



Establishing core collections for enhanced use of germplasm in crop improvement

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ABSTRACT

Plant genetic resources are the basic raw materials and their use in breeding is the most sustainable way to conserve biodiversity. Low use of germplasm in crop improvement programs has resulted large gap between the number of germplasm preserved in genebanks and the number of germplasm used in crop breeding across the globe. Breeders are reluctant to use germplasm largely either due to lack of reliable information on economic traits besides linkage drag or due to breakdown of co-adapted gene complexes, which may prolong cultivar development time. The reduced subsets, representing diversity in the germplasm collection of a given species preserved in genebank, in the form of core or mini core collections are the ideal genetic resources for discovering new sources of variations for use in crop improvement programs. Two decades of research at ICRISAT has led to the establishment of core and mini core collections and their subsequent evaluations has resulted in identification of new sources of variations, for example, resistance to abiotic and/or biotic stresses in chickpea, groundnut, pigeonpea, pearl millet, sorghum, finger millet and foxtail millet. Likewise, a number of nutritionally dense (high protein, Ca, Fe and Zn) germplasm have been identified in finger millet, foxtail millet, groundnut, pearl millet, and sorghum. A few groundnut germplasm with improved oil quality, as determined by variation in oleic and linoleic fatty acids, were also identified. Many of these germplasm were agronomically at par or even superior over controls and showed specific and wide adaptation. The identified sources may be used in genomics and breeding to broaden the cultigen's gene pool in these crops.

Keywords: association mapping, cereals, core and mini core collections, legumes, germplasm, population structure and diversity

Introduction

Plant genetic resources (PGR) are the basic raw materials for use in crop improvement programmes. The use of PGR in crop improvement is one of the most sustainable ways to conserve valuable genetic resources for the future, and simultaneously to increase food and nutritional security (McCouch et al. 2013). Key to successful crop improvement is a continued supply of genetic diversity in breeding programs, including new or improved variability for target traits. Globally, 7.4 million accessions are preserved in over 1750 genebanks, with cereals and food legumes, respectively, constituting 45% and 15% collection (Figure 1). Eleven percent of

these accessions are preserved in CGIAR Centers' genebanks. ICRISAT has the largest collections of its mandate crops (chickpea, groundnut, pigeonpea, pearl millet, and sorghum) and of small millets, totaling 120,454 accessions from 144 countries (Table 1), with mandate crops germplasm constituting 91.4% and small millets 8.6% of its collections. Of these, 2.3% constitute wild and weedy relatives of the mandate crops and of small millets. Managing and utilizing such large diversity in germplasm collections are greatest challenge to germplasm curators and crop breeders.

Breeders are often reluctant to use germplasm and prefer to use their own working collections,

Table 1. Current status of germplasm collections preserved at ICRISAT genebank (accessed on Jan 01, 2014) and safety duplicates preserved at Svalbard Global Seed Vault, Norway.

| Crop | ICRISAT | | Svalbard Global Seed Vault |
|-----------------|----------------|---------------|----------------------------|
| | Accessions (#) | Countries (#) | Accessions (#) |
| Barnyard millet | 749 | 10 | 726 |
| Chickpea | 20,268 | 60 | 16,931 |
| Finger millet | 6,084 | 24 | 5,828 |
| Foxtail millet | 1,542 | 26 | 1,458 |
| Groundnut | 15,446 | 92 | 13,900 |
| Kodo millet | 665 | 2 | 653 |
| Little millet | 473 | 5 | 460 |
| Pearl millet | 22,658 | 50 | 19,685 |
| Pigeonpea | 13,771 | 74 | 9,519 |
| Proso millet | 849 | 30 | 813 |
| Sorghum | 37,949 | 92 | 34,027 |
| Total | 120,454 | 144 | 104,000 |

