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Conserving and characterizing the genetic diversity of grain legumes

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1 Introduction

Pulses or grain legumes are crops that belong to species in the *Fabaceae* family (Smykal et al., 2015). These include globally important pulses such as beans (*Phaseolus vulgaris*), chickpeas (*Cicer arietinum*), lentils (*Lens culinaris*), cowpeas (*Vigna unguiculata*), peas (*Pisum sativum*), faba beans (*Vicia faba*) and pigeon peas (*Cajanus cajan*). There are also more regional or local pulses such as grasspea (*Lathyrus sativus*), tepary beans (*Phaseolus acutifolius*), scarlet runner beans (*Phaseolus coccineus*), lima beans (*Phaseolus lunatus*), mung beans (*Vigna radiata*), adzuki beans (*Vigna angularis*), Bambara groundnuts (*Vigna subterranea*), black gram (*Vigna mungo*), moth bean (*Vigna acontifolia*), creole bean (*Vigna reflexopiloxa*) and rice bean (*Vigna umbellata*). All of these crops are mainly used in local diets for their high protein content. When they are grown, they contribute to soil health with their nitrogen-fixation properties due to their symbiotic relationship with nitrogen-fixing bacteria (Foyer et al., 2016). Groundnuts (*Arachis hypogaea*) and soybeans (*Glycine max*) are also grain legumes but not pulses, due to their high oil content as well as protein content. All of these grain legumes are critical components of sustainable cropping systems (Siddique et al., 2001), diversified protein balanced diets and the health of humans and soils (Foyer et al., 2016).

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The year 2016 was declared as the 'International year of pulses' to bring needed attention to this undervalued commodity (FAO, 2014b). These crops are prevalent in subsistent farming systems globally but are viewed as specialty crops in the broader agricultural and market context. FAO (2014b) concluded that from 1961 to 2012, there were massive gains in yield and production for maize, wheat, rice and soybean, from 200 to 800% growth versus only 54% for pulses. The pulses are made up of a variety of species that are grown in a wide range of environments globally. There is much less investment in international and national research on crop improvement, agronomic practices, as well as market or product development for pulses. Both the production and consumption have increased over time since 1961 but recently production and consumption have been slowing down due to competition with meat and milk as income increases. This slowdown does not predict a surplus of pulses in the future or a drop in demand since, in many of the consuming countries, the population is growing at a rate that exceeds farming output. Approximately 84% of the pulse production in the world is used to meet domestic consumption and only 16% is traded internationally. Countries like India and China are being forced to import pulses; thus international trade in pulses is expected to continue to grow much more rapidly than production.

Generally there is a need to increase the production and marketing of these crops, not only for dietary diversification and improved nutritional and health benefits but also for the sustainability of the future cropping systems and long-term soil health (Foyer et al., 2016). This will be a challenge given the impact of change in the climate in many of the current production areas, especially given the more local adaptation and use of these crops. One of the key resources for adapting to these challenges through crop improvement will be the genetic diversity conserved *ex situ* in gene banks and *in situ* with farmers or in protected areas. Thus, Foyer et al. (2016), Upadahyaya et al. (2011a) and Smykal et al. (2015) reviewed the status of the genetic resources of the grain legume crops currently conserved in some of the key gene banks. From these reviews it is clear that there is a need to secure the conservation of these genetic resources for use now and for future generations. Thus efforts being made to increase our characterization and exploitation of these genetic resources will be reviewed for chickpeas and pigeonpeas, as an example. The new approaches being taken for enhancing the use of these resources will also be considered.

2 Conservation of grain legume genetic resources: overview and global assessment of germ plasm collections

2.1 Overview

The global nature of grain legume production and consumption means that there is a global interdependency for food supply and genetic resources. This interdependency was recognized in the First and Second Global Plan of Actions for Plant Genetic Resources for Food and Agriculture (FAO, 1996, 2011a) and was the basis for the International Treaty for Plant Genetic Resources for Food and Agriculture. Khoury et al. (2015) assessed this interdependency based upon the nutritional value, productivity, food supplies and gross domestic product to determine the degree to which international exchange of plant genetic resources underpins national food supplies, production and economies. The assessment demonstrated that national food supplies and production systems are highly interdependent in relation to plant genetic resources. The result was consistent across all regions and continents, even in countries located in areas of high indigenous crop diversity. This interdependence has increased in the past 50 years due to globalization of food systems. Thus, a global system for the conservation of this diversity as a global public good is critical to ensure availability of and access to the resource for all future generations.

Gepts (2006) concluded that a number of technical and financial issues are key challenges in the future to *ex situ* conservation but also a clear long-term political agenda and commitment to these collections in the future. To maintain these commitments, a much greater effort is needed to raise public awareness of the value of these conserved resources and the needs for sustained conservation and use of these global public goods. The Global Crop Diversity Trust was established to secure the resources needed to ensure sustained support for the long-term conservation of *ex situ* collections forever, https://www.croptrust.org, and facilitate the development of a global system for *ex situ* conservation of the crop diversity the world depends upon for food security. In 2006, the Global Crop Diversity Trust facilitated the development of a global *ex situ* conservation strategy with the conservation and use community. The Second Global Plan of Action for PGRFA (FAO, 2011a) served as a framework for the actions needed to promote the development of an efficient system for the conservation and use of plant genetic resources for food and agriculture through better management, cooperation and coordination. The eighteen priority actions of the Global Plan of Action for PGRFA formed the elements of the global strategies for the key grain legumes.

The Global Conservation Strategy for *ex situ* conservation of chickpeas, lentils, lathyrus (grass pea) and faba bean was finalized in 2008 with the leadership of Dr Geoffrey Hawtin and the support of the GCDT and GRDC in Australia (Hawtin, 2008a,b,c,d). The Global Conservation Strategy for *ex situ* conservation of cowpeas was finalized in 2010 with the leadership of IITA and the support of the GCDT and GRDC in Australia (Dumet and Fatokun, 2011). A draft Global Conservation Strategy for *ex situ* conservation of pigeonpeas was finalized in 2011 with the leadership of ICRISAT and the support of the GCDT (Singh et al., 2011). A draft global conservation strategy has been developed for Phaseolus (bean) genetic resources with the leadership of Dr Daniel Debouck of CIAT, mainly based upon expert consultations and was supported by GCDT and GRDC in Australia (Debouck, 2014). All of these grain legume crops are listed in Annex I of International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) (FAO, 2009).

All of these strategies were the result of a survey of genebank curators and a consultation process with experts. Most of these global strategies are nearly ten years old, but when developed were seen as a dynamic process with a need for monitoring and updating. These strategies have been used as a guidance for the global community to ensure actions are taken to address the future needs for conservation and use in these crops. Global *ex situ* conservation strategies have not been developed for soybeans and groundnuts as well as for minor grain legumes, such as green gram, black gram, adzuki beans, Bambara groundnuts and among others. Some of these are not explicitly included in the multilateral system (FAO, 2009), so their global access and benefit sharing for exchange are not clear.

Frankel (1977) categorized crop genetic resources into six broad groups that include the wild relatives of crop species, landraces, genetic stocks, obsolete cultivars, modern cultivars and breeding lines. These genetic resources represent the gene pool potentially available to breeders and other users of collections. This broad pool can be further subdivided into primary, secondary and tertiary gene pools (Harlan and de Wet, 1971). The primary pool consists of the biological species, including cultivated, wild and weedy forms of the crop, and gene transfer within this group is considered to be easy. In the secondary gene pool are the species from which gene transfer is possible but difficult, while the tertiary gene pool is composed of species from which gene transfer is possible only with great difficulty. Clearly the boundaries on these groups are fuzzy and also change with technology. Consequently, Smartt (1984) have suggested the gene pools concept of Harlan and De Wet (1971) be modified to increase the number of gene pools from three to four to coincide with, respectively, populations, species, genera and tribes.

