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Exploiting genomic resources for efficient conservation and utilization of chickpea, groundnut, and pigeonpea collections for crop improvement

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Abstract

Both chickpea and pigeonpea are important dietary source of protein, while groundnut is one of the major oil crops. Globally, ~1.1 million grain legume accessions are conserved in genebanks, of which, ICRISAT genebank holds ~50,000 accessions of cultivated species and wild relatives of chickpea, pigeonpea, and groundnut from 133 countries. These genetic resources are reservoirs of many useful genes for the present and future crop improvement programs. Representative subsets in the form of core and mini core collections have been used to identify trait-specific genetically diverse germplasm for use in breeding and genomic studies in these crops. Chickpea, groundnut and pigeonpea have moved from 'orphan' to 'genomic resources rich crops'. The chickpea and pigeonpea genomes have been decoded, and the sequences of groundnut genome will soon be available. With the availability of these genomic resources, the germplasm curators, breeders and molecular biologists will have abundant opportunities to enhance the efficiency of genebank operations, mine allelic variations in germplasm collection, identify genetically diverse germplasm with beneficial traits, broaden the cultigen's genepool, and accelerate the cultivar development to address new challenges to production, particularly with respect to climate change and variability. Marker-assisted breeding approaches have already

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been initiated for some traits in chickpea and groundnut, which should lead to enhanced efficiency and efficacy of crop improvement. Resistance to some pests and diseases has been successfully transferred from wild relatives to cultivated species.

Key words: Grain legumes, genetic and genomic resources, mini core collection, inter-specific gene transfer

Legumes are the primary source of dietary protein in semi-arid tropic (SAT) regions of Asia and Africa. They have a great potential in alleviating protein hunger and malnutrition prevalent amongst the poor in SAT regions. The fodder is a valuable source as animal feed. It is an integral part of the cropping systems for improving and sustaining soil fertility. The legumes in SAT regions are mostly grown in marginal environments and their productivity is seriously constrained due to low soil fertility, unpredicted weather conditions, and susceptibility to abiotic and biotic stresses. The use of plant genetic resources (PGR) is one of the most efficient ways to develop high yielding and climate resilient cultivars. PGR usually refers to the sum total of genes, gene combinations, or genotypes available for the genetic improvement of crop plants and comprise of landraces, traditional and modern cultivars, mutants, and wild and weedy relatives that provide raw materials for cultivars development.

Since green revolution, large scale cultivation of genetically uniform high-yielding modern cultivars has replaced most of the landraces and traditional cultivars, which resulted genetic erosion and vulnerability of the crops to new pests and diseases. This has led to several epidemics in the past, e.g., the Irish famine of 1850 caused by large-scale cultivation of a genetically uniform potato crop susceptible to late blight (Stevens, 1933), the 1943 famine caused by the brown spot disease of rice in India (Padmanabhan, 1973), or the Southern corn blight epidemic that caused large-scale destruction of maize crop in USA (Horsfall, 1972). This necessitates identifying new and diverse sources of germplasm possessing beneficial traits to

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broaden the genetic base of the crops capable of withstanding frequent climatic fluctuations. PGR are the reservoir of many useful genes, and the efficient conservation and judicious use of these resources in crop improvement programs would help to sustain and stabilize grain legume production for the present and future generations.

1. Current status of grain legume genetic resources maintained at ICRISAT genebank

Globally, about 7.4 million germplasm accessions of different crops have been collected and/or assembled and conserved in over 1750 genebanks, of which, the grain legumes genetic resources constitute about 1.1 million germplasm accessions. Large proportions of these germplasm have been preserved globally in many national and international genebanks (FAO, 2010). International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) has the global mandate to collect, conserve, characterize, and distribute germplasm of its mandate crops including three grain legumes, chickpea, pigeonpea, and groundnut. Currently, it holds ~50,000 accessions of cultivated species and wild relatives of chickpea, pigeonpea, and groundnut from 133 countries (Table 1). Of these, 38,596 germplasm accessions were assembled through donations from 187 genebanks/institutions globally, while 10,889 accessions were collected through joint missions organized in 62 countries. The existing collections represent 70 - 80% of the available diversity and are an insurance against genetic erosion; and serve as resource to identifying variations for agronomic traits including resistance to abiotic and biotic stresses for future crop improvement programs. The genebank at ICRISAT ensures effective management of germplasm at international standards and continue to supply good quality seeds to researchers globally. The main activities in management of PGR include conservation and characterization, regeneration and evaluation, documentation and distribution. Over 98% of the grain legume germplasm accessions, maintained at ICRISAT, have been characterized for morpho-agronomic

