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Chapter 2
Linking of Genebank to Breeding and Food Security

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Abstract Genebanks have the responsibility of collecting, maintaining, characterizing, evaluating, documenting and distributing plant genetic resources for research, education and breeding purposes globally. About 7.4 million germplasm accessions are conserved ex situ in the genebanks globally. Efficient use of germplasm in crop improvement is depending on the availability of accession-level information on the traits of interest. For the majority of accessions, only basic passport and characterization data are available, while data on unique traits is generally lacking that limits their utilization in crop improvement. Development of germplasm diversity and trait-specific subsets enhanced availability of accessions-level information. Researchers can search in the global plant genetic resources database called Genesys PGR which contains passport data, characterization and evaluation data sets and trait-specific subsets developed on various crops (https://www.genesys-pgr.org/). The impact of germplasm for contributing to increased yield, adaptation, nutrition and improved health and sustainable agriculture has been demonstrated in many crops. There are many instances where a single plant genetic resource has proved to have large commercial value by conferring a specific trait. With the availability of new technologies such as high-throughput large-scale phenotypic assessment for key traits and use of multi-omic tools could accelerate rapid identification of traits and genes for breeding improved cultivars. This chapter details about ex situ germplasm conservation, discovering climate resilient germplasm following different approaches such as diversity and trait-specific subsets, focused germplasm

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identification strategy, molecular characterization of germplasm and trait discovery, access to germplasm and the impact of genebank contributing to the global agriculture sustainability.

Keywords Plant Genetic Resources · Germplasm · Ex situ genebank · Conservation · Breeding · Genomics · Food Security

2.1 Introduction

Plant genetic resources (PGR) are the key to crop improvement and have an important role to play to address food security and nutrition. The unprecedented rate of biodiversity loss is one of the major challenges that erode the resilience of the agricultural system and threatens food and nutrition security. Although the number of plant species used for food by pre-agricultural humans is estimated to be around 7000, only a small fraction (~250) of them has been fully domesticated (von Wettberg et al. 2020). The trends in diversity across crops and regions with a 50-year perspective indicate the increased dominance of mega crop varieties in agricultural landscapes and displacement of traditional landraces. This trend occurs at a faster rate in Asia than in Africa (Gatto et al. 2021). The main focus of Sustainable Development Goal 2.5 is to maintain the genetic diversity of crops and their wild relatives in the genebanks, ensuring access to that diversity following international laws. To minimize the biodiversity loss due to the replacement of landrace by improved varieties (Gepts 2006; Van de Wouw et al. 2009; Khoury et al. 2014), national and international genebanks have been established to conserve and distribute germplasm globally for sustainable agriculture. Globally, about 7.4 million accessions are conserved ex situ in the genebanks. Germplasm resources with information on key traits aid in the selection and their use in crop improvement. The trait of importance to most users includes productivity, stress tolerance, and quality traits. However, for the majority of germplasm accessions, only basic passport and characterization data are available, while data on unique traits is generally lacking. This chapter details about ex situ germplasm conservation, discovering climate-resilient germplasm following different approaches such as diversity and trait-specific subsets, focused germplasm identification strategy, molecular characterization of germplasm and trait discovery, access to germplasm and the impact of genebank contributing to the global agriculture sustainability.

2.2 Ex Situ PGR Conservation

Plant genetic resources for food and agriculture (PGRFA) comprise the diversity of genetic materials, including landraces, breeding or modern cultivars, genetic stocks, crop wild and weedy relatives, that can be used now or in the future for food and agriculture. PGR includes any materials of plant origin including reproductive and

vegetative propagating materials, which contain the functional units of heredity. The biological diversity of the PGR is mainly conserved within or away from their natural habitats called in situ and ex situ conservation, respectively. Global threats to PGRFA in situ and on farm have increased in the last few decades because of many reasons including global climate change and the increased impact of human activities. Therefore, ex situ conservation in the genebank is the most common approach either semi-controlled (field genebank) or under controlled conditions (seeds, tissues, seedlings, pollens, and DNA). Seed genebanks are the easiest way to store germplasm at low temperature, while field genebank is for the conservation of genetic resources under normal growing conditions. To safeguard against the loss of plant biological diversity, intensive collection of different crop species was undertaken by the global community (Upadhyaya et al. 2010). As a result, over 7.4 million PGR conserved ex situ in over 1750 genebanks globally (https://www.fao.org/wiews) and the International Agricultural Research Centres (IARC) conserve about 10% of the total accessions accounts (Table 2.1). As of December 31, 2021, the 11 IARC Centers conserve over 722,000 accessions of crop, forage, and tree germplasm and make them available under the standard material transfer agreement (SMTA). These IARCs account for about 94% of the germplasm distributed within the guidelines of the International Treaty on Plant Genetic Resources for Food and Agriculture (Plant Treaty). During the 15 years (January 2007 to December 2021), the IARC's genebanks and breeding programs distributed over six million samples under 61,000 SMTAs.

The conservation of plant genetic resources (PGR) has gained significant importance. This is demonstrated by the impressive number of nations that have ratified the Convention on Biological Diversity, endorsed the International Undertaking on Plant Genetic Resources, or both. Despite this very encouraging development, many genebanks face financial and operational difficulties. According to the FAO report 'State of the World's Plant Genetic Resources for Food and Agriculture' (FAO 1998), many genebanks may not at present be capable of performing their basic conservation role. In the case of seed genebanks, where the technology of storing germplasm samples is relatively easy to apply under most operational circumstances, the problems relate more to resource constraints that impact the performance of essential operations. This is critical in the case of the core activities of maintaining the viability and genetic integrity of the stored accessions, as well as sufficient stocks, to meet user demands. Consequently, the importance of efficient and cost-effective genebank management has increased over the years and has become a decisive element in the long-term ex situ conservation of PGR.

