAUQn5wMahcKEwjgtfrR5OeFAxUAAAAAHQAAAAAQBA (accessed on 29 April 2024).
73. ECPGR: European Accessions. Available online: www.ecpgr.cgiar.org/aegis/european-collection/european-accessions (accessed on 23 March 2024).
74. Mastrangelo, A.M.; Hartings, H.; Lanzanova, C.; Balconi, C.; Locatelli, S.; Cassol, H.; Valoti, P.; Petruzzino, G.; Pecchioni, N. Genetic Diversity within a Collection of Italian Maize Inbred Lines: A Resource for Maize Genomics and Breeding. *Plants* **2024**, *13*, 336. [CrossRef]
75. McDonald, B.A. Population Genetics of Plant Pathogens. *Plant Health Instr.* **2004**, *4*.
76. Frankham, R.; Ballou, J.D.; Briscoe, D.A. *Introduction to Conservation Genetics*, 4th ed.; Cambridge University Press: Cambridge, UK, 2007; pp. 52–75.

77. Lamkey, K.R.; Smith, O.S. Performance and Inbreeding Depression of Populations Representing Seven Eras of Maize Breeding. *Crop Sci.* **1987**, *27*, 695–699. [CrossRef]
78. Bezançon, G.; Pham, J.L.; Deu, M.; Vigouroux, Y.; Sagnard, F.; Mariac, C.; Kapran, I.; Mamadou, A.; Gérard, B.; Ndjeunga, J.; et al. Changes in the diversity and geographic distribution of cultivated millet (*Pennisetum glaucum* (L.) R. Br.) and sorghum (*Sorghum bicolor* (L.) Moench) varieties in Niger between 1976 and 2003. *Genet. Resour. Crop Evol.* **2009**, *56*, 223–236. [CrossRef]
79. Hammer, K.; Knupffer, H.; Xhuveli, L.; Perrino, P. Estimating genetic erosion in landraces—two case studies. *Genet. Resour. Crop Evol.* **1996**, *43*, 329–336. [CrossRef]
80. Bitocchi, E.; Bellucci, E.; Rau, D.; Albertini, E.; Rodriguez, M.; Veronesi, F.; Attene, G.; Nanni, L. European flint landraces grown in situ reveal adaptive introgression from modern maize. *PLoS ONE* **2015**, *10*, e0121381. [CrossRef] [PubMed]
81. Cassani, E.; Puglisi, D.; Cantaluppi, E.; Landoni, M.; Giupponi, L.; Giorgi, A.; Pilu, R. Genetic studies regarding the control of seed pigmentation of an ancient European pointed maize (*Zea mays* L.) rich in phlobaphenes: The "Nero Spinoso" from the Camonica valley. *Genet. Resour. Crop Evol.* **2017**, *64*, 761–773. [CrossRef]
82. Heslot, N.; Rutkoski, J.; Poland, J.; Jannink, J.L.; Sorrells, M.E. Impact of marker ascertainment bias on genomic selection accuracy and estimates of genetic diversity. *PLoS ONE* **2013**, *8*, e74612. [CrossRef] [PubMed]
83. Zhao, D.; Sapkota, M.; Lin, M.; Beil, C.; Sheehan, M.; Greene, S.; Irish, B.M. Genetic diversity, population structure, and taxonomic confirmation in annual medic (*Medicago* spp.) collections from Crimea, Ukraine. *Front. Plant Sci.* **2024**, *15*, 1339298. [CrossRef]
84. Dou, T.; Wang, C.; Ma, Y.; Chen, Z.; Zhang, J.; Guo, G. CoreSNP: An efficient pipeline for core marker profile selection from genome-wide SNP datasets in crops. *BMC Plant Biol.* **2023**, *23*, 580. [CrossRef] [PubMed]
85. Peakall, R.; Smouse, P.E. GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Mol. Ecol. Notes* **2006**, *6*, 288–295. [CrossRef]