largely due to non-availability of reliable information on traits of economic importance (Upadhyaya et al. 2011e), fear of carrying linkage drag, and possibly due to breakdown of co-adapted gene complexes (Ortiz et al. 1998). The other reasons for the underutilization of germplasm include i) lack of accurate and precise large-scale multi-location evaluation of germplasm, 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 exotic germplasm in crop breeding (Dwivedi et al. 2009; Upadhyaya et al. 2011e). Clearly, there is a need to increase the use of genetically diverse germplasm with beneficial traits to meet the emerging challenges in agricultural production. Reduced subsets in the form of core (~10% of entire collection) or mini core (~1% of entire collection or ~10% of core collection) collections, representing diversity of the entire collection of a given species in the genebank, have been suggested as a resource to study population structure and diversity, discover new sources of variation and identify agronomically beneficial and genetically diverse germplasm for use in crop improvement programs.

This short article provides the current status of PGR preserved in ICRISAT genebank and the formation of reduced subsets (core or mini core collections) for assessing population structure and diversity and discovering new sources of variations, which may be used in cultivar development.

Forming reduced subsets to promote use of germplasm in crop breeding

Reduced subsets such as core (Frankel

1984) and mini core (Upadhyaya and Ortiz 2001) collections, representing diversity of the entire collection of a given species, has been suggested as a gateway to enhance utilization of germplasm in crop improvement programs.

A. Methodology for forming core and mini core germplasm collections

Upadhyaya et al. (2009a) elaborated the standard procedure that they used for developing core and mini core collections. Essentially, this includes stratifying entire collection by taxonomic groups and country of origin, with accessions from smaller and adjacent countries with similar agro-climates grouped together. The standardized data (to eliminate scale differences) is then subjected to hierarchical cluster algorithm of Ward (1963), which optimizes an objective function because it minimizes the sums of squares within groups (clusters) and maximizes the sums of squares among groups. Following this, from each cluster, ~10% of the accessions are randomly selected for inclusion into the core collection. In situation, where the accessions are less than ten in a cluster, at least one accession is included. The means, the variances, the Shannon-Weaver diversity index (H'), and the frequency distribution of traits between the entire collection and core collection is used to test the validity of the latter. To know whether phenotypic associations, which may be under genetic control, were conserved in the core collection, the phenotypic correlations in the entire collection and core collection is estimated and compared. Similar procedure is used to develop mini core collection using the core collection which is further evaluated

for agronomic and/or nutritional traits and data from such evaluations is subjected to statistical analysis as detailed above (Figure 2).

B. Core and mini core collections

Both core and mini core collections are available in chickpea, finger millet, foxtail millet, groundnut, pigeonpea, pearl millet and sorghum, and core collections in barnyard-, kodo-, little- and proso millets (Tables 2 and 3). The core collection constituted about 10% of the accessions of the entire collection of a given species preserved in genebank, while the mini core collection constituted 1% of the accessions of entire collection or 10% of the accessions of the core collection representing diversity of the core collection and entire collection of a given species preserved in a genebank.

C. Genotype-based reference sets

ICRISAT in collaboration with the Generation

Challenge Programme (GCP) developed global composite collections of its mandate crops and of finger and foxtail millets, which were subsequently SSR-genotyped to form genotype-based reference sets (Table 4). The reference sets in sorghum consists of 383 accessions, 200 accessions in foxtail millet, while in chickpea, groundnut, pigeonpea, pearl millet and finger millet 300 accessions each.

Core and mini core collections as resource to discovering new sources of variations

Research to date suggests that core and mini core collections or genotype-based reference sets (Glaszmann et al. 2010) have been found useful in extracting germplasm with agronomically beneficial traits for use in crop improvement programs. The researchers at ICRISAT and elsewhere have extensively evaluated these subsets for resistance to abiotic and biotic stresses and for agronomic and nutritional traits, and reported a number of germplasm

Table 2. Core collections of ICRISAT mandate crops and small millets.