2.2.1 Seed Genebank

Seed genebanks conserve crop diversity mostly in the form of seeds. Every genebank in the world follows some basic core activities/operations such as germplasm collection and acquisition, conservation, distribution, characterization,

Table 2.1 Plant genetic resources for food and agriculture conserved and made available by the International Agricultural Research Centres (IARC) genebanks

CGIAR Centre	Crop	Number of accessions	CGIAR Centre	Crop	Number of accessions available with SMTA
AfricaRice	Rice	19,696	ICRAF	Multipurpose trees	6744
Bioversity	Banana	1682		Fruit trees	8246
CIAT	Beans	37,934	ICRISAT[a]	Chickpea	20,838
	Forages	22,662		Groundnut	15,360
	Cassava	5965		Pigeon pea	13,559
CIMMYT	Maize	28,694		Pearl millet	24,663
	Wheat	135,021		Small millets	11,797
CIP	Andean roots and tubers	1173		Sorghum	42,880
	Potato	7367	IITA	Cowpea	17,051
	Sweet potato	6143		Cassava	3184
ICARDA	Lentils	14,295		Maize	1561
	Grass pea	4301		Miscellaneous legumes	6747
	Forages	25,358		Banana	392
	Faba bean	9594		Yam	5929
	Chickpea	15,230	ILRI	Forages and fodder	3918
	Barley	31,843	IRRI	Rice	127,413
	Pea	4593			
	Wheat	41,967			

Source: Global Crop Diversity Trust/CGIAR Online Reporting Tool, covering the period up to December 31, 2021

[a]ICRISAT genebank data as on Jan 2023

regeneration, viability testing and monitoring, safety duplication and documentation. New accessions are collected or assembled to enrich the diversity of the genebank collections considering the geographical and taxonomical gaps in the collection. A comprehensive technical guide on collecting plant genetic resources providing many practical and managerial suggestions has been published (Guarino 1995). It is important that collected or harvested germplasm material is processed as soon as possible to avoid loss in viability or decrease in longevity. Seed moisture content (SMC) is one of the most important factors determining longevity of the stored seeds. Before the seeds are stored, they should be properly dried and the seed moisture content should be accurately determined. A small change in SMC can greatly affect the storage life of the seeds (Roberts 1973). Different SMC determination methods and equipment are available, the principles and methodology of which are presented by Ellis et al. (1985), and the procedures by Hanson (1985). The recommended levels of SMC are between 3% and 7% for long-term conservation

(FAO–IPGRI. 1994). The genebank curator has to accurately assess the initial viability of each accession to be stored and monitor the viability of an accession during its storage life to reduce or avoid the loss of genetic diversity within and between the accessions. Details on genebank standards for viability monitoring were proposed by a panel of experts and were subsequently endorsed by the FAO Commission on Plant Genetic Resources (FAO 2014). Knowing the precise storage behaviour of the species is essential for a seed sample before it can be stored, in order to ensure its optimum storage conditions are used, that is, the optimum moisture content and storage temperature. A protocol to determine seed storage behaviour has been published by IPGRI (Hong and Ellis 1996), in conjunction with a seed storage behaviour compendium (Hong et al. 1996), which contains storage behaviour information on more than 7000 species. When the optimum seed moisture content is accurately determined and the seeds have been packaged, they should be stored at the best available temperature. The genebank standards recommend a preferred temperature of −18 °C or below for long-term storage also called as base collections and 5–10 °C for medium-term storage also known as active collections. This two-tiered storage concept of the base collection for long-term storage and active collection for accessions for distribution or research is largely based on experiences with the storage of orthodox seeds. Regeneration of accessions is one of the most crucial processes involved in genebank management, since during regeneration accessions are particularly vulnerable to loss or change of genetic diversity. It is also a costly process in which practical compromises are frequently made, the consequences of which might only be observed much later. It was for these reasons that IPGRI published a scientific background paper for the regeneration and multiplication of germplasm resources in seed genebanks (Breese 1989). Regeneration in genebanks is carried out after the accessions show viability that is below threshold level or the quantity of accession reaches a critical level after which it cannot be distributed. Most genebanks have computerized documentation systems which greatly facilitate the storage and maintenance of data, as well as its retrieval. A helpful overview of the various aspects of genebank documentation can be found in the guidebook for genetic resources documentation (Painting et al. 1995). Most of the routine genebank operations described above generate information which is key to the efficient functioning of the genebank operations.

2.2.2 Field Genebanks and in Vitro Conservation

Germplasm from clonal crops which are either vegetatively propagated and/or do not produce seeds, or for species with short-lived recalcitrant seeds are usually conserved in the field genebanks and/or as in vitro conservation. The field genebank has limitations regarding efficiency, cost, security and long-term maintenance. In vitro conservation involves maintenance of explants in a protected environment, aseptic plants and supports safe and easy international exchange of plant materials and lower conservation cost. Techniques for collecting species which produce recalcitrant

seeds have been developed which enable the collector to grow the material in vitro, under aseptic conditions. This approach will allow germplasm collections to be made in remote areas (e.g. for highly recalcitrant cacao seeds), or when the transport of the collected fruits would become prohibitively expensive (e.g. coconut collecting in the South Pacific) where the target species would not have seeds or other storage organs to be collected. A good overview of such techniques has been presented by Withers (1995).