2.2 Global assessment of grain legume crop germ plasm collections

Smykal et al. (2015) did an extensive review of the phylogeny, crop evolution, domestication and status of conserved germ plasm of economical important species of the *Fabaceae* family. The review included an inventory of the key genetic resources conserved *ex situ*, cultivated as well as wild, for the grain and forage legumes. This inventory included the collections that are held in *ex situ* gene banks as well as in botanical gardens and herbariums. They also reviewed the status of *in situ* reserves, especially for the wild relatives.

Smykal et al. (2015) reported that there were an estimated 985 452 accessions of the major grain legumes held in *ex situ* collections globally due to the importance of grain legumes to many countries. One of the major sources of information on genebank holdings in Smykal et al. (2015) was public databases such as FAO-WIEWS (http://www.fao.org/wiews-archive/wiews.jsp) and Genesys (https://www.genesys-pgr.org). Foyer et al. (2016) also presented a systematic inventory of grain legume collections that had published accession-level information in publically available databases such as Genesys, the Chinese Crop Germ Plasm Information System, the National Institute of Agrobiological Sciences database in Japan and the National Bureau of Plant Genetic Resources database in India. In their assessment they also listed the main holding institutes with the percentage of the global accessions held. Both of these assessments are given in Table 1, although the number of accessions given in Foyer et al. (2016) was much less for the main grain legumes since they involved a smaller sample of the gene banks globally.

The most comprehensive inventory is given in FAO-WIEWS (2011b) but this is based on reporting from 2009. So for the assessment in Table 1, the inventory was updated for specific gene banks from up-to-date accession-level information given in Genesys. In Table 1, institutes who reported holding more than 100 accessions in FAO-WIEWS and Genesys were designated as holders of significant collections for eighteen major and minor legume crops. The total number of accessions held in significant collections was 970 064 or 94.2% of all the accessions reported in these two databases. The largest collections held were for crops such as soybeans, common beans, groundnuts and chickpeas. Overall these four crops account for 61% of all the accessions conserved globally *ex situ* for the major and minor legumes reported in Table 1. The smallest numbers of institutes and accessions were found for the local legumes crops such as Grasspea, tepary beans, scarlet runner beans, lima beans, Bambara groundnuts, black gram, adzuki beans and moth beans.

Major *ex situ* collections were defined as institutes that held more than 500 accessions (Table 1). The number of institutes who hold major collections ranged from 1 for Bambara groundnuts to 61 for common beans, while the proportion of the accessions held by

Table 1 The number of accessions cited in Smykal et al. (2015) and Foyer et al. (2016), the number of institutes and number or proportion of accessions held that reported in FAO-WIEWS and Genesys as holding significant major or the largest collections for eighteen major and minor legume crops

		FAO-WIEWS and Genesys					
	Cited in literature	Significant (>100 accessions)		Major (>500 accessions)		Largest (>1000 accessions)	
Crop	No. of accessions	No. of institutes	No. of accessions	No. of institutes	% of accessions	No. of institutes	% of accessions
Pea	98947[1]	56	71357	31	93.0%	24	85.8%
Chickpeas	98313[1]	41	96029	22	94.7%	11	86.3%
Faba beans	38000[1]	45	40514	21	85.6%	9	65.4%
Lentils	58407[1]	44	56188	21	88.5%	11	77.3%
Grasspea	38360[1]	17	15962	8	86.4%	5	70.7%
Common beans	261968[1]	103	190671	61	94.4%	41	87.9%
Tepary beans	1257[2]	4	809	0	0.0%	0	0.0%
Scarlet runner beans		14	4677	3	52.2%	0	0.0%
Lima beans	6420[2]	15	12961	7	89.3%	0	0.0%
Soybeans	156849[1]	92	206993	35	89.4%	20	79.2%
Cowpeas	65323[1]	52	72461	20	89.7%	13	82.6%
Bambara groundnuts	2183[2]	10	3878	1	52.4%	1	52.4%
Mung bean	23658[2]	29	42091	14	93.4%	9	84.9%
Black gram	1668[2]	11	7032	5	81.5%	2	52.2%
Adzuki beans	9978[2]	7	9500	3	90.4%	3	91.5%
Moth bean		3	3517	2	93.1%	2	93.1%
Pigeonpea	40820[1]	22	37828	7	92.3%	6	90.5%
Groundnuts	128435[1]	61	98136	27	87.4%	18	81.3%
Total	**1030586**		**970604**		**90.6%**		**80.8%**

[1] Smykal et al. (2015).

[2] Table 2 of Foyer et al. (2016).

the major collections ranged from 52.2% for scarlet runner beans to 94.7% for chickpeas. Overall crops, about 91% of the accessions are conserved in major collections. Five International gene banks (ICARDA, ICRISAT, CIAT, IITA and ILRI) and 72 national institutions conserve these major collections.

A large collection holder was defined as an institute that held more than 1000 accessions. Overall, about 81% of the accessions are conserved by the largest collections. There are six National Institutes who hold major collections of most of the grain legumes assessed in Table 1. These are the Australia Grains Genebank in Australia, CENARGEN in Brazil, ICS-CAAS in China, NBPGR in India, VIR in Russia and USDA-ARS in the United States. Institutes in Bulgaria, Chile, Ecuador, Ethiopia, France, Germany, Hungary, Indonesia, Iran, Japan, Mexico, Philippines, Ukraine and Zambia hold large collections of at least four of these grain legumes. Foyer et al. (2016) also assessed the proportion of the global accessions held in the main institutions that are included here.

The assessment in Table 1 is based solely on the size of the collections. This illustrates that the global system for these crops is made up of a few large collections that conserve the vast majority of the accessions in *ex situ* conservation. This does not infer that these larger collections hold the most important genetic diversity since many of the collections have accessions in common and are held outside the centre of origin for the crop. There may be a number of important small collections in terms of the unique nature of the accessions held. Foyer et al. (2016) concluded that several accessions are duplicated across collections but determining this based upon the inventory list is not easy. This assessment of the exact nature of the accessions held in the collections was done as part of the global conservation strategies and will be discussed later.

A key component of the global *ex situ* system will be a global linkage of all these substantial national, regional and international collections, especially those that contain significant numbers of unique accessions not duplicated elsewhere, such as the genetic stock and mutant collections described in Upadhyaya et al. (2011a). These unique genetic resources are held outside of the national gene banks by individual researchers, so one goal of any global system would be to locate those collections, determine their longer-term value, assess their risk status and, if necessary, safety duplicate these into more publically held collections.

3 Conservation of grain legume genetic resources: status of *ex situ* conservation of grain legume genetic resources

As part of the various global grain legume conservation strategy developments, a survey was distributed to curators of collections for chickpea, lentil, grasspea, faba bean, and cowpea to gather basic information on the numbers and types of accessions held, the conditions under which they were stored, the routine operations for maintenance of the accession, the accession-level documentation, safety duplication and their accessibility (Hawtin, 2008a,b,c,d; Dumet and Fatokun, 2011). No survey was done for the Phaseolus strategy or for any of the other crops given in Table 1. Upadhyaya et al. (2011a) also described these global strategies and concluded that the surveys provided valuable information on who holds what, how it is maintained and shared, the gaps in collections and the common issues faced by these collection holders. They described the baseline assessment that was made by the crop community of actions that needed to be taken forward.

In relation to the adequacy of storage facilities and conditions, gene banks generally have adequate storage facilities for medium- and long-term storage. In general, the larger seed size of many of the legumes results in the need for more space for long-term storage and this can significantly affect the cost for conservation. For cowpeas in gene banks in Africa, there are significant constraints to storage due to the lack of consistent electricity supplies.