| Crop | Accessions used (#) | Traits used (#) | Core collection accessions (#) | Reference |
|-----------------|---------------------|-----------------|--------------------------------|------------------------|
| Chickpea | 16,991 | 13 | 1,956 | Upadhyaya et al. 2001 |
| Finger millet | 5,940 | 14 | 622 | Upadhyaya et al. 2006b |
| Foxtail millet | 1,474 | 23 | 155 | Upadhyaya et al. 2008b |
| Groundnut | 14,310 | 14 | 1,704 | Upadhyaya et al. 2003 |
| Pearl millet | 20,844 | 22 | 2,094 | Upadhyaya et al. 2009b |
| Pigeonpea | 12,153 | 14 | 1,290 | Reddy et al. 2005 |
| Proso millet | 833 | 20 | 106 | Upadhyaya et al. 2011c |
| Sorghum | 22,474 | 21 | 2,247 | Grenier et al. 2001 |
| Barnyard millet | 736 | 21 | 89 | Upadhyaya et al. 2014c |
| Kodo millet | 656 | 20 | 75 | |
| Little millet | 460 | 20 | 56 | |

Table 3. Mini core collections of ICRISAT mandate crops and small millets.

| Crop | Entire collection (#) | Mini core collection accession (#) | % of entire collection | Traits used (#) | Reference |
|----------------|-----------------------|------------------------------------|------------------------|-----------------|--------------------------|
| Chickpea | 16,991 | 211 | 1.24 | 16 | Upadhyaya and Ortiz 2001 |
| Finger millet | 5,940 | 80 | 1.34 | 18 | Upadhyaya et al. 2010 |
| Foxtail millet | 1,474 | 35 | 2.37 | 21 | Upadhyaya et al. 2011b |
| Groundnut | 14,310 | 184 | 1.28 | 34 | Upadhyaya et al. 2002 |
| Pearl millet | 20,844 | 238 | 1.14 | 12 | Upadhyaya et al. 2011a |
| Pigeonpea | 12,153 | 146 | 1.2 | 16 | Upadhyaya et al. 2006a |
| Sorghum | 22,473 | 242 | 1.08 | 21 | Upadhyaya et al. 2009c |

accessions with agronomically beneficial traits, as detailed below.

A. Resistance to abiotic and biotic stresses

A number of accessions resistance to abiotic and/or biotic stresses have been reported in chickpea (Gaur et al. 2013; Krishnamurthy et al. 2013a, b; Upadhyaya et al. 2013a), finger millet (Kiran Babu et al. 2013; Krishnamurthy et al. 2014a), foxtail millet (Sharma et al. 2013a; Krishnamurthy et al. 2014b), groundnut (Upadhyaya et al. 2014a), pigeonpea (Krishnamurthy et al. 2012; Sharma et al. 2012a), pearl millet (Sharma et al. 2013b), and sorghum (Vadez et al. 2011; Sharma et al. 2010, 2012b). Some germplasm accessions, for example in chickpea and groundnut, showed multiple resistance to abiotic and biotic stresses, with a few having good agronomic and seed quality traits (Upadhyaya et al. 2013a, 2014a). In addition, a number of agronomically superior but susceptible to abiotic and/or biotic stresses have also been reported in chickpea and groundnut (Upadhyaya et al. 2013a, 2014a) which may be used as resource for agronomic traits in resistance breeding programs.

B. Agronomic and nutritional traits

A number of chickpea and groundnut mini core accessions had no resistance to abiotic or biotic stresses, however, some of these were agronomically superior and adapted to diverse environments (groundnut: rainy and /or postrainy environments; chickpea: irrigated and /or rainfed environments), which form the good source for use as parents in resistance breeding programs (Upadhyaya et al. 2013a, 2014a). Likewise, a number of seed-nutrient dense (Oil and protein, Fe and Zn) accessions have been identified in finger millet, foxtail millet, groundnut, and sorghum (Kumar et al. 2009; Upadhyaya et al. 2011b,d, 2012a,b). Early maturity and seed size are agronomically important traits, and accessions with early maturity and large seed size have been reported both in chickpea (Kabuli types) and groundnut (Spanish type) (Upadhyaya et al. 2006c, 2007; Gowda et al. 2011). Small millet grains are rich in Ca, and some accessions with high Ca content have been reported both in finger and foxtail millets (Upadhyaya et al. 2011b,d). The legume kernel-derived fibers stimulate the growth of colonic bifidobacteria, which contributes to improved colon health (Smith et al. 2006; Fernando et al. 2010). More recently, large variability was observed among chickpea mini core accessions for oligosaccharides such as sucrose (3.6 to 54.1 mg g⁻¹), raffinose (0.2 to 15.1 mg g⁻¹), stachyose (2.8 to 59.4 mg g⁻¹), and ciceritol (4.8 to 90.7 mg g⁻¹) (ICRISAT unpublished data). Clearly, there is a need to screen mini core