2.2.3 Cryopreservation

The cryopreservation technique ensures long-term and safe storage of those species which are difficult to conserve as seed. This can be achieved by storing the samples at ultra-low temperatures, either above −150 °C or at −196 °C liquid nitrogen. For several species (e.g. potato, apple, banana and cassava), procedures have been developed which allow this technique to be applied routinely for conservation (Engelmann and Takagi 2000). Cryopreservation is also being used for the long-term storage of orthodox seed having short longevity. Cryopreservation mostly involves the two-step cooling process which is based on the induction of explant 'vitrification' during a very fast decrease in temperature. Vitrification of cells and tissues is the physical process, which avoids intracellular ice crystallization, during ultra-freezing, by the transition of the aqueous solution of the cytosol into an amorphous, glassy state. As a result of this process, plant tissues are protected from damage and remain viable during their long-term storage at −196 °C. For different plant species, a number of vitrification-based techniques have been developed such as vitrification, encapsulation-dehydration, encapsulation-vitrification, desiccation (Reed 2008) and, more recently, droplet vitrification and D/V cryoplate (Yamamoto et al. 2011; Niino et al. 2013) but the techniques are continuously modified and improved to produce higher plant recovery rates, to expand the number of the cryopreserved species and, above all, working on the species, which are still hard to process with the cryopreservation. Cryopreservation techniques are now used for plant germplasm storage in many institutes around the world (Niino 2006; Malik et al. 2012).

2.2.4 DNA Banking

DNA banking is an efficient and long-term method to conserve the genetic information. DNA banks are now considered as a means of complimentary conservation. DNA storage is particularly useful for those species that cannot be conserved in traditional seed or field genebanks nor conserved in situ due to high risk in that area. DNA storage has so far been undertaken with objectives other than conservation in mind, usually to allow genetic material to be made readily available for molecular

applications, for distribution or training. The DNA Data Bank of Japan (DDBJ, http://www.ddbj.nig.ac.jp) (Mashima et al. 2017) is a public database of nucleotide sequences established at the National Institute of Genetics (NIG, https://www.nig.ac.jp/nig). Since 1987, the DDBJ has been collecting annotated nucleotide sequences as its traditional database service. The data at DDBJ primarily accumulated via submissions of sequence data by the researchers. This endeavour has been conducted in collaboration with GenBank (Benson et al. 2017) at the National Center for Biotechnology Information (NCBI) and with the European Nucleotide Archive (ENA) (Toribio et al. 2017) at the European Bioinformatics Institute (EBI). The collaborative framework is called the International Nucleotide Sequence Database Collaboration (INSDC) (Cochrane et al. 2016) and the product database from this framework is called the International Nucleotide Sequence Database (INSD). In 2020, the DDBJ accepted 6836 submissions of annotated nucleotide sequences, and 59.3% were submitted by Japanese research groups. The DDBJ has periodically released all public DDBJ/ENA/GenBank nucleotide sequence data in the flat-file format.

Plant DNA Bank in Korea (PDBK) is responsible for collection of the Korean vascular plants and useful plants mainly in East Asia and establishing the genomic DNA database from those plants with its voucher information. The PDBK is one of the largest plant genomic DNA bank in the world and has various plant genomic DNAs including about 2950 domestic species and many foreign species in its collection that mostly belong to Korean endemic, rare, and endangered plant species. The PDBK is having approximately 22,000 accessions of the purified and concentrated genomic DNA from other countries in East Asia. All the DNA materials are well characterized and handled according to the standard procedures and are being dispatched under the material transfer agreement for research purpose. PDBK dispenses approximately 500 accessions of genomic DNAs per year to researchers globally with very high purity of each DNAs. The quality is monitored randomly by the qualitative and quantitative tests (https://pdbk.korea.ac.kr/about.asp).

2.3 Safety Duplication

Safety duplication involves duplication of a genetically identical sub-sample of the accession to mitigate the risk of its partial or total loss caused by natural or man-made catastrophes. The safety duplicates are genetically identical to the base collection and are referred to as the secondary most original sample (Engels and Visser 2003). Safety duplicates include both the duplication of material and its related information, and are deposited in a base collection at a different location, usually in another country. The location is chosen to minimize possible risks and provides the best possible storage facilities. Safety duplication is generally under a 'black-box' approach. This means that the repository genebank has no entitlement to the use and distribution of the germplasm. It is the depositor's responsibility to ensure that the deposited material is of high quality, to monitor seed viability over time and to use their own base collection to regenerate the collections when they

begin to lose viability. The germplasm is not touched without permission from the depositor and is only returned on request when the original collection is lost or destroyed.

The Svalbard Global Seed Vault (SGSV) in Norway is an example of a secure facility for safety duplication of crop genetic resources. SGSV is the world's largest safety stock for seeds from the earth's diversity of cultivated crops. Located far beyond the Arctic Circle and 130 m deep inside a frozen mountain, permafrost provides an environmentally friendly solution to long-term secure conservation of crop diversity as a safety duplicate that is only accessed in case of disaster or loss of the samples from the main safety backup. The vault can hold 4.5 million seed samples of crop diversity. The seeds are stored at −18 °C which is required for optimal storage of the seeds and the seeds are stored and sealed in custom-made three-ply foil packages. The packages are sealed inside boxes and stored on shelves inside the vault. The low temperature and moisture levels inside the SGSV ensure low metabolic activity, keeping the seeds viable for long periods of time (https://www.croptrust.org/our-work/svalbard-global-seed-vault).NordGen. Together with the Norwegian Ministry of Agriculture and Food, the organization Global Crop Diversity Trust (GCDT) is responsible for the operation of the SGSV. It offers free storage of seed specimens conserved by international, national, regional genebanks as well as institutions and organizations. Ownership of the seeds never changes. They are stored under so-called black box conditions, which means, among other things, that only the institution that puts in the seeds can take them out. The SGSV currently conserves more than 1.1 million seed samples of 5934 species that have been deposited by 89 national and international genebanks worldwide (https://seedvault.nordgen.org/).