Grain legumes have evolved various physiological and physical processes to manage the timing of germination in their natural environments and many of these have gone through modification from selection during domestication (Kigel et al., 2015). A review of the nature of physiological and physical dormancy in legumes is given in Kigel et al. (2015). In general, one of the key issues for conservation is the impact of seed coat impermeability or seed hardness on seed viability monitoring. For some legumes, such as lentils, the low seed moisture that is recommended for long-term storage can result in greater seed hardness. The seed coat can be fragile in some of the crops and this will increase the possibility of damage during post-harvest seed handling. This damage reduces viability and also increases the risk of pathogen infestation. The high oil content of crops like groundnuts and soybeans can impact the long-term viability of these seeds. Seed health is a greater challenge for grain legumes than cereals (Girish et al., 2001), and not just from the risk of pathogen attack with the seed coat damage. Grain legumes suffer more from seed-borne pathogens and viruses than cereals. Thus, the longer-term impact of these challenges to conservation is the difficulty in determining the timing and methodology for the routine monitoring of seed viability; the larger packaging and greater space requirement for seed storage; the lower seed longevity that requires more frequent regeneration and the increased risk of seed health issues for regeneration, distribution and seed longevity. The only crop for which the survey asked for information on the seed viability or germ plasm health monitoring was cowpeas. Many of the gene banks surveyed for cowpeas did not do an initial germination test after harvest, nor did they do routine monitoring of germination of the stored seeds. There was also very little routine monitoring of seed health.

There are established international standards for the conservation of orthodox seeds such as grain legumes (FAO, 2014a). In the future, to assure the security of the conservation of grain legume accessions, it will be necessary to implement quality-management systems for some of these key routine processes such as seed viability testing, germ plasm health testing, regeneration and accession-level documentation. The respondents for the cowpea survey concluded the main constraints to *ex situ* conservation in Africa collections were the unreliability of electricity supply, the lack of appropriate storage containers, high humidity storage conditions, prevalence of biotic stresses and sustainability of funding for routine operations of the gene banks. Over all gene banks, problems in maintaining standards arise because of lack of sufficient and consistent annual operating budgets to meet the costs of routine operations such as regeneration, characterization and documentation, especially in developing countries. This lack of funding can lead to a number of outstanding issues including:

- Backlogs in regeneration, which is threatening the viability and safety of a significant number of genebank accessions (summary of status of respondents given in Table 2)
- Formal arrangements for safety duplication of accessions which is also contributing to the threat to the safety of unique accessions (summary of respondents given in Table 2)

Table 2 The number of respondents and the key results of the surveys completed as part of the development of the global *ex situ* conservation strategies for chickpea, lentil, grasspea, faba bean and cowpea

Crop	No. of respondents	Quality of collections	Regeneration backlog	Safety duplication
Chickpea	21	12 institutions with more than 50% landraces. 9 institutions have 1–4% wild species while 1 has more than 20% wild species. 6 institutions have more than 50% of the accession collected from within the country	Significant regeneration needs for 8 countries who responded	14 collections indicate more than 40% safety duplicated but not all to site outside country. Some very important collections like NBPGR, Turkey, Nepal and Ethiopia have no or very little safety duplication
Lentil	19	13 institutes with more than 50% landraces, 8 institutes with wild accessions. 7 institutes with accession mainly collected from within the country	Important collections in Iran, Russia, Chile, Turkey, Syria, Hungary, China, Pakistan, Bangladesh, Ukraine, Nepal, Morocco, Slovakia and Mexico have significant backlogs	10 institutions with adequate safety duplication but many important collection like those in NBPGR, Turkey, Spain and Ethiopia have no safety duplication
Grasspea	13	NBPGR, VIR, Ethiopia, Nepal, Portugal, Spain and Turkey hold majority of their accessions of national origin	7 institutes have more than 50% regeneration needs	The strategy came up with action plan to securely safety duplicate the unique accessions
Faba bean	29	20 institutions have majority landraces, 15 institutions have wild relatives, 17 collections with majority from original country, large number of collections with unique locally adapted germ plasm	Adequate information on regeneration backlog missing for many collections, 12 institutions have more than 50% regeneration needs	10 institute with more than 50% safety duplicated. Many important collections are not safety duplicated and very few with adequate formal arrangements
Cowpea	15	Quality was assessed based upon age of collection and 13 holding diverse species and overall 60% landraces and 2% wild species	Backlog not assessed	8 institutes with no safety duplication

- Inability to provide seed and information of accessions on request
- Lack of passport, characterization and other documentation of accessions in collections.

The long-term conservation and distribution of accessions requires that seeds are periodically screened for viability, and when viability falls below an internationally accepted level, the accession is regenerated and fresh seed is placed in the genebank. Regeneration is a relatively expensive procedure and is often one of the first activities postponed when funds supporting genebank activities decline. It is clear that regeneration of accessions is probably the single greatest threat to the safety of grain legume accessions held in globally important gene banks. Regeneration needs are generally greater in developing countries but there are even backlogs in some major developed countries. In the various surveys given in Table 2, there were significant regeneration backlogs, and some of the main collections with unique, local accessions had not responded to the survey question, such as for lentils. From 2008 to 2012, the Global Crop Diversity Trust managed a large-scale effort to address the urgent regeneration need for about 80 000 accessions of crops held in 77 national collections (https://www.croptrust.org/what-we-do/supporting-the-global-system/rescuing-diversity). The accessions to be regenerated were identified with the finding of the crop strategies.

The faba bean strategy concluded that there is a need to conduct a survey of regeneration protocols globally to ensure security. Upadhyaya et al. (2011a) reviewed the few studies that had been done on allelic changes in an accession that had undergone periodic regeneration. They concluded that these studies did imply that there is a need to review and improve regeneration protocols to accommodate larger population sizes as well as monitor the genetic integrity of the accession over time to reduce the risk of erosion.

Another major deficiency identified in this survey was the lack of safety duplication of many important collections (Table 2). Safety duplication refers to the need for duplicate samples of accessions in a collection to be maintained in more than one genebank as a form of insurance against disastrous loss (for example, due to fire, earthquake or war). 'Black box' duplication – whereby the genebank of origin is responsible for the quality of the stored samples and their regeneration, and the recipient genebank merely holds the samples in long-term storage – is considered the most convenient and cost-effective method of safety duplication. Duplication of accessions for safety is a requirement of agreed international standards for genebank management. Yet, there are many globally important collections that do not have unique accessions duplicated elsewhere for safety, for example, in key collections for chickpeas and lentils. In the strategy for grasspea, an action plan was developed to safety duplicate unique accessions in some of the largest gene banks. Clearly, this is a major area of concern, and full safety duplication, of at least unique accessions, must be the goal of an efficient rationalized global system of collections. The lack of safety duplication is linked, in part, to the need for urgent regeneration of samples in many collections. Curators sensibly see no point in sending seed of accessions that have low viability to duplicate black box storage. Consequently, these two safety issues, the regeneration of accessions and the safety duplication, need to be considered simultaneously with samples of newly regenerated seed also used for safety duplication.

The inability of collections to distribute accessions upon request can be due to the lack of availability of the seed. Availability of an accession for distribution is related to the viability of the seed, its health and its legal status. National governmental phytosanitary regulations can significantly hamper germ plasm exchange. This is related to the plant and seed health. The legal status of an accession is related to accessibility under either the internationally agreed terms of access and benefit sharing provided for in the multilateral system as set out in the SMTA by the International Treaty on Plant Genetic Resources for Food and Agriculture (FAO, 2009), by national or local regulations, or term-limited material transfer agreements (MTA) or patents. The conditions for distribution, use and benefit sharing should be made available to potential users. As the multilateral system expands, the access to many major collections should improve, but realistically, some collections or accessions may continue to be governed by local regulations, or by term-limited MTA or patents, and not by the SMTA of the ITPGRFA. Collection holders should make more of the accessions available in the multilateral system as the implementation of the ITPGRFA expands. In the survey on faba beans and cowpeas, the respondents were asked to give more details on distributions; domestic versus international users, type of user, farmer versus researcher; and type of agreement used, SMTA versus institutional MTA. ICARDA, IITA, USDA and European gene banks did most of the international distributions. Farmers were significant recipients of accession from the national gene banks.