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traits following internationally accepted list of descriptors (IBPGR/ICRISAT, 1992, 1993; IBPGR, ICRISAT, ICARDA, 1993). Germplasm collection at ICRISAT genebank is available to global research community for research and training under the terms and conditions of the Standard Material Transfer Agreement.

Regeneration is the crucial process in genebank management. Breeding behavior of the crop (self- or cross-pollinated) and sample size are the two key factors affecting efficient regeneration. Both chickpea and groundnut are self-pollinated crops. Regeneration is therefore carried out in field without any control on pollination by using at least 80 plants for regenerating an accession. Pigeonpea is often cross-pollinated crop with out-crossing ranging from 20-70%, depending on visit by bees (Saxena et al., 1990). The pigeonpea germplasm accessions are regenerated during the rainy season under insect proof field cages (Figure 1) at Patancheru, India. Specialized facility such as *Arachis* house, a space fixed with large-cylindrical concrete structure (75 cm height, 90 cm inner ring diameter, 5 cm ring thickness) with ring-to-ring distance of 52.5 cm (Figure 2), has been established for regeneration of wild *Arachis* species. These rings are filled with about 0.5 m^3 pasteurized (3 cycles of 1 hour each at 82°C and 34.5 x 10^3 Pa (5 psi) soil mixture (soil, sand and FYM in 3:2:1 ratio) and can accommodate 5-6 plants per accession. Some of the wild *Cicer* species need long day length and cool weather to grow and produce seeds. Such species are also regenerated under controlled greenhouse conditions (Figure 3). Chickpea, groundnut and pigeonpea seeds are orthodox that can be dried to low seed moisture content (about 5-7%) for efficient conservation. A two-tier system (active or base collection) is followed for conservation of germplasm accessions. The active collection is maintained in medium-term storage (4°C, 20-30% RH) in aluminum cans, while the base

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collection in long-term storage (-20°C) after packing the seeds in vacuum-sealed aluminum foil pouches (Upadhyaya and Gowda, 2009).

2. Germplasm utilization for grain legume improvement

The role of plant genetic resources in the improvement of cultivated plants has been long recognized (Frankel and Hawkes, 1975). Many germplasm accessions have performed significantly better and produce higher grain yield when evaluated in different environments, and hence were released directly as cultivars. Globally, 15 chickpea germplasm accessions in 15 countries, 10 pigeonpea germplasm accessions in seven countries and 11 groundnut germplasm accessions in 15 countries have been directly released as cultivars from the material supplied from ICRISAT genebank. These cultivars have greatly benefited the farmers by contributing to increase in production and productivity in those countries.

Studies have shown scanty use of germplasm in crop improvement programs globally. India has one of the largest grain legume breeding programs and has released 229 cultivars of chickpea, lentil, pigeonpea, blackgram and mungbean through hybridization and selection (data up to 2003). Pedigree analysis of these cultivars revealed that Pb-7 in chickpea, L-9-12 in lentil, T-1 and T-90 in pigeonpea, T-9 in blackgram, and T-1 in mungbean were the most frequently used parents (Kumar et al., 2004). Low use of germplasm has also been reported in soybean (Mikel et al., 2010). The chickpea researchers at ICRISAT (1978-2004) used 12,887 parents (586 unique accessions) to develop 3548 advanced varieties (ICCV#), which included only 91 unique germplasm accessions and five wild *Cicer* species (Upadhyaya et al., 2006a). Similarly in groundnut, the researchers used 986 parents (during 1986-2002) to develop 8279 advanced varieties (ICGV#). However, those included only 132 unique germplasm and 10 wild *Arachis*

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species (Upadhyaya et al., 2006a) from about 15,000 germplasm accessions available in its genebank. This may be attributed to the use of working collections, consisting mostly of elite breeding lines and some improved trait specific lines by most of the breeders, which results in re-circulation of the same germplasm and hence narrow genetic base of the resulting cultivars. Hence, there is a need to identify genetically diverse trait-specific germplasm for use in legumes breeding to meet the production challenges as a result of climate change and variability.