2.4 Germplasm Exchange

The introduction of germplasm for conservation and use is an important function for most genebanks. At the same time, many genebanks also play an important role in distributing germplasm samples to potential users, thus linking conservation directly with use. As germplasm is never free of pests and diseases, great care has to be given to quarantine aspects to avoid the transfer of harmful pathogens together with the germplasm. When exchanging germplasm accessions, the curator has to adhere to existing plant quarantine regulations for both legal and biological reasons. Furthermore, the curator can actively contribute to the safe exchange of germplasm samples by following the technical guidelines which are jointly being produced by FAO and IPGRI. Since the early 1990s, the availability of germplasm has become more restricted. Several countries have introduced access legislation, as part of the implementation of the Convention on Biological Diversity (CBD), and many have implemented the Prior Informed Consent provision of the CBD. The latter requires a mutual agreement on the conditions under which the germplasm material is allowed to be taken out of the country. Both these measures have led to the

development and use of material transfer agreements and germplasm acquisition agreements which spell out the conditions under which germplasm can be used and acquired.

2.5 Discovering Climate-Resilient Germplasm

Characterization of germplasm following the crop-specific descriptors provide first-hand information for selection of desirable germplasm based on the traits of interest for which the data is available. The global plant genetic database called Genesys is an online platform on PGR conserved in genebanks globally, contains passport data, characterization and evaluation data sets and trait-specific subsets developed on various crops (https://www.genesys-pgr.org/).

2.5.1 Germplasm Diversity and Trait-Specific Subsets

Ex situ germplasm collections have grown enormously in size and number over the years as a result of global efforts to conserve plant genetic resources for food and agriculture (Odong et al. 2013). The larger size of the germplasm collection and limited information on traits of importance have been highlighted as significant issues hindering their effective utilization in crop improvement programs (Gollin et al. 2000; Koo and Wright 2000; FAO 2010). To overcome this situation, a small set of accessions be selected from the collection containing as much genetic diversity as possible and these types of selections would offer a good starting point when targeting new traits of interest. Considering this, Frankel (1984) proposed a 'core collection' which would 'represent with a minimum of repetitiveness, the genetic diversity of a crop species and its relatives.' A core collection consists of a limited set of accessions (about 10%) derived from an existing germplasm collection, chosen to represent the genetic spectrum in the whole collection. The available data on the geographic origin, specific plant characteristics, trait data, and molecular data are utilized to develop core subsets. There are many methods and free software packages available such as PowerCore (Kim et al. 2007), CoreHunter (De Beukelaer et al. 2018), ccChooser (Studnicki et al. 2012), and MSTRAT (Gouesnard et al. 2001) and GenoCore (Jeong et al. 2017) are few examples that could help to construct core subsets using molecular marker data, genetic distances, phenotypic traits, geographic origin, or integration of these various data types. The accessions remaining after selecting core accessions are considered as the *reserve collection* (Brown 1989). Due to reduced size, the core collections can be evaluated extensively and more economically for important traits. Following this approach, core collections have been constituted in several crops species, including rice (Yan et al. 2007), groundnut (Upadhyaya et al. 2003), pearl millet (Upadhyaya et al. 2009a), sorghum (Grenier et al. 2001), and other crops. In many cases, the germplasm collections conserved by

most of the genebanks are very large in size. For example, the size of the ICRISAT sorghum core collection is 2242 accessions that was developed from 22,473 accessions (Grenier et al. 2001), which is still large in size and limits their utilization. To overcome this, Upadhyaya and Ortiz (2001) developed the concept of mini-core collection (10% of core or 1% of the entire collection). Following this approach, mini-core collection has been developed in many crops including rice (Agrama et al. 2009), sorghum (Upadhyaya et al. 2009b), chickpea (Upadhyaya and Ortiz 2001), and other crops (Table 2.2). The important point is that core collection should be dynamic, not static; thus a periodic review and modification of the core collection is necessary considering the increase in size and information of collection, to add new diversity.

Once the core and/or mini core are available, researchers would have a manageable number of accessions to evaluate extensively and identify new variability and traits combinations. For example, the evaluation of 242 accessions of sorghum mini core resulted in the identification of promising germplasm sources resistance to biotic stress (70 accessions), abiotic stress (12 accessions), and other traits such as bioenergy (13 accessions) and nutritional traits (27 accessions) (Upadhyaya et al. 2019). Similarly, in the groundnut mini-core collection (184 accessions), 28 accessions were identified as resistant to abiotic stress and 30 to biotic stress (Upadhyaya et al. 2014a); and in the chickpea mini-core collection, 40 accessions were reported as resistant to abiotic stress and 31 to biotic stress (Upadhyaya et al. 2013a). When we require additional or new source variability for a given trait, researchers can refer back to the clusters from which the core collection accession came to select similar accessions from the entire collection. This approach will increase the probability of identifying specific traits from a large ex situ collection.

2.5.2 Focused Identification of Germplasm Strategy (FIGS)

FIGS is a tool that supports researchers to identify promising germplasm traits-specific sources from large ex situ collections more accurately and efficiently. The FIGS approach matches plant traits with geographic and agro-climatic information of the places where germplasm accessions were collected as the environment strongly influences natural selection, thus it increases the chances of finding the adaptive trait of interest. The main aim of this method is to develop trait-specific subsets rather than capturing all the genetic variation present in the genetic resources. Thus, it is one of the efficient strategies to explore and sort out the plant genetic resources for climate change adaptive traits. FIGS can be developed either by following filtering and modelling strategies. FIGS following filtering requires a deep understanding of the ecology and the optimal conditions of the expression of the traits under study and how these conditions affect the crop. Filters can be applied in the search process to narrow down from a large collection to a small subset considering geographic locations of a given stress occurrence, climatic conditions favouring stress occurrence, and long-term-climatic and/or soil characteristics of the