collections to identify germplasm with nutraceuticals properties for use as ingredients in functional foods for improving human health.

C. Bioenergy trait

Of recent, sweet sorghum has gained importance as smart bioenergy (Ethanol) crop. Sweet sorghum stalks are rich in sugar. When sorghum mini core collection accessions were evaluated for stalk-sugar content, as measured by Brix (%) for two seasons, a number of accessions with high Brix (%) and some with good agronomic traits were identified. For example, IS# 13294, 13549, 23216, 23684, 24139, 24939, and 24953 with significantly greater mean brix (14.0% to 15.2%) than the best control, IS 33844 (12.4%) across environments; however, these were found low yielder and had lower 100-seed weight. On contrary, IS# 1004, 4698, 23891, and 28141 significantly outyielded IS 33844 by 11.7% to 22.7%, but were comparable to controls for Brix (~13%) (Upadhyaya et al. 2014b). These accessions are therefore ideal genetic resource for use in breeding programs to develop sweet sorghum as bioenergy crop.

Core and mini core collections as diversity panel for assessing population structure, diversity, allelic richness and association genetics

The reduced subsets (both conventional mini core collections and reference sets) are ideal genetic resource for use as diversity panels for studying the population structure and diversity, and conduct association genetics for agronomically beneficial traits.

A. Dissecting population structure, diversity, and allelic richness

The analysis of allelic richness revealed that these reference sets captured between 78 to 95% allelic diversity of the global composite collections (Table 4), which sufficiently discriminated the majority of the accessions (with few exceptions) clustered based on biological and/or geographical diversity (Figure 3). For examples, the sorghum accessions structured according to geographic regions and race within region (Billot et al. 2013), the chickpea accessions separated based on seed types, with desi types differentiating from kabuli types (Upadhyaya et al. 2008a), the groundnut accessions largely clustered at subspecies level, and most of the wild relatives of chickpea, groundnut and pigeonpea grouped separately from cultivated types (Generation Challenge Program Project abstracts 2008; www.generationcp.org/component/docman/doc_download/281-2008?Itemid=15). More importantly, a

Table 4. Global composite collections and reference sets formed for ICRISAT mandate crops and small millets.

| Crop | SSRs used (#) | Composite collection | | Reference set | | Reference |
|----------------|---------------|----------------------|------------|---------------|-------------|------------------------------------|
| | | accession (#) | Allele (#) | accession (#) | Allele (#) | |
| Chickpea | 48 | 2915 | 1683 | 300 | 1315 (78.1) | Upadhyaya et al. 2008a |
| Finger millet | 20 | 959 | 231 | 300 | 206 (89.2) | GCP Project Report 2008 (abstract) |
| Foxtail millet | 19 | 452 | 362 | 200 | 315 (87) | GCP Project Report 2008 (abstract) |
| Groundnut | 21 | 852 | 490 | 300 | 466 (95.1) | GCP Project Report 2008 (abstract) |
| Pearl millet | 19 | 1021 | 230 | 300 | 218 (94.8) | GCP Project Report 2008 (abstract) |
| Pigeonpea | 20 | 952 | 197 | 300 | 164 (83.2) | GCP Project Report 2008 (abstract) |
| Sorghum | 41 | 3367 | 783 | 383 | 613 (78.3) | Billot et al. 2013 |

Figures in parenthesis represent proportion of reference set alleles representing composite collection alleles.

number of rare (frequency $\leq 1\%$), common (frequency from 1% to 20%), most frequent (frequency $> 20\%$) and unique (present in one or group of accessions but absent in other accessions or group of accessions) alleles were detected in all the crops investigated. Unique alleles together with DUS (distinct, uniform and stable) (Rathinavel et al. 2005) test may be used for identifying for propriety germplasm. Rare alleles are rich-resource to associate these allelic differences with new variations arising as a result of mutations or environment-induced variations in germplasm.