3 Forming representative sets as a means to enhance utilization of germplasm in grain legume improvement

3A. Core and mini core collection

The core and mini core collections are now available in chickpea, groundnut, and pigeonpea (Table 2). Standard procedure was used to develop these representative subsets (Figure 4). Validation studies revealed that these reduced subsets represented >80 % diversity in the core (or mini core) of the entire collection of germplasm of a given species, maintained in a genebank. These representative subsets are ideal resource to identifying new sources of variation for use in crop improvement programs.

3B. Composite collection and reference set

As a part of the Generation Challenge Programme (www.generationcp.org), the global composite collections have been developed in chickpea, groundnut, and pigeonpea to create a public platform that use molecular methods to unlock genetic diversity, which may be used to improve the genetic potential of these crops. The chickpea global composite collection consists of 3,000 accessions, while global composite collections in groundnut and pigeonpea, each consists of 1,000 accessions (Upadhyaya et al., 2006 b, c, d). These composite collections were further characterized for molecular diversity using 20-50 SSR markers to form genotype-based reference

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sets, which contains 300 genetically most diverse accessions each in chickpea, groundnut and pigeonpea (Upadhyaya et al., 2008a, b, c). These subsets are ideal resource to conduct population structure and diversity, association mapping, identifying new sources of variation, and mining allelic variation associated with agronomically beneficial traits.

4. Trait-specific germplasm for use in breeding and genomic studies

Identifying trait-specific genetically diverse germplasm is prerequisite to the success of any breeding programs. Representative subsets are the ideal resource, instead of large collections, for discovering new sources of variation. Research to date at ICRISAT and elsewhere suggests that the representative subsets, when evaluated for agronomic traits and resistance to abiotic and biotic stresses, have resulted in identification of new sources of genetically diverse germplasm with beneficial traits (Upadhyaya et al., 2009a, 2010, 2012c). For example, chickpea germplasm with early maturity and/or large seed size; early-maturing groundnuts (90 days) with high pod yield or those with large variability in pod/seed characteristics, oil and oil quality (as measured by O/L ratio), and grain Fe and Zn contents; early-maturing pigeonpea or those with large-seed size, more pods per plant, and high in grain protein, Fe and Zn contents (Table 3). Abiotic and biotic stresses are the major yield reducing constraints to crop production, and identification of resistant sources to these stresses is the first step to developing stresses resistant/tolerant elite germplasm/cultivars. For abiotic stresses, several germplasm with resistance to drought, salinity and heat stresses in chickpea; to drought (intermittent and terminal drought), salinity and low temperature in groundnut; or to salinity and water logging in pigeonpea have been reported (Table 4). Likewise, resistance to fusarium wilt, ascochyta blight, botrytis gray mold, dry root rot, and pod borer in chickpea; resistance to early leaf spot, late leaf spot, rust, *Aspergillus flavus*, peanut bud necrosis, tomato spotted wilt virus, root-knot nematode, and soil born fungal diseases

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in groundnut; and resistance to fusarium wilt, sterility mosaic, and pod borer in pigeonpea (Table 5) have been reported. Many of these germplasm have shown multiple resistances to both abiotic and biotic stresses.

5. Wild relatives and introgression to broaden cultigen's genepool

For some of the stresses, either resistance in cultivated genepool is not available or cultivated genepool have only low to moderate resistance. In contrast, very high levels of resistance to many of the biotic stresses have been reported in wild relatives of chickpea, groundnut, and pigeonpea (Dwivedi et al., 2008a). Wild relatives can also enhance the agronomic traits in cultigens (Upadhyaya, 2008). However, it is not easy to introgress resistance from wild to cultivated genepool, which may require novel techniques and protocols to affect such introgression. Research to date suggests that researchers at ICRISAT and elsewhere were successful in introgressing beneficial traits from wild relatives to cultivated genepool, for example, resistance to root-knot nematode and late leaf spot in groundnut (Simpson and Starr, 2001; Simpson et al., 2003; Mallikarjuna et al., 2012), resistance to phytophthora blight, pod borer, bruchid, and podfly resistance in pigeonpea (Mallikarjuna et al., 2011; Jadhav et al., 2012), and resistance to botrytis gray mold in chickpea (Ramgopal et al., 2012). Chromosome segment substitution lines (CSSLs) are useful genetic resources for QTL mapping to elucidate the genetic and molecular basis of interesting traits of wild species. Foncéka et al. (2012) reported the development of 122 CSSLs from the cross between the wild synthetic allotetraploid (*A. ipaensis* × *A. duranensis*)$^{4\times}$ and the cultivated Fleur 11 cultivar, which offer a broad coverage of the groundnut genome, with target wild chromosome segments averaging 39.2 cM in length. Such genetic resources may be useful in deciphering the molecular basis of trait expression and