Table 2.2 Core and mini-core subset developed in different field crops globally

Crop	Core/ mini core	Number of accessions used	No. of traits used	Number of accessions in core/mini core	Reference
Rice	Core	4310	50 phenotypic traits and 36 SSRs	932	(Zhang et al. 2011)
Sorghum	Core	22,474	21	2247	(Grenier et al. 2001)
Sorghum	Core	33,100	7	3475	(Prasada Rao and Ramanatha Rao 1995)
Groundnut	Core	14,310	14	1704	(Upadhyaya et al. 2003)
Groundnut	Core	7432		831	(Holbrook et al. 1993)
Groundnut (Valencia)	Core	630	26	77	(Dwivedi et al. 2008)
Groundnut	Asian Core		15	504	(Upadhyaya et al. 2005)
Soyabean	Core	15,558	18	1600	(Oliveira et al. 2010)
A worldwide bread wheat	Core	3942	38 SSRs	372	(Balfourier et al. 2007)
Pearl millet	Core	16,063	11	1600	(Bhattacharjee et al. 2007)
Pearl millet (augmented)	Core	20,844	22	2094	(Upadhyaya et al. 2009a)
World sesame	Core	1724	17	172	(Mahajan et al. 2007)
West African yam *Dioscorea* spp.	Core	1724	18	172	(Mahalakshmi et al. 2007)
USDA rice	Core	18,412	14	1790	(Yan et al. 2007)
Korean sesame	Core	2246	12	475	(Kang et al. 2006)
Pigeon pea	Core	12,153	14	1290	(Reddy et al. 2005)
Iberia peninsula common beans	Core	388	34	52	(Rodiño et al. 2003)
Safflower	Core	5522	12	570	(Dwivedi et al. 2005)
China sesame	Core	4251	14	453	(Xiurong et al. 2000)
Indian mung bean	Core	1532	38	152	(Bisht et al. 1998)

(continued)

Table 2.2 (continued)

Crop	Core/ mini core	Number of accessions used	No. of traits used	Number of accessions in core/mini core	Reference
Perennial *Medicago*	Core	1100	50	200	(Basigalup et al. 1995)
Annual *Medicago*	Core	1240	16	211	(Diwan et al. 1995)
Saccharum spontaneum	Core	342	11	75	(Tai and Miller 2001)
Chickpea	Core	3350		505	(Hannan et al. 1994)
Chickpea	Core	16,991	13	1956	(Upadhyaya et al. 2001)
Finger millet	Core	5940	14	622	(Upadhyaya et al. 2006b)
Foxtail millet	Core	1474	23	155	(Upadhyaya et al. 2009c)
Proso millet	Core	833	20	106	(Upadhyaya et al. 2011a)
Barnyard millet	Core	736	21	89	(Upadhyaya et al. 2014b)
Kodo millet	Core	656	20	75	(Upadhyaya et al. 2014b)
Little millet	Core	460	20	56	(Upadhyaya et al. 2014b)
Rice	Mini core	1794	26 phenotypic traits and 70 molecular markers	217	(Agrama et al. 2009)
Sorghum	Mini core	2247	21	242	(Upadhyaya et al. 2009b)
Japanese rice landraces	Mini core	236	32 SSRs	50	(Ebana et al. 2008)
Pearl millet	Mini core	2094	12	238	(Upadhyaya et al. 2011b)
Chickpea	Mini core	1956	16	211	(Upadhyaya and Ortiz 2001)
Pigeon pea	Mini core	1290	16	146	(Upadhyaya et al. 2006a)
Groundnut	Mini core	1704	34	184	(Upadhyaya et al. 2002)
Groundnut	Mini core	831	16	112	(Holbrook and Dong 2005)
Finger millet	Mini core	5940	18	80	(Upadhyaya et al. 2010)
Foxtail millet	Mini core	1474	21	35	(Upadhyaya et al. 2011c)

Table 2.3 A few examples of promising germplasm sources identified following FIGS approach for biotic and abiotic stress tolerance

S. No	Crop	Trait	Reference
1.	Wheat	Powdery mildew (*Blumeria graminis* (DC) Speer f.sp. *tritici*)	(Bhullar et al. 2009)
2.	Wheat	Powdery mildew (*Blumeria graminis* (DC) Speer f.sp. *tritici*)	(Vikas et al. 2020)
3.	Wheat	Sunn pest (*Eurygaster intergriceps* put.)	(Bouhssini et al. 2009)
4.	Wheat	Russian wheat aphid (*Diuraphis noxia* Kurdj.)	(Bouhssini et al. 2011)
5.	Wheat	Stem rust (*Puccinia graminis* Pers.)	(Endresen et al. 2012)
6.	Wheat	Stripe (yellow) rust (*Puccinia striiformis*)	(Bari et al. 2014)
7.	Barley	Net blotch (*Pyrenophora teres* Drechs.)	(Endresen et al. 2011)
8.	Faba bean	Drought tolerance	(Khazaei et al. 2013)

collection site, etc. When evaluation data is available for adaptive traits, FIGS can explore the mathematical relationship between the adaptive trait of interest and the long-term climatic and/or soil characteristics of collection sites to choose a small set from a large collection. Further, the small FGIS set can be evaluated to identify promising germplasm sources for use in crop improvement. A few examples of promising germplasm sources identified following FIGS approach for biotic and abiotic stress tolerance are presented in Table 2.3.