It is clear from the surveys of collections that there is a great variation in the systems used to record, store and distribute information. A major step in developing an integrated global accession-level information system is to ensure adequate information storage and retrieval infrastructure and to rectify the deficiencies in the documentation of accession in collections. This will involve, at a minimum, to improve the accession-level documentation of collections to enhance accessibility and as a tool to assist in the management and rationalization of collections, using GRIN-Global or other genebank management systems; utilize globally agreed protocols for data ontology and quality; and publish all key accession-level data, such as passport, characterization, evaluation and other relevant data from individual collections to global portals, such as Genesys, to allow for easy access by all collection holders and the collections' user clientele.

A global portal to publish and share accession-level information exist through Genesys (https://www.genesys-pgr.org). Currently, the main contributors of accession-level information in Genesys are the CGIAR international gene banks, USDA-ARS in the United States, the Australian Grains Genebank, and the national institutes in European countries who make their data available through EURISCO (http://eurisco.ipk-gatersleben.de). For example, this global portal currently host 100 239 accessions of common beans that are held mainly by CIAT, USDA-ARS in the United States, IPK in Germany and VIR in Russia. These three institutions hold about 60% of the accessions reported in Genesys. The accessions in Genesys are 66% landraces or farmers varieties. The information on the accession includes collection management such as safety duplication, availability and germ plasm storage type. It also includes passport and characterization data but the quality of this information varies. The quality of the passport information is scored for each holding institute using the passport data completeness index (PDCI) as described by van Hintum et al. (2011). For example, the collection held by the CIAT has an average PDCI of 4.51.

The development of genebank management software packages, like GRIN-Global, will facilitate the secure conservation and use of the accession held globally (http://www.grin-global.org/index.php/Main_Page). This system can be used by an individual genebank to capture a diversity of accession-level information as well as enhance the management of the collection. It allows for automation of data collection and of monitoring. It allows for greater sharing of this information internally and externally. It would facilitate the publishing of accession-level information from individual gene banks to an integrated web-based system like Genesys that allows the searching of the databases of all cooperating gene banks. This has clearly been one of the major challenges facing the development of an integrated global conservation system for grain legume genetic resources.

4 Conservation of grain legume genetic resources: *ex situ* collections of grain legumes

4.1 Composition of accession held in *ex situ* collections of grain legumes

In most of the surveys and in the consultation workshop, an assessment was made for the quality of the collections based on the type of accessions conserved (wild species, landraces, breeding material or others) as well as the source of the accessions (collected within country, outside country, introduced from others or other sources). A summary of the quality of the collections, based on the type and source of accessions, is also given in Table 2. Cowpeas used a different approach to assessing the quality of collections. This analysis identified key collections that hold a high percentage of unique local accessions.

For example in chickpeas (Hawtin, 2008a), NBPGR in India reported that their accessions were 1% wild species and 65% landraces or farmer's varieties and 89% were collected from within India. PGRI in Pakistan had 1% wild species and 98% landraces with 70% of the accession collected from within Pakistan. AARI in Turkey had 1% wild relatives and 98% landraces but 100% of the accession in their collections was originally collected from within Turkey. ABD in Nepal has a smaller collection but 100% is landraces and 97% collected from Nepal. BCRI in Ethiopia has 75% landraces with 71% of the accessions collected from Ethiopia. Even CRF-INIA in Spain had 1% wild species, and 60% landrace with 58% of the accession collected from Spain. Thus for chickpeas, these collections would be considered as important in terms of the conservation of unique local diversity. The same Institutions in India, Turkey, Ethiopia, Nepal and Spain held key collections of lentils (Hawtin, 2008d). In both crops, VIR in Russia held a collection with more than 90% landraces but almost 95% of these were collected from outside Russia. In their case, given the age of the collections, there is a high probability that these are unique accessions.

For grasspea and faba beans, the local nature of these fairly minor grain legume can be seen in the high proportion of collections that held mainly landraces collected within the country (Hawtin, 2008b,c). There has been little breeding effort in this crop and not that much germ plasm exchange so the national origin of most collections would have been expected. The ICRISAT, ICARDA and IITA collections for all of these crops have a high percentage of landraces but these have been assembled and collected from many countries. They also hold a higher percentage of breeding material than the national gene banks.

4.2 Redundancies in *ex situ* collections of grain legumes

The task of rationalizing collections and identifying duplicates is difficult and time consuming. Over time, a variety of names and numbering systems have been used for the same accessions in different gene banks. Single plant selections can be made from an accession and given new accession numbers. Landraces may have been collected from several geographically distinct sites and represent genetically different samples but may have the same name. Many accessions have limited passport data available that makes the determination of their origin and uniqueness difficult. Hazenkamp et al. (2014) discussed the use of a central crop registry that was developed using the main wheat and rice collections. They compiled accession-level information on passport data for the collections, established standardized data fields for comparison, assessed similarity of accession within and amongst collections, and feedback the results to the institutes to improve data quality. They described a methodology to assess similarities in collections using this crop registry of accessions. For wheat in the CIMMYT, USDA-ARS and ICARDA collections, they found over 50% of the similarities were direct donor–recipient relationships or due to the subdivision of an accession into further new accession based on single plant selection.

The crop registries could be expanded to include other crops to gain a much better understanding of the duplicates within and between collections globally. This is a key first step in rationalization and it will empower curators to identify unique and duplicated accessions that are held in other collections. Curators can make decision about reducing redundancy and negotiate with other gene banks to insure safety duplication. It will allow them to focus on the conservation of the unique accessions and establish safety duplication for these. Upadhyaya et al. (2011a) concluded that the development of crop registries was a recurrent recommendation in the grain legumes conservation strategies. They indicated that ICRISAT was developing a registry for chickpeas.

The European Cooperative Programme for Plant Genetic Resources (ECPGR) involves 43 countries that aim to facilitate long-term conservation on a cooperative basis and to increase utilization (http://www.ecpgr.cgiar.org). AEGIS was established by ECPGR to create a European-wide integrated system to securely conserve unique and important accessions for Europe and make them available (Engels and Maggioni, 2011). This effort would lead to increased security of long-term conservation with a more systematic safety duplication, facilitated access, improved quality standards for conservation, cost-efficient conservation, reduced redundancies and improved information sharing from the greater collaboration of European collection holders. So far 34 countries have become member of AEGIS (http://www.ecpgr.cgiar.org/aegis/aegis-homepage). For example, within the European collection, there are currently 124 accessions from *Phaseolus* and 527 accessions from *Pisum*.

It must be noted, however, that the identification of genetic duplicates within and between collections is not a trivial exercise. Genetic drift within accessions due to out-crossing, founder effect from restricted sampling, genotypes by environment interactions, unrecognized selection pressures and mechanical mixtures have been observed. Upadhaya et al. (2011a), Smykal et al. (2015) and Foyer et al. (2016) concluded that genotyping collections with greater genomic saturation and precision and with reduced per-sample costs will aid in the characterization of genetic diversity, both unique and common. It can be used to detect redundancies within and between accessions in collections. It will also help in the monitoring and management of genetic integrity of the accessions during regeneration. It will also be a tool to enhance the use of accessions.