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further exploitation in enhancing the genetic potential of groundnut crop. More such resources are needed, which are being developed in all three crops at ICRISAT.

6. Genomic resources in conservation and utilization of germplasm in grain legume improvement

6. A. Efficient and cost-effective conservation of germplasm

DNA markers and other genomic resources may be used for efficient conservation of plant genetic resources, with a potential to enhance the efficiency of genebank operations in following ways: i) optimizing genebank management strategies; ii) improving sampling strategies; iii) identifying gaps in germplasm collection and improving the collection composition; iv) measuring and reducing genetic drift/shift; v) identifying contamination and duplicates; vi) knowing population structure and diversity and assessing geographic and biological representation in collection; vii) forming genotype-based, region-based and trait-based representative subsets and identifying trait-based genetically diverse accessions; viii) marker-trait associations and mining allelic variations associated with beneficial traits; ix) identifying new alleles and enlarging the allelic diversity in germplasm collection; x) fingerprinting to identify the unique germplasm with specific attributes. The chickpea, groundnut and pigeonpea are no more orphan or less-studied crops but are genomic resources rich crops. Researchers at ICRISAT and elsewhere have used these resources to dissect the population structure and diversity in global composite collection to form genotype-based reference sets in chickpea, groundnut and pigeonpea (Upadhyaya et al., 2008a, b, c), assess genetic diversity in Bolivian germplasm (Husain and Mallikarjuna, 2012), assess genetic relationships among species in genus *Arachis* (Koppolu et al., 2010); and monitor introgression of genomic segments from wild *Arachis* to the cultivated genepool in groundnut (Foncéka et al., 2009). Clearly, more such

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studies are needed to improve the efficiency of genebank operations and for effective utilization of genetic resources in crop improvement.

6B. Developing elite germplasm/cultivars with specific attributes

Speedy developments in the last decade has witnessed the fast development of large-scale genomic resources in chickpea, groundnut and pigeonpea, which have provided a platform for dissecting the complex traits controlling crop productivity and resilience to accelerate efficiency and precision in breeding programs. ICRISAT in collaboration with national and international partners played an important role in developing huge genomic resources (large numbers of SSR and SNP markers, and high density consensus genetic maps) and cost-effective high throughput genotyping platforms to accelerate application of these resources to increase grain legumes productivity (Varshney et al. 2013a and references cited therein). In addition, chickpea and pigeonpea genomes have been decoded (Varshney et al., 2012, 2013b), and the sequencing of groundnut genome is underway (http://www.peanutbioscience.com). All these developments will facilitate study of population structure and diversity in germplasm collection, forming trait-based diversity panels, genome-wide association mapping, and effect genomic selection to developing new cultivars with specific characteristics in grain legumes. The re-sequencing of germplasm/breeding lines with unique traits will enable us to detect variation at nucleotide level and, if such variation is found associated with beneficial traits, to enhance trait value in breeding programs. Marker-QTL information may be used towards introgressing genomic region(s) associated with beneficial traits into improved genetic background. For example, the chickpea breeders and molecular biologists at ICRISAT have identified a major QTL associated with terminal drought tolerance in chickpea, which they have introgressed into three elite cultivars, JG11, Chefe and KAK2. Multi-location field evaluation of the backcross progenies demonstrated

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on average 24% and 11% increase in grain yield under irrigated and rainfed conditions, respectively. Chickpea researchers in national programs in India and sub-Sahara Africa have initiated the introgession of this genomic region into several elite cultivars. Likewise, diagnostic markers in groundnut have been used to segregate groundnut germplasm with variation in O/L ratio (Chu et al., 2009; Wang et al., 2011a, b), introgression of wild-genome segments in advanced backcross populations involving a popular cultivar, Fleur 11, from west Africa (Foncéka et al., 2009), and successfully introgressed high oleate trait into a root-knot nematode resistant cultivar, "Tifguard High O/L" (Chu et al., 2011).