2.5.3 Molecular Characterization and Trait Discovery

Advances in genome sequencing technologies have made a significant contribution to the next-generation genebanking for the efficient conservation and enhanced use of germplasm in crop improvement. Genomics and gene editing technological interventions could enable a new era of de novo domestication through the introduction of domestication genes into non-domesticated plants (Van Tassel et al. 2020). Large-scale high-density genotyping helps in understanding the genetic diversity and population structure of the germplasm collection and linking DNA sequence variants to the phenotypes of interest (Varshney et al. 2021). There are several large-scale genotyping efforts in different crops. For example, in chickpea, 3366 accessions including 3171 cultivated and 195 wild species accessions were sequenced at an average coverage of around 12×, and constructed a pan-genome to describe the genomic diversity of chickpea (Varshney et al. 2021). This study identified superior haplotypes for improvement-related traits in landraces that can be introgressed into elite breeding lines through haplotype-based breeding, and also

found targets for purging deleterious alleles through genomics-assisted breeding and/or gene editing (Varshney et al. 2021). In wheat, Sansaloni et al. (2020) sequenced about 80,000 wheat accessions using DArTseq technology and identified over 300,000 high-quality SNPs and SilicoDArT markers, provides great opportunity for developing wheat varieties utilizing allelic diversity missing in the current breeding program. In rice, resequencing of a core collection of 3000 accessions originating from 89 countries resulted in the identification of about 29 million single nucleotide polymorphisms (SNPs), 2.4 million small indels, and over 90,000 structural variations that contributed to within and between-population variation (3000 Rice Genome Project 2014; Wang et al. 2018). The phylogenetic analysis based on SNP data confirmed the presence of five varietal groups in *O. sative* gene pool, namely, indica, aus/boro, basmati/sadri, tropical japonica and temperate japonica, and also suggest several subpopulations that correlate with genographic locations (3000 Rice Genome Project 2014; Wang et al. 2018). In addition, using pan-genome analysis, over 10,000 novel full-length protein-coding genes and also presence-absence variations were reported (Wang et al. 2018). From the USDA soybean collection, 14,430 soybean accessions were selected from the whole set of about 22,000 were genotyped using the Illumina Infinium SoySNP50K BeadChip (Bandillo et al. 2015). The results indicated that the accessions originating from Japan were relatively homogenous and distinct from the Korean accessions, while both Japanese and Korean accessions diverged from the Chinese accessions. The GWAS performed using 12,000–13,000 accessions identified SNPs signals for seed protein and oil (Bandillo et al. 2015), and also for ten key phenotypic descriptive traits (Bandillo et al. 2017). Such large-scale genotyping of genebank collections support in gene discovery, genomic prediction, genome-wide association mapping, marker development, and other applications.

2.5.4 *Contribution of Plant Genetic Resources for Global Food Security and Nutrition, and Environmental and Economic Benefits*

Breeding of high-yielding, resistance/tolerance to biotic and abiotic stresses, and climate-resilient crops is important for meeting the food demand of the increasing population globally. Plant genetic resources contribute significantly for addressing the food security, malnutrition and environmental sustainability. Impact of germplasm for contributing to increased yield, adaptation, nutrition and improved health and sustainable agriculture have been demonstrated in many crops. There are many instances where a single plant genetic resources has proved to have large commercial value by conferring a specific trait. Well-known examples include *Rht1* and *Rht*2 dwarfing genes in wheat, the dwarfing genes of the green revolution, originated in Japan, by crossing a semi-dwarf wheat variety called Daruma with American high-yielding variety to produce Norin 10, which was further used to develop number of

semi-dwarf cultivars. The dwarfing alleles are named *Rht1* (*Rht-B1b*) and *Rht2* (*Rht-D1b*) (Gaur et al. 2020). In rice, the semi-dwarfing gene, *sd1* first identified in the Chinese variety 'Dee-geo-woo-gen' was utilized to develop the semi-dwarf cultivars such as Taichung Native 1 (TN1) and IR8, and later it formed the basis for the development of new high-yielding, semi-dwarf cultivars (Spielmeyer et al. 2002). The semi-dwarfing gene in rice (sd1) is a recessive allele that confers lodging resistance through shortened culm and highly responsive to nitrogenous fertilizers. Groundnut is an important oil seed crop, originated in southern Bolivia to northern Argentina region of South America. Recent study revealed that the contribution of a wild species accession, *Arachis cardenasii* GKP 10017 originating from Bolivia for the development of groundnut cultivars resistant to foliar fungal disease. The ICRISAT genebank assembled the GKP 10017 accession from USDA-ARS, registered as ICG 8216. From ICRISAT it reached globally and contributed as a source for developing groundnut cultivars resistance to late leaf spot and rust in Africa, Asia, Oceania, and the Americas, and provided widespread improved food security and environmental and economic benefits (Bertioli et al. 2021). Table 2.4 shows a few examples of ICRISAT-supplied germplasm that impacted global crop productivity.

Globally, the burden of malnutrition in all its forms remains a major challenge to the humanity. Thus, there is an urgent need to transform food systems to sustainably deliver better quality diets for improved nutrition and health. Breeding staple crops by mainstreaming nutrition as a key component could deliver biofortified crop cultivars for different nutrients. Globally, HarvestPlus program focuses on biofortification of major staples (rice, wheat, maize, beans, cassava, sweet potato, and pearl millet) through conventional plant breeding methods to increase the micronutrients content of staple food crops, works with several CGIAR research centres and national agriculture research systems in collaboration. Between 2004 and 2022, 262 biofortified cultivars in 12 crops have been released in 30 countries. For example, in pearl millet, utilizing the intra-population variability within ICTP 8203, the high Fe and Zn biofortified varieties of pearl millet 'Dhanshakti' and 'Chakti' were released in India and Africa (Rai et al. 2014; Govindaraj et al. 2019). The ICTP 8203 is a large-seeded and high-yielding open-pollinated variety derived from *iniadi* landrace from northern Togo, bred at ICRISAT, Patancheru. Currently, India is growing >70,000 ha of biofortified pearl millet, and many more cultivars are under various stages of testing for a possible release. There are several such examples on the impact of germplasm globally for addressing food security and nutrition.