4.3 Gaps in *ex situ* collections of grain legumes

An important aim for the global grain legume conservation strategy was to identify gaps in existing collections. For most of the crops, further collection of landraces that were likely to still exist in the field in areas that are poorly represented in gene banks were identified as priority areas. For example, the chickpea global conservation strategy identified key under-represented areas for landraces in the Hindu Kush-Himalayan region, the west and north of China, Ethiopia for desi chickpeas, Uzbekistan, Armenia and Georgia (Hawtin, 2008a). Collecting priorities should be decided in conjunction with national genebank and experts who have knowledge of the local material.

Though the collections of grain legumes at ICRISAT genebank are large, they cannot be considered as complete. Critical assessment of grain legume collections revealed several potential gaps. The genebank at ICRISAT conserves more than 20 600 accessions of chickpea from 59 countries. Major chickpea-growing countries were explored during collection missions launched by ICRISAT for its mandate crops during 1974–1993. Russia and CIS, Colombia, Peru, Western Nepal, Eritrea and Myanmar were considered as the geographical gaps in cultivated chickpea collection. Singh et al. (1997) identified geographical gaps for wild species in the ICRISAT collection from Russia and CIS, Turkey, Afghanistan, Iraq and Ethiopia. The Mediterranean region, which is one of the two primary centres of diversity, contributed only 1240 accessions (7.4%) and was under-represented. Ethiopia, which is the secondary centre of diversity, was also under-represented with only 928 accessions (Upadhyaya, 2003).

The pigeonpea germ plasm collection at ICRISAT genebank consists of more than 13 700 accessions from 74 countries. Germ plasm collection has covered the major geographic areas of importance. However, West Africa and SouthEast Asia (Philippines, Indonesia, Thailand, Malaysia and China) and to some extent Latin America are not yet covered by collections (Singh et al., 2006). The areas that are yet to be surveyed for pigeonpea collection in India include Uttar Pradesh and Uttaranchal (districts of Mirzapur, Bundelkhand region, Tarai), Madhya Pradesh (Rewa, Sidhi, Gwalior, Morena, Bhind region), Maharashtra (Nasik, Dhule) and adjoining North Karnataka, southeastern districts of Bihar, parts of Orissa, Rajasthan, Gujarat, Nilgiri and Ragan hills and the northeast region. In Uganda, emphasis would be in eastern and northern parts of the country. The focus for the future should be more on trait-based collections and wild species (Singh et al., 2006).

Results of studies using passport and characterization data and GIS software such as FloraMap and ArcGIS revealed several geographical and trait-diversity gaps in the pigeonpea germ plasm from East and Southern Africa (Upadhyaya et al., 2015). A total of 84 districts located in 35 provinces of four East African countries and 54 districts located in 18 provinces of three Southern African countries were found as the important geographical gaps. Five districts of three provinces in Ethiopia, 12 districts of three provinces in Kenya, 37 districts of 14 provinces in Tanzania, 30 districts of 15 provinces in Uganda, six districts of three provinces in Malawi, 28 districts of eight provinces in Mozambique and 20 districts of seven provinces in Zambia were identified as the geographical gaps in the collections from ESA countries. Eight districts in Eastern, Central and Coast provinces in Kenya were identified as gaps in phenotypic diversity for all traits. A total of 22 districts located in five countries were identified as gaps in diversity for one or the other trait (Upadhyaya et al., 2015).

Collection of wild species has been given less emphasis than the collection of land races in the past, as they were considered to be under less threat given their unaided survival and reproduction.However, there is a need to substantially increase the genetic diversity of the wild species conserved *ex situ*. More and more wild species are being threatened by changing patterns of land use and climate change. There is also an increased demand for readily available samples of wild species for researchers and breeders. Table 2 indicates that few institutions hold a significant number of wild accessions. Singh et al. (2014) reviewed the collections of the wild *Lens* held globally. From their assessment, they identified key gaps where there is an urgent need to collect and protect the populations *in situ*.

Upadhyaya et al. (2011b) studied the occurrence of *C. scarabaeoides* near the sites of origin for pigeonpea. They reported a total of 790 districts located in 118 provinces in Bangladesh, Cambodia, India, Indonesia, Laos, Malaysia, Myanmar, Nepal, Papua New Guinea, Philippines, Thailand and Vietnam as geographical gaps in the collection. Among wild species, those endemic to Australia are inadequately represented in the global collection. The species *C. cinerius, C. confertiflorus*, *C. lanceolatus*, *C. marmoratus* and *C. latisepalus*, which were considered to be important for their drought tolerance, are yet to be collected. Therefore, under- and unexplored districts for cultivated and wild species of pigeonpea should be the target of future collection missions for pigeonpea germ plasm.

The conservation of genetic and mutant stocks is important to ensure that this historic research legacy is not lost and that this significant gap in collections is addressed (Upadhyaya et al., 2011a). Obsolete and new cultivars are often more rigorously characterized and broadly evaluated due to release practices and regulations, resulting in a higher short-term 'value' for immediate, direct use in grain legume breeding programmes. In many cases these are not conserved for the long term, but as more data, such as phenotyping, genotyping and nutritional profiles, are generated from this germ plasm, there is a need to fill this conservation gap for the future users.

5 Global *ex situ* conservation strategy for grain legumes

The global conservation strategies concluded that the current system for *ex situ* conservation of grain legumes is not global but consists of poorly linked international and national collections which hold some unknown degree of common and unique accessions that are conserved with limited sustained support and are not very well known or available to users. Therefore, this global system should ensure availability of these genetic resources for future generation users so that they will be able to resolve new challenges of production. However, since the current system does not meet the need of users, nor is it able to take advantage of new technologies and tools to enhance its use, a new model for managing global resources in a secure, cost-effective, sustainable global system will need to be adopted to benefit research, production and consumption (Bramel, 2017).

Bramel (2017) recognized some basic principles for this global conservation and use system. There is a need to ensure secure conservation with quality-management system and safety backups. Viable, healthy, fully documented accessions, including the information, should be available to users in a timely manner upon request. A rational, cost-effective global system should have centralization of some specialized activities, such as international distribution or safety duplication, and decentralization of a few other activities at the regional or national level, such as local regeneration, distributions, evaluations, plant breeding and outreach to farmers. A sustainable global system should have multiple types of collections such as international or global collections, national or local collections, specialized collections for wild relatives or genetic stocks, and research collection from breeders. Collections should be linked to better meet user needs to ensure that the genetic diversity conserved is appropriate with minimum redundancies to reduce collection size and complexity. The documentation on accessions that are shared in an integrated system should include passport, characterization, evaluation and genomic data. Users should be able to access this information from a global platform that integrates accession-level data from individual collections into a single portal with tools to enhance search and selection. Finally, a legal framework that facilitates access and the sharing of benefits should be used, such as a fully functioning multilateral system under the ITPGRFA.

Each institution holding a collection in this global system should have primary responsibility for the long-term conservation of the germ plasm that is unique to their collection. The institution may choose to outsource some of the functions associated with the long-term conservation of their unique accessions (e.g. regeneration), but those decisions remain their own responsibility. All the unique accessions would be conserved and managed in accordance with agreed international scientific and technical standards utilizing a quality-management system. All the unique accessions would also be 'black box' safety duplicated in at least one other gene bank to avoid loss due to mishap or catastrophe. Complete passport and characterization data would be available for all the accessions and accessible online. The databases of all the collections in the network would be linked through an integrated information platform that would allow public web access.