7. Conclusion

Grains legumes are important for food and nutritional security as well for improving soil fertility. Legumes including chickpea, groundnut and pigeonpea have narrow genetic base due to bottlenecks associated with the origin and domestication of these crops. The susceptibility of these crops to abiotic and biotic stresses, cultivation on marginal lands, and limited response to high input agriculture has further constrained productivity, particularly in developing countries. Genetic resources and the variability are the key to the success of any crop improvement programs. Large collection of chickpea, groundnut and pigeonpea germplasm, both cultivated and wild types are maintained in many national and international genebanks globally. ICRISAT's strategic research on development of representative subsets, in the form of core and mini core collections or genotyping-based reference sets, in these crops and subsequent to their extensive evaluation have resulted in identification of several germplasm with specific traits, i.e., resistance to abiotic and/or biotic stresses or those with superior agronomic and/or nutritional traits. The use of such germplasm in crop improvement programs would transform these crops more responsive to new and emerging production challenges, particularly with respect to climate

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change and variability. Chickpea, groundnut and pigeonpea are no more orphan crops but genomic resources rich crops, which have enabling effects towards identifying and tracking allelic variants associated with beneficial traits and identifying segregants with specific attributes, thus accelerating molecular breeding in grain legume improvement. Genomic resources and associated genotyping platforms have also enabled researchers to monitor introgression of wild segments carrying useful genes in cultivated groundnut.

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Figure captions

Figure 1. Pigeonpea germplasm accessions grown under insect-proof cages at ICRISAT, Patancheru, India

Figure 2. Wild *Arachis* accessions grown under *Arachis* House at ICRISAT, Patancheru, India

Figure 3. Wild *Cicer* accessions grown under controlled green house conditions at ICRISAT, Patancheru, India

Figure 4. Flow diagram to establish a mini core collection

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Table 1. Status of chickpea, groundnut and pigeonpea genetic resources conserved at ICRISAT genebank and the number of samples distributed worldwide.

Crop	Accessions held in RS Paroda genebank at Patancheru, India				Number of samples distributed (1974 to April 15, 2013)	
	Cultivated	Wild	Total	Country of origin	Number of samples	Number of countries
Chickpea	19,960	308	20,268	60	131,720	88
Groundnut	14,968	478	15,44 6	92	99,287	96
Pigeonpea	13, 216	555	13, 771	74	71,040	112
Total	48,144	1,341	49,485	133 (unique)	302,047	136 (unique)

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Table 2. Core and mini core collections reported in chickpea, groundnut and pigeonpea.

Accessions used in developing representative set	Representative subset developed	Accessions in subset	Reference
	Chickpea		
3350	Core collection	505	Hannan et al., 1994
16,991	Core collection	1,956	Upadhyaya et al., 2001a
-	Core collection (Kabuli type)	103	Pouresmael et al., 2009
1,002	Core collection	158	Kibret, 2011
1956	Mini core collection	211	Upadhyaya and Ortiz, 2001
482	Mini core collection	39	Biabani et al., 2011
	Groundnut		
7,432	Core collection	831	Holbrook et al., 1993
4,738	Asian core collection	504	Upadhyaya et al., 2001b
14,310	Core collection	1,704	Upadhyaya et al., 2003
6,390	Core collection	576	Jiang et al., 2008
630	Valencia core collection	77	Dwivedi et al., 2008b
1704	Mini core collection	184	Upadhyaya et al., 2002
831	Mini core	111	Holbrook and Dong, 2005
	Pigeonpea		
12,153	Core collection	1,290	Reddy et al., 2005
1,290	Mini core collection	146	Upadhyaya et al., 2006e

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Table 3. Source of early maturity, yield and nutritional quality traits identified using core and mini core collections of chickpea, groundnut and pigeonpea.