Landraces, crop wild relatives, and specifically adapted ecotypes are generally heterogeneous, adapted to specific local environments, and often low/or no market preference, they can be endowed with rich sources of genes for crop improvement. Advances in plant genomics is opening a new era in germplasm research such as deployment of desirable alleles originating from the germplasm (landrace) in the crop improvement programs. For instance, genes can now be edited in situ such that alleles conferring desirable traits or phenotype can be reintroduced into elite cultivars without disturbing the genetic background that confers valuable traits including

Table 2.4 Germplasm lines that impacted ICRISAT mandate crops productivity globally

Crop	Accessions	Origin	Contribution	Selection from germplasm directly released as variety
Pigeon pea	ICP 7035	India	Source for resistance to sterility mosaic disease (SMD) and a large seed size	*Kamica* in Fiji, *Guimu 4* in China, *JK Sweety* in India and ICP 7035 in both Nepal and Philippines
Pigeon pea	ICP 8863	India	Source for resistance to *fusarium* wilt	*Maruthi* in India
Chickpea	ICC 4958	India	Donor for drought tolerance, used as parents in chickpea improvement for drought tolerance	Transferred through MAS in several varieties recently
Groundnut	ICG 12991	India	Source of resistance to rosette virus disease	Baka in Malawi, as Serenut 4 T in Uganda, Nematil in Mozambique and Msandile in Zambia
Sorghum	IS 2205	India	Source for resistance to shoot fly and stem borer resistance	Used as national check in India for shoot fly and stem borer resistance
Sorghum	IS 33844	India	It is an excellent maldandi-type with large and lustrous grains and high yield (predominant post-rainy sorghum landrace in Maharashtra and Karnataka states of India). This was selected from a germplasm collection from Maharashtra by ICRISAT genebank staff in 1989.	Parbhani Moti in India
Pearl millet	IP 17862	Togo	An *Iniadi* pearl millet landrace was the important source material for the development of improved cultivars	ICTP 8203, MP 124, PCB 138 in India; *Okashana 1* and *Okashana 2* in Namibia and Nyankhombo in Malawi.
Barnyard millet	IEc 542	Japan	High grain and fodder yielding, most popular in Uttarakhand, India	PRJ 1 in India

yield, quality and stress tolerance traits. A few examples of genes that contributed to enhancing productivity, quality and stress tolerance in crop cultivars are listed in Table 2.5.

Table 2.5 Examples of genes that contributed to enhancing productivity, quality and stress tolerance in crop cultivars

S. No	Crop	Genes	Traits	Reference
1	Rice	*Sd1*	Semi-dwarf	(Spielmeyer et al. 2002)
2	Wheat	*Rht-B1b (Syn. Rht1)*	Semi-dwarf	(Peng et al. 1999)
3	Wheat	*Rht-D1b (Syn. Rht2)*	Semi-dwarf	(Peng et al. 1999)
4	Sorghum	*Sh1*	Seed shattering	(Lin et al. 2012)
5	Sorghum	*SbWRKY*	Seed shattering	(Tang et al. 2013)
6	Sorghum	*Wx*	Endosperm texture	(McIntyre et al. 2008, Sattler et al. 2009)
7	Sorghum	*Ma1/SbPRR37*	Maturity	(Murphy et al. 2011)
8	Sorghum	*Ma3*	Maturity	(Childs et al. 1997)
9	Sorghum	*Ma6*	Maturity	(Murphy et al. 2014)
10	Sorghum	*SbSUC9*	Maturity	(Upadhyaya et al. 2013b)
11	Sorghum	*LD*	Maturity	(Upadhyaya et al. 2013b)
12	Sorghum	*SbMED12*	Maturity	(Upadhyaya et al. 2013b)
13	Sorghum	*Dw1/Sbht9.1*	Plant height	(Hilley et al. 2016)
14	Sorghum	*Dw2*	Plant height	(Hilley et al. 2017)
15	Sorghum	*Dw3*	Plant height	(Multani et al. 2003)
16	Sorghum	*bmr2*	Brown midrib	(Saballos et al. 2012)
17	Sorghum	*bmr6*	Brown midrib	(Saballos et al. 2009)
18	Sorghum	*bmr12*	Brown midrib	(Sattler et al. 2012)
19	Sorghum	*Glossy 15*	Shoot fly resistance	(Satish et al. 2009); (Aruna et al. 2011)
20	Sorghum	*Rf1/SbPPR13*	Fertility	(Klein et al. 2005)
21	Sorghum	*Rf2*	Fertility	(Madugula et al. 2018)
22	Sorghum	*Rf6*	Fertility	(Praveen et al. 2015)
23	Sorghum	YELLOW SEED1 (Y1)	Grain mould resistance	(Nida et al. 2019)
24	Sorghum	YELLOW SEED3 (Y3)	Grain mould resistance	(Nida et al. 2019)
25	Barley	*Short clum1 (hcm1)*	Short clum	(Lundqvist et al. 1997)
26	Rye	*Dw1 (Ddwl)*	Dwarf	(Tenhola-Roininen and Tanhuanpää 2010)

2.6 Access to Genebank Collection

The legal landscape for biodiversity and genetic resources has changed dramatically over the last 40 years, and continues to evolve. Arguably, the biggest changes that took place were the shift from the common heritage concept in the International Undertaking (1983) to national sovereignty in the Convention on Biological Diversity (1993) and then countries choosing to exercise that national sovereignty to

create an international multilateral system for PGRFA under the Plant Treaty (2004). Many peoples' perceptions of genetic resources and their value have been influenced by advances in science and technology and their potential for commercial exploitation. One policy response was to strengthen intellectual property laws to protect commercial investments. This development catalysed a call for national control over genetic resources that are relied upon as 'inputs' into research and development chains with commercial potential. These policy responses are, in turn, influencing the ability of research and development organizations (both public and private) to access and use genetic resources conserved in genebanks worldwide and also to exploit new technologies, and share benefits created through their work. Genebanks worldwide are mostly facilitating access to their collections through the Multilateral System (MLS) of the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGFRA).