86. Šajgalík, M.; Ondreičková, K.; Hauptvogel, P.; Mihálik, D.; Glasa, M.; Kraic, J. Higher Effectiveness of New Common Bean (*Phaseolus vulgaris* L.) Germplasm Acquisition by Collecting Expeditions Associated with Molecular Analyses. *Sustainability* **2019**, *11*, 5270. [CrossRef]
87. Nagy, S.; Poczai, P.; Cernák, I.; Gorji, A.M.; Hegedus, G.; Taller, J. PICcalc: An online program to calculate polymorphic information content for molecular genetic studies. *Biochem. Genet.* **2012**, *50*, 670–672. [CrossRef] [PubMed]
88. Anderson, J.A.; Churchill, G.A.; Autrique, J.E.; Tanksley, S.D.; Sorrells, M.E. Optimizing parental selection for genetic linkage maps. *Genome Dyn.* **1993**, *36*, 181–186. [CrossRef]
89. Galli, Z.; Halász, G.; Kiss, E.; Heszky, L.; Dobránszki, J. Molecular identification of commercial apple cultivars with microsatellite markers. *Hortscience* **2005**, *40*, 1974–1977. [CrossRef]
90. Vietina, M.; Agrimonti, C.; Marmiroli, M.; Bonas, U.; Marmiroli, N. Applicability of SSR markers to the traceability of monovarietal olive oils. *J. Sci. Food Agric.* **2011**, *91*, 1381–1391. [CrossRef] [PubMed]
91. Registro Varietà Vegetali. Available online: https://cns.sian.it/portale-sian/sottosezione.jsp?pid=6 (accessed on 8 March 2024).
92. Giupponi, L.; Leoni, V.; Colombo, F.; Cassani, E.; Hejna, M.; Rossi, L.; Pilu, R. Characterization of "Mais delle Fiorine" (*Zea mays* L.) and nutritional, morphometric and genetic comparison with other maize landraces of Lombardy region (Northern Italy). *Genet. Resour. Crop Evol.* **2021**, *68*, 2075–2091. [CrossRef]
93. Petroni, K.; Pilu, R.; Tonelli, C. Anthocyanins in corn: A wealth of genes for human health. *Planta* **2014**, *240*, 901–911. [CrossRef] [PubMed]
94. Revilla, P.; Soenga, P.; Malvar, R.A. Effects of antioxidant activity of black maize in corn borer larval survival and growth. *Spanish J. Agric. Res.* **2018**, *16*, 1004–1011. [CrossRef]
95. Casas, M.I.; Duarte, S.; Doseff, A.I.; Grotewold, E. Flavone-rich maize: An opportunity to improve the nutritional value of an important commodity crop. *Front. Plant Sci.* **2014**, *5*, 440. [CrossRef] [PubMed]
96. Landoni, M.; Puglisi, D.; Cassani, E.; Borlini, G.; Brunoldi, G.; Comaschi, C.; Pilu, R. Phlobaphenes modify pericarp thickness in maize and accumulation of the fumonisin mycotoxins. *Sci. Rep.* **2020**, *10*, 1417. [CrossRef] [PubMed]
97. Lago, C.; Cassani, E.; Zanzi, C.; Landoni, M.; Trovato, R.; Pilu, R. Development and study of a maize cultivar rich in anthocyanins: Coloured polenta, a new functional food. *Plant Breed.* **2014**, *133*, 210–217. [CrossRef]
98. Suriano, S.; Balconi, C.; Valoti, P.; Redaelli, R. Comparison of total polyphenols, profile anthocyanins, color analysis, carotenoids and tocols in pigmented maize. *LWT* **2021**, *144*, 111257. [CrossRef]
99. Revilla, P.; Alves, M.L.; Andjelkovic, V.; Balconi, C.; Dinis, I.; Reis Mendes-Moreira, P.M.; Redaelli, R.; Ruiz De Galarreta, J.I.; Vaz Patto, M.C.; Vaz Patto, M.C.; et al. Traditional maize (*Zea mays* L.) foods in Europe. *Front. Nutr.* **2022**, *8*, 683399. [CrossRef]