Trait	Summary of identified sources	Reference
	Chickpea	
Early maturity and grain yield	28 early maturing accessions yielding more than controls over five environments	Upadhyaya et al., 2007a
	11 large-seeded kabuli germplasm	Gowda et al., 2011
	Several promising accessions with yield attributing traits	Upadhyaya et al., 2007b; Meena et al., 2010; Parameshwarappa et al., 2011
	Groundnut	
Yield and yield attributing traits and oil content	Several accessions with high pod yield, shelling percentage, and 100-seed weight in *fastigiata*, *vulgaris*, and *hypogaea* botanical types	Upadhyaya et al., 2005
Early maturity	Good diversity for early maturity	Upadhyaya et al., 2006f
Oil and protein contents and oil quality	Accessions with high oil and protein and oil with better oil quality	Upadhyaya et al., 2012a
Grain Fe and Zn	Forty eight accessions with higher Fe, 43 with higher Zn and 23 with high Fe and Zn together with superior agronomic traits	Upadhyaya et al., 2012b
	Pigeonpea	
Early maturity	Eight accessions	Upadhyaya et al. 2010, 2012c
Large-seed size	Three accessions (>15 g 100 seed^{-1})	Upadhyaya et al. 2010, 2012c
High pod number per plant	Three accessions with high pods per plant (>200 pod plant^{-1})	Upadhyaya et al. 2010, 2012c
Grain protein, Fe and Zn	Six accessions for high seed protein, 8 accessions with high Fe and four accessions with high Zn	Upadhyaya et al. 2013

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Table 4. Source of resistance to abiotic stresses identified using core and mini core collections of chickpea, groundnut and pigeonpea.

Trait	Summary of identified sources	Reference
	Chickpea	
Drought	Large genetic variation for drought avoidance root traits, harvest index (HI), drought tolerant index or surrogate traits such as SCMR, SPAD, and Δ^{13}	Kashiwagi et al. 2005, 2006; Parameshwarappa and Salimath, 2008; Krishnamurthy et al., 2010; Parameshwarappa et al., 2010, 2011
Salinity	Several accessions with varying levels of tolerance to salinity; some with specific adaptation in both alfisol and vertisol saline soils	Serraj et al., 2004; Vadez et al., 2007; Krishnamurthy et al., 2011a
Heat	Several heat tolerant germplasm with high yield potential	Upadhaya et al., 2011; Krishnamurthy et al., 2011b
	Groundnut	
Drought	18 accessions tolerant to terminal drought	Upadhyaya, 2005
Salinity	Six salinity tolerant accessions	Srivastava, 2010
Low temperature at germination	Several accessions tolerant to low temperature	Upadhyaya et al., 2009b
	Pigeonpea	
Salinity	16 salinity tolerant accessions	Srivastava et al., 2006
Water logging	23 accessions tolerant to water logged conditions	Krishnamurthy et al., 2012

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Table 5. Source of resistance to biotic stresses identified using core and mini core collections of chickpea, groundnut and pigeonpea.

Trait	Summary of identified sources	Reference
	Chickpea	
Diseases	Several accessions either with single or multiple resistance to usarium wilt, Ascochyta blight, Botrytis gray mold, and dry root rot	Pande et al., 2006
Legume pod borer	Several accessions with low damage to legume pod borer	ICRISAT 2009, 2010
Beetle	One accession resistant to pulse beetle	Erler et al., 2009
	Groundnut	
Leaf spots and rust	Several accessions with resistance to rust, early leaf spot and late leaf spot and/or *Aspergillus flavus*	Kusuma et al., 2007
Peanut bud necrosis disease (PBND)	Four accessions resistant to PBND	Ahmed, 2008
Root-knot nematode, tomato spotted wilt virus (TSWV), soilborn fungal diseases	Several accessions resistant to root-knot nematode, TSWV and soil born fungal diseases	Isleib et al., 1995; Anderson et al., 1996; Holbrook et al., 1998; Franke et al., 1999; Damicone et al., 2010; Chamberlin et al., 2010
	Pigeonpea	
Fusarium wilt (FW), Sterilty mosaic disease (SMD)	Six accessions resistant to FW and 24 resistant to SMD	Sharma et al., 2012
Pod borer	11 accessions with moderate resistance to pod borer	ICRISAT, 2010

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Figure 1. Pigeonpea germplasm accessions grown under insect-proof cages at ICRISAT, Patancheru, India