2.6.1 The International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA)

To address PGRFA in the post-CBD era, the FAO drafted and adopted the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA, www.fao.org/plant-treaty), which came into force on 29 June 2004 (FAO 2002). The objectives of the ITPGRFA are very similar to those of the CBD but focus on the conservation and sustainable use of PGRFA and the sharing of the benefits arising from their use (FAO 2002). PGRFA are defined as: "any genetic material of plant origin of actual or potential value for food and agriculture" (FAO 2002). The ITPGRFA confirms the sovereign rights of countries over their genetic resources but aims to facilitate the exchange of PGRFA by the establishment of a Multilateral System of Access and Benefit-Sharing (MLS) in which PGRFA are exchanged under a Standard Material Transfer Agreement (SMTA), instead of under the prior informed consent and mutually agreed terms prescribed by the CBD.

The MLS is a global pool of PGRFA, meant to facilitate access to these PGRFA as well as to achieve fair and equitable sharing of the benefits arising from their utilization. PGRFA may be added to this pool by countries and the institutions under their control, by natural and legal persons in the contracting parties and by international institutes (Manzella 2013). The MLS does not extend to all PGRFA but covers a set of 35 food crops and 29 forages, which are listed in Annex I of the ITPGRFA. The selection of this set of crops and forages was based on criteria of food security and interdependence and was a negotiated compromise between countries favouring the inclusion of all PGRFA and countries favouring the inclusion of only a limited number of crops (Visser 2013). According to Article 11 of the ITPGRFA, the MLS is to include all PGRFA of the food crops and forages listed in Annex I that are "under the management and control of the Contracting Parties and in the public domain" (FAO 2002). PGRFA that belong to the food crops and forages listed in Annex I but

do not fulfil the other conditions are not automatically included in the MLS but can be included on a voluntary basis by natural and legal persons holding these PGRFA. Access to materials in the MLS under the SMTA is granted only for their use in research, breeding and training for food and agriculture; other uses are explicitly excluded (FAO 2002). With regard to benefit sharing, the Contracting Parties to the ITPGRFA recognize that facilitated access itself is an important benefit, but also underline the importance of other forms of benefit sharing, such as the exchange of information, technology transfer, capacity building, and the sharing of commercial benefits. If material received under an SMTA is used to create PGRFA that are not freely available for research and breeding by others, the recipients must pay 0.77% of the sales of those PGRFA (or 0.5% of all sales of PGRFA belonging to the same crop) to an international benefit-sharing fund (www.fao.org/plant-treaty/areas-of-work/benefit-sharing-fund), which is used to support conservation and sustainable utilization of PGRFA. The Contracting Parties to the ITPGRFA undertake to include in the MLS those PGR of the crops and forages in Annex I that are in the public domain and under their management. However, even if material is not part of the MLS, providers of PGR can distribute their material under the SMTA.

2.6.2 Article 15 of ITPGRFA

Article 15 deals with ex situ collections of PGRFA held by the CGIAR genebanks and other international institutions. The Treaty called on the CGIAR Centres to sign agreements with the Governing Body to bring their collections under the Treaty. PGRFA listed in Annex I that are held by the CGIAR Centres are to be made available as part of the MLS. In 2006, all Centres of the CGIAR System holding collections of Plant Genetic Resources for food and agriculture (PGRFA) signed Agreements with the Governing Body of the International Treaty on Plant Genetic Resources for Food and Agriculture (the Treaty) placing them in-trust collections of PGRFA within the purview of the Treaty. In accordance with these Agreements, all shipments of PGRFA of crops listed in Annex 1 to the Treaty (shipments of PGRFA under the Multilateral System) were subjected to the terms and conditions of the Standard Material Transfer Agreement (SMTA) adopted by the Governing Body of the International Treaty on Plant Genetic Resources for Food and Agriculture in June 2006. The CGIAR centres make more than 750,000 accessions available under the MLS (FAO 2019).

2.7 Summary

The key to sustainable agriculture is genetic material that is better adapted to withstand biotic and abiotic stresses. In order to address present and forthcoming threats to food and nutritional security, it is imperative to preserve the genetic

diversity that is especially crucial. One of the primary concerns, however, is the enormous rate of biodiversity loss, which threatens food and nutrition security, weakens the agricultural system's resilience, and jeopardises crop improvement. Hence, national and international genebanks that hold more than 7.5 million accessions of crops have been established in order to reduce the biodiversity loss caused by the replacement of landraces by improved cultivars. Efficient use of germplasm in crop improvement is depending on the availability of accession-level information on the traits of interest. Thus, core collection, mini-core collection, and FIGS approaches have been created to successfully find novel variations and trait recombinants. Molecular characterization, which includes activities like high-density genotyping, phylogenetic analysis, pan-genome analysis, etc., can be further combined with the selection of germplasm and the construction of subsets and mining novel alleles for use in traits improvement. Impact of germplasm for contributing to increased yield, adaptation, nutrition and improved health and sustainable agriculture have been demonstrated in many crops. There are many instances where a single plant genetic resource has proved to have large commercial value by conferring a specific trait. With the availability of new technologies such as high-throughput large-scale phenotypic assessment for key traits and use of omic tools could accelerate rapid identification of traits and genes for breeding improved cultivars.

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