The global conservation strategies for chickpea, lentil, grasspea and faba bean describe a global approach to conserving the genepool. They recognized that gene banks differed in the degree of genetic variation they conserved for the global community (quality and quantity of their accessions), the status of the routine operations in terms of meeting international standards, the international availability of the accession to users and the completeness of accession-level information that is shared globally. Based on these criteria they classified gene banks into three groups that would take on different roles in the global systems for *ex situ* conservation. These groups consisted of gene banks that meet international standards and make their accession available globally; gene banks that need to upgrade their operations to better meet the conservation and user needs locally; and gene banks that should be supported to safety duplicate their unique accession within the gene banks of the first two groups. Many of the gene banks in the last group were found in countries where the crop had limited production or where the information on the collections was not well known. In all cases, there was a recognition of the need for all gene banks to share accession-level information at the global level in order to facilitate the global system collaboration.

There is an example of an integrated regional genebank system for cooperation and collaboration in the conservation and use of grain legume genetic resources. ECPGR is a collaborative programme among most European countries that aims at contributing to a rational and effective conservation of plant genetic resources and facilitates their utilization in Europe (http://www.ecpgr.cgiar.org). In Europe, an ECPGR Working Group on Grain Legumes was established in 1991 (http://www.ecpgr.cgiar.org/working-groups/grain-legumes/). Since its inception, the Working Group has covered a range of species (*Arachis*, *Cicer*, *Glycine max*, *Lens*, *Lupinus*, *Phaseolus*, *Pisum*, *Vicia faba* and *Vigna*). Despite the large number of crops, countries and collections involved, ECPGR provides a very useful model and source of experience for global grain legume conservation.

The secure conservation of grain legume genetic resources will also depend upon secure annual resources. The mission of the Global Crop Diversity Trust is to both facilitate the development of this global system for wheat and support the long-term conservation of the most important global collections (https://www.croptrust.org). Consistent support of the routine conservation needs for these global collections is too important to be left to the vagaries of the annual funding cycle of international donors or national governments. Thus a Crop Diversity Trust fund has been established not only to generate annual income to maintain these collections at agreed standards but also to ensure the long-term availability, security and use of these collections. Currently, the Crop Trust supports part of the routine operating cost for the long-term conservation of grain legumes at CIAT, ICRISAT, ICARDA and IITA.

6 Characterizing the genetic diversity of chickpeas and pigeonpeas

The use of PGR in crop improvement is one of the most sustainable ways to conserve valuable genetic resources for the present and future use in crop improvement programmes (McCouch et al., 2013). Extensive use of fewer and closely related parents in crop improvement programmes could result in new cultivars with increased vulnerability to pests and diseases. Breeders prefer to use their own working collections including mostly improved materials (Nass and Paterniani, 2000). They are often reluctant to use wild species and landraces available in gene banks, thereby increasing the gap between available genetic resources and their use in breeding programmes (Marshall, 1989). The reasons for the low use of germ plasm include i) lack of accurate and precise large-scale multilocation evaluation data of germ plasm; ii) lack of rational systematic entry points into the vast international collections and iii) lack of robust, cost-effective tools to facilitate the efficient utilization of germ plasm in crop breeding (Dwivedi et al., 2009; Upadhyaya et al., 2011a). The identification of useful germ plasm for breeding programmes is a basic need to enhance the use of conserved accessions. This could be achieved by two approaches: (i) identifying regions from where germ plasm that has a higher mean for desired traits as well as genetic diversity and (ii) developing and systematically evaluating representative core collections of legumes.

6.1 Diversity in *ex situ* collections

Studies on phenotypic diversity in germ plasm collections are very limited. Studies at ICRISAT revealed a wide variation in the germ plasm collections of chickpeas and pigeonpeas for morphological, agronomic and nutritional traits as well as stress resistance. Narayan and Macefield (1976) assessed the diversity of a large collection of 5466 accessions. Moreno and Cubero (1978) studied 150 accessions from five geographical regions. Murthy (1975) used 459 accessions from 16 countries. At ICRISAT, an assessment was made for the entire collection of chickpea, covering 16 820 accessions from 43 countries that had been characterized for seven morphological and 13 agronomic traits and wilt reaction during 1974–1998 (IBPGR, ICRISAT and ICARDA, 1993; Upadhyaya, 2003). The ICRISAT collection has a high representation from India in South Asia with (7174 accessions) and Iran (4838 accessions) in West Asia. The Mediterranean region, which is one of the two primary centres of diversity, contributed only 1240 accessions (7.4%) and was under-represented in the collection at ICRISAT. Ethiopia, which is the secondary centre of diversity, was also under-represented (928 accessions) although this was 83% of the accession from the Africa region.

None of the morphological descriptors was monomorphic and most showed at least two relatively frequent phenotypic classes. The kabuli types that are characterized by no anthocyanin, white flower, owl's head seed shape, a smooth seed surface and beige seeds were more frequent in the collection from East Asia, the Mediterranean region and Europe (Upadhyaya, 2003). Desi-type accessions with pink flowers, brown or yellow-brown seeds, angular seed shape and rough seed surface were abundant in South Asia, Southeast Asia and Africa. Erect and spreading growth habits were found in high frequencies in East Asia. Semi-erect and semi-spreading growth habits were evenly distributed in South Asia, whereas in the rest of the regions, except South-East Asia, semi-erect accessions were predominant. Significant differences were observed among regions for all 13 agronomic traits (Upadhyaya, 2003). Accessions from Africa were early to flower (54.4 days), while those from South-East Asia had a later maturity (109.7 days). On the other hand, collections from East Asia were late to flower (70.1 days) and those from Europe were late to mature (124.6 days), produced the highest number of pods per plant (53.2) and larger seeds (25.7 g 100 seeds^{-1}), and had the highest plot yield (1469 kg ha^{-1}).

Significant variability for most of the traits in all the 25 years was maintained to the ICRISAT chickpea collection (Upadhyaya, 2003). The variances among regions were heterogeneous for all the 13 agronomic traits. East Asia had the highest variances for days to 50% flowering, plant height, plant width and days to maturity; Africa for apical primary branches, pods per plant and seeds per pod and Europe for basal primary branches and basal secondary branches, apical secondary branches, and plot yield. West Asia region had the highest pooled Shannon-Weaver diversity index (H`) for morphological (0.39 ± 0.070) and agronomic traits (0.57 ± 0.012) (Upadhyaya, 2003). Seed colour (0.62 ± 0.083) among morphological traits and days to 50% flowering (0.58 ± 0.025) among agronomic traits had the highest pooled H`. Over all the 21 traits, West Asia (0.50 ± 0.033) had the highest H` and South-East Asia the least pooled H` (0.31 ± 0.035). This may be because the southwest Asia, which is one of the primary centres of diversity, was classified as West Asia.

A hierarchical cluster analysis conducted on the first three principal component scores (total variation accounted for was 91%) resulted in two clusters (Upadhyaya, 2003). Africa, South Asia and South-East Asia grouped together to form Cluster 1 and the Americas, Europe, West Asia, Mediterranean region, and East Asia formed Cluster 2. The accessions from Cluster 1 were predominantly of desi type with a low 100-seed weight, whereas those from Cluster 2 were predominantly of kabuli type with a high 100-seed weight. Accessions in Cluster 2 took more days to flower and mature with taller plants, more tertiary branches and higher 100-seed weight than the accessions in Cluster 1. Accessions in Cluster 1 had wider plants, more basal primary branches, apical primary branches, basal secondary branches, apical secondary branches, pods per plant, seeds per pod and higher plot yield than in Cluster 2. Accessions resistant to Fusarium wilt were available from all regions but their proportion was slightly higher amongst the accessions from South Asia, South-East Asia and West Asia. This may be due to the fact that while Fusarium wilt is a widespread disease in large chickpea-growing countries (Nene et al., 1991), it is a more serious disease in these three regions (Nene and Reddy, 1987).