Figure 2. Wild *Arachis* accessions grown under *Arachis* House at ICRISAT, Patancheru, India

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Figure 3. Wild chickpea accessions grown under controlled green house conditions at ICRISAT, Patancheru, India

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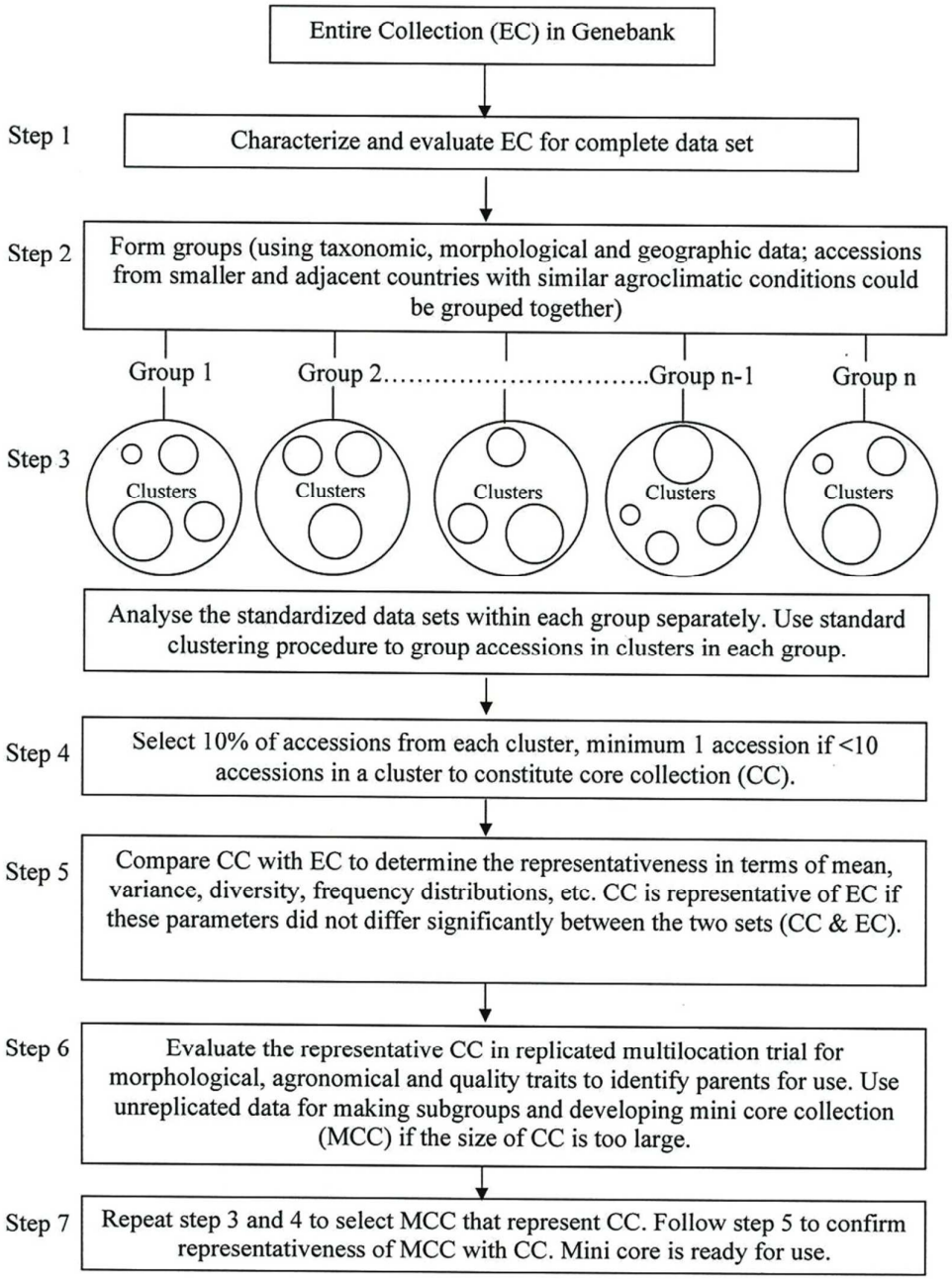

Figure 4. Flow diagram to establish a mini core collection