The reports on pigeonpea germ plasm characterization are not exhaustive and mainly focused on smaller germ plasm sets. For example, 320 accessions were characterized for five traits by Dhari et al. (1997), 168 accessions for 20 traits by Satpute et al. (1994), 75 accessions for seven traits by Singh et al. (2002), 67 accessions for 10 traits by Hazarika et al. (1986) and 40 accessions for 11 traits by Kyi et al. (2001). The ICRISAT genebank conserves 13 778 pigeonpea germ plasm accessions from 74 countries. Studies using

11402 accessions characterized during 1976–2001 revealed a large variation in the collection for 14 qualitative and 12 quantitative traits (Upadhyaya et al., 2007). Pigeonpea is an often cross-pollinated crop with an average out-crossing of 13.1% and range of 9.7–24.1% (Githiri et al., 1991). Therefore a lot of heterogeneity (more than one descriptor state occurring in an accession) exists in the accession that have been collected from the farmers' fields. This genetic variability within accessions has been maintained, but the variance component amongst accessions is still significant for most of the traits in all the 22 years of germ plasm characterization, indicating that over the years significant diversity has been maintained in the germ plasm collection.

The accessions were grouped based on geographical proximity and similarity of the climate, and this grouping revealed a large variation for the region of South India, Maldives and Sri Lanka but minimum variation for Europe and Oceania (Reddy et al., 2005; Upadhyaya et al., 2005). Accessions from Oceania were earliest to flower (121 days) and mature (179 days) with short stature (138 cm), whereas those from Africa took the maximum number of days for flowering (162 days) and maturity (226 days) and were tall (203 cm). The number of primary branches was the highest in accessions from America (14.84) and Europe (14.70), while the accessions from South India, Maldives and Sri Lanka produced more secondary branches and racemes and had high harvest index, shelling percentage and seed yield (76.87 g per plant).

Shannon-Weaver diversity index (H`) (Shannon and Weaver, 1949) indicated that the accessions from Indonesia, Philippines and Thailand had the highest pooled H` for qualitative traits (0.349 + 0.059) and accessions from Africa for quantitative traits (0.613 + 0.006) (Upadhyaya et al., 2005). African accessions also had the highest pooled H` (0.464 + 0.039) over all the traits, while those from Oceania had the lowest pooled H` (0.337 + 0.037).

A hierarchical cluster analysis conducted on the first three PC scores (92.28% variation) resulted in three clusters (Upadhyaya et al., 2005). Semi-spreading growth habit, green stem colour, indeterminate flowering pattern and yellow flower were predominant among the qualitative traits. Primary seed colour had maximum variability, with orange colour followed by cream being two of the most frequent secondary colours in the collection.

6.2 Developing core and mini core collections

Reduced subsets, such as core collections (Frankel, 1984), that represent a high proportion of the diversity of the entire collection of a given species have been suggested as an approach to enhance the utilization of germ plasm in crop improvement programmes. Reddy et al. (2005) and Upadhyaya et al. (2001) have developed core collections of the ICRISAT crops, including chickpea and pigeonpea. When the entire collection is very large, even a 10% core collection is still unwieldy for meaningful evaluation and convenient exploitation. Upadhyaya and Ortiz (2001) postulated the concept of the mini core collection and described a two-stage strategy to develop the mini core collection. The first stage involves developing a representative core collection (about 10%) from the entire collection using all the available information, such as origin, geographical distribution and characterization data of accessions. The second stage involves an evaluation of the core collection for various morphological, agronomic and quality traits, and selection of a further subset of about 10% accessions of the original core collection. Thus the mini core collection will contain 10% of the core, or ~1% of entire collection, that represents the diversity of the entire collection (Upadhyaya and Ortiz, 2001). Upadhyaya and Ortiz (2001), Upadhyaya et al. (2006c, 2010) and Upadhyaya (2015) reported on mini core collections of chickpea and pigeonpea. A summary of these efforts is given in Table 3.

Mini core collections developed for the ICRISAT genebank serve as a gateway for accessing the genetic diversity of the entire collection by the global research community. The mini core collections are a resource for in-depth evaluation of the diversity within a collection for increased use of accessions in crop improvement programmes. So far, the genebank has provided 296 sets of mini core collections including those of chickpea and pigeonpea to scientists in 36 countries and 115 sets to scientists at ICRISAT for the evaluation and identification of promising accessions. Extensive multi-discipline evaluation of mini core collections identified new promising sources of resistance to biotic stresses, abiotic stresses, nutritional traits and grain yield in chickpea (Gaur et al., 2013; Upadhyaya et al., 2013a) and pigeonpea (Sharma et al., 2012; Upadhyaya et al., 2006a; Upadhyaya, 2015) for use in crop improvement programmes.

Some of the chickpea mini core accessions showed multiple resistances to abiotic and biotic stresses but only a few had good agronomic and seed-quality traits (Upadhyaya et al., 2013a). Early maturity and seed size are agronomically important traits, and accessions with early maturity and large seed size have been reported in chickpea (Upadhyaya et al., 2006b). In contrast, a number of accessions had no resistance to abiotic or biotic stresses but were agronomically superior and adapted to diverse environments. Both of these accession types would be a good parent in breeding programmes (Upadhyaya et al., 2013a).

More recently, large variability was observed among chickpea mini core accessions for oligosaccharides such as sucrose (3.6–54.1 mg g^{-1}), raffinose (0.2–15.1 mg g^{-1}), stachyose (2.8–59.4 mg g^{-1}) and ciceritol (4.8–90.7 mg g^{-1}). Clearly, there is a need to screen mini core collections to identify germ plasm with nutraceuticals properties for use as ingredients in functional foods for improving human health (Upadhyaya et al., 2006b).

6.3 Composite collections and reference sets for assessing molecular diversity

Assessing the range of diversity and understanding the genetic structure of gene pools is critical for the effective use of genetic resources. Past studies indicated abundant diversity among wild *cicer*, but limited variation in cultivated chickpea (Kazan and Muehlbauer, 1991; Shan et al., 2005; Rao et al., 2007) and pigeonpea (Burns et al., 2001; Odeny et al., 2007). As part of Generation Challenge Program (GCP), ICRISAT had developed global composite collections of chickpea (3000 accessions) and pigeonpea (1000 accessions)

Table 3 Summary of core and mini core collections of legumes developed at ICRISAT genebank, India

	Entire collection	No. of accessions used	No. of accessions in core	No. of accessions in mini core	Reference
Chickpea	20 764	16 991	1956	211	Upadhyaya and Ortiz, 2001
Pigeonpea	13 778	12 153	1256	146	Upadhyaya et al., 2006c

that include mini core collections (Upadhyaya et al., 2006b, 2011c,d). The GCP aimed at exploiting the genetic diversity housed in existing germ plasm collections with a focus on improving the drought tolerance in crops (www.generationcp.org). The reduced subsets (both conventional mini core collections and reference sets) are an ideal as diversity panels for studying population structure, population diversity, and association genetics for agronomically beneficial traits.

Molecular characterization of composite sets using 50 SSRs in chickpea and 20 SSRs in pigeonpea has helped in the understanding of genetic diversity and population structures in each species. In chickpea, the 48 SSR markers detected 1683 alleles in 2915 accessions, of which 935 were considered rare, 720 common and 28 most frequent. The alleles per locus ranged from 14 to 67and the polymorphic information content ranged from 0.467 to 0.974. Marker polymorphism varied between groups of accessions in the composite collection and reference set. A number of group-specific alleles were detected: 104 in Kabuli, 297 in desi, and 69 in wild *Cicer*; 114 each in Mediterranean and West Asia, 117 in South and South-East Asia and 10 in African region accessions. Desi and kabuli shared 436 alleles, while wild *Cicer* shared 17 and 16 alleles with desi and kabuli, respectively. Desi chickpea contained a higher proportion of rare alleles (53%) than kabuli (46%), while wild *Cicer* accessions were devoid of rare alleles. A genotype-based reference set captured 1315 (78%) of the 1683 composite collection alleles of which 463 were rare, 826 common and 26 the most frequent alleles (Upadhyaya et al., 2008).

In pigeonpea, analysis using 20 SSR markers data on 952 accessions detected 197 alleles, of which 115 were rare and 82 were common alleles. Gene diversity varied from 0.002 to 0.726. Sixty group-specific alleles were detected in 45 wild accessions and 64 unique alleles in 907 cultivated accessions. Geographically, 32 unique alleles were found in Southern Indian provinces, Maldives and Sri Lanka, seven in Indonesia, Philippines and Thailand, four in Northwestern Indian provinces, Iran and Pakistan, and two in Africa. Wild types as a group were genetically more diverse than the cultivated types (Upadhyaya et al., 2011c). A genotype-based reference set of 300 genetically diverse accessions have been established. The reference set captured 95% of the 197 alleles found in the composite collection. The genotype-based reference sets are ideal for allele mining, association genetics, mapping and cloning gene(s), and in applied breeding for the development of broad-based elite breeding lines/cultivars with superior yield and enhanced adaptation to diverse environments. The genetically diverse reference set offer breeders the opportunity to combine multiple resistances into an agronomically improved genetic background for genetic mapping populations for trait mapping and cultivar development.

6.4 Germ plasm enhancement through wide crosses

The narrow genetic diversity in cultivated germ plasm has hampered the effectiveness of conventional breeding as well as the development and utilization of genomic tools. Wild relatives of grain legumes are reported to harbour beneficial alleles and genes for resistance/tolerance to biotic and abiotic stresses and for increased grain yield and quality. For example some of the wild relatives of chickpea have also been reported to possess high seed protein ((Dwivedi et al., 2005; Nguyen et al., 2005; Sharma et al., 2007). Singh et al. (2005) and Dwivedi et al. (2007) found that some of the interspecific derivatives with *C. reticulatum* or *C. echinospermum* in their pedigree showed high degree of resistance to wilt, foot rot and root rot diseases and produced greater biomass and/or seed yield.

A number of the wild *Cajanus* species, especially from the secondary gene pool, that are cross compatible with cultivated pigeonpea have been used for the genetic improvement of pigeonpea (Upadhyaya et al. 2013b). The most significant achievement is the development of a unique cytoplasmic nuclear male sterility system (CMS). Saxena et al. (2010) described eight unique CMS systems with cytoplasm derived from wild *Cajanus* species. A4 cytoplasm derived from *C. cajanifolius* (Saxena et al., 2005) is stable across environments with a good fertility restoration system and has been used to develop the world's first commercial pigeonpea hybrid, ICPH 2671 (Saxena et al., 2010).

Evaluation of wild *Cajanus* species has identified accessions having resistance to alternaria blight (Sharma et al., 1987), Phytophthora blight (Rao et al., 2003), sterility mosaic virus (SMV) (Kulkarni et al., 2003), pod fly (Sharma et al., 2003), root-knot nematodes (Sharma et al., 1993; Sharma 1995), tolerance to salinity (Rao et al., 2003; Srivastava et al., 2006), drought (Rao et al., 2003) and photoperiod insensitivity (Rao et al., 2003). Wild *Cajanus* species, especially, *C. scarabaeoides, C. acutifolius, C. platycarpus, C. reticulates, C. sericeus* and *C. albicans* have been reported to have resistance to pod borer, *Helicoverpa armigera* (Mallikarjuna et al., 2007; Sujana et al., 2008; Sharma et al., 2009). Some wild *Cajanus* species namely *C. scarabaeoides, C. sericeus, C. albicans, C. crassus, C. platycarpus* and *C. cajanifolius* have higher seed protein content (average 28.3%) compared to pigeonpea cultivars (24.6%) (Singh and Jambunathan, 1981).

Utilization of wild *Cajanus* species has not only led to the development of the CMS systems and to cultivars with resistant/tolerant to biotic/abiotic stresses but also contributed significantly towards the improvement of agronomic performance and nutritional quality of cultivated pigeonpea (Upadhyaya et al., 2013b). Breeding lines with high protein content have been developed from crosses with *C. sericeus, C. albicans*, and *C. scarabaeoides*. A high protein line, ICPL 87162 with a 30–34% protein content, was developed from the cross *C. cajan* x *C. scarabaeoides* (Reddy et al., 1997).

6.5 Past use and impact of accessions conserved in *ex situ* collections

The collections of two important grain legumes, chickpea and pigeonpea, conserved at the ICRISAT genebank are a major source of variability for researchers globally. Seeds of the conserved accessions are available free of cost at the ICRISAT genebank under the Standard Material Transfer Agreement (SMTA) of International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA), for use in research and for training purposes. To date, the ICRISAT genebank provided 151 939 samples of chickpea to researchers in 88 countries and 74 783 samples of pigeonpea to researchers in 113 countries. Within ICRISAT, 190 636 samples of chickpea and 86 900 samples of pigeonpea were provided to researchers.

Globally the collections in gene banks are large but only a few accessions (<1%) have been used in crop improvement programmes. A large gap exists between the availability and actual utilization of the conserved accessions. This is true for CGIAR institutes as well as for the national institutes. At ICRISAT, during 35 years, only eight pigeonpea accessions were supplied more than 200 times. In chickpea, only 91 accessions were used in developing breeding lines (Upadhyaya et al., 2006a).

Despite the constraints to the use of the conserved accessions, samples of grain legumes supplied from ICRISAT genebank have been used as raw materials in crop improvement programmes and academic studies globally. Overall crops that ICRISAT conserves, national institutes have released more than 1000 varieties in 81 countries utilizing germ plasm and breeding lines from ICRISAT that are contributing to global food security. Some of the elite accessions have been released directly as cultivars, 22 chickpea accessions in 23 countries and 11 pigeonpea accessions in 7 countries. ICC 11879, a chickpea landrace, was released as a variety in eight Mediterranean countries and ICC 13816 was released as a variety in seven countries. A vegetable pigeonpea landrace from India (ICP 7035) has been released as cultivar in India, Fiji, Nepal, China and Philippines. Wilt-resistant pigeonpea landrace ICP 8863 that was released as Maruti in India generated benefits of US$ 75 million by 1996 with a 73% internal rate of return (Bantilan and Joshi 1996). In addition, the grain legume collections at ICRISAT genebank have served as an insurance against the genetic erosion globally. The genebank was able to restore chickpea accessions to Ethiopia (931) and India (7488) and pigeonpea accessions to India (5977) and Sri Lanka (71), when national gene banks have lost their local collections due to natural calamities, strife, etc.

7 Conclusion

To ensure that future users will have this diversity available for grain legumes, such as chickpeas and pigeonpeas as well as all the other crops, there are urgent needs to better secure their conservation. The crop communities for beans, chickpeas, cowpeas, pigeonpeas, grasspeas, and faba beans have developed global *ex situ* conservation strategies. The global conservation strategies concluded that the current system for *ex situ* conservation of grain legumes is not global but consist of poorly linked international and national collections which hold some unknown degree of common and unique accession, that are conserved with limited sustained support and not very well known or available to users. Some of the key needs for the global conservation system related to the regeneration of accessions, safety duplication of collections, a global information system to enhance the use of conserved germ plasm, global rationalization of accessions, and collections to fill significant gaps. A review of the characterization and utilization of diversity within chickpeas and pigeonpeas found that *ex situ* collections are currently underutilized but with the identification of subsets, more intense evaluation, and the application of the new tools from genomics, the exploitation of these collections is projected to increase. The conserved collections of grain legumes are critical resources for future food systems. Thus, there is a need to consider a new model for managing global resources in a secure, cost-effective, sustainable global system.

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