B. Association mapping

Advances in genomics have provided greater insights to the germplasm users into the structure of the germplasm diversity and mine allelic variation associated with beneficial traits for use in crop breeding. Understanding how diversity is structured to unlock its potential for crop improvement is an emerging area worldwide made possible by the rapid advances in scale, robustness and reliability and the sharp fall in unit costs of deploying marker technology. The genomes of several food crops including chickpea, foxtail millet, pigeonpea and sorghum have been sequenced (Patterson et al. 2009; Zhang et al. 2012; Varshney et al. 2012, 2013). More importantly, the resequencing of diverse germplasm with agronomically beneficial traits may provide researcher opportunities to associate these sequence differences with trait variation, as evidenced in maize and sorghum (Lai et al. 2010; Zheng et al. 2011). The deployment of new generation sequencing technologies will further accelerate efficient and

precise identification and tracking of thousands of genetic variants in the populations at much reduced cost (Hamilton and Buell 2012).

The mini core collection of sorghum has been extensively genotyped using large number of SSRs and SNPs markers and together with phenotyping data, a number of significant marker-trait associations have been discovered. For example, six and ten SNPs linked to plant height and maturity, with some having close proximity to previously mapped height/maturity QTL or candidate genes for plant height and inflorescence architecture (Upadhyaya et al. 2013b; Morris et al. 2013); eight SNPs associated with resistance to anthracnose (*Colletotrichum sublineolum*) with candidate genes for disease resistance found in most of the detected SNPs (Upadhyaya et al. 2013c); two to five SNPs linked to leaf rust (*Puccinia purpurea*) and grain mold (*Fusarium* sps.) (Upadhyaya et al. 2013d); one to two SSR alleles linked to kernel weight and tiller numbers localized with previously mapped QTL (Upadhyaya et al. 2012c).

Multi-trait genetically diverse germplasm for use in crop improvement

The genetically diverse germplasm with multiple resistant traits offer breeders opportunity to develop breeding and genetic mapping populations combining multiple resistances into an agronomically improved genetic background for cultivar development. A number of genetically diverse germplasm pairs with resistance to abiotic and/or biotic stresses, those possessing good seed quality and resistance to abiotic and/or biotic stresses, or those that are agronomically

desirable but susceptible have been identified both in chickpea and groundnut (Upadhyaya et al. 2013a, 2014a) for enhancing trait values. Clearly, more research is needed to identify genetically diverse germplasm pairs with agronomically beneficial traits to support crop breeding.

Conclusions

Core and mini core collections representing diversity in the entire collection of the germplasm of a given species preserved in the genebank are ideal resource for efficient conservation and utilization of plant genetic resources in crop improvement programs. Core and mini core collections or genotype-based reference sets are available in chickpea, finger millet, foxtail millet, groundnut, pigeonpea, pearl

millet, and sorghum. New sources of variations for resistance to abiotic and/or biotic stresses and of agronomic and nutritional (oil and protein, Ca, Fe and Zn, O/L ratio) traits have been reported for use in crop breeding. More importantly, a number of accessions with multiple resistance and nutrient dense types, some with specific adaptation (rainy and/or post-rainy seasons), are available in ICRISAT genebank, which can be accessed after signing with ICRISAT the *Standard Material Transfer Agreement* (www.icrisat.org/icrisat-ip-mta-htm). With the availability of abundant genomic resources on these crops, it is visualized that there will be increased use of genomics-based germplasm analysis to enhance use of germplasm and make impact in breeding programmes in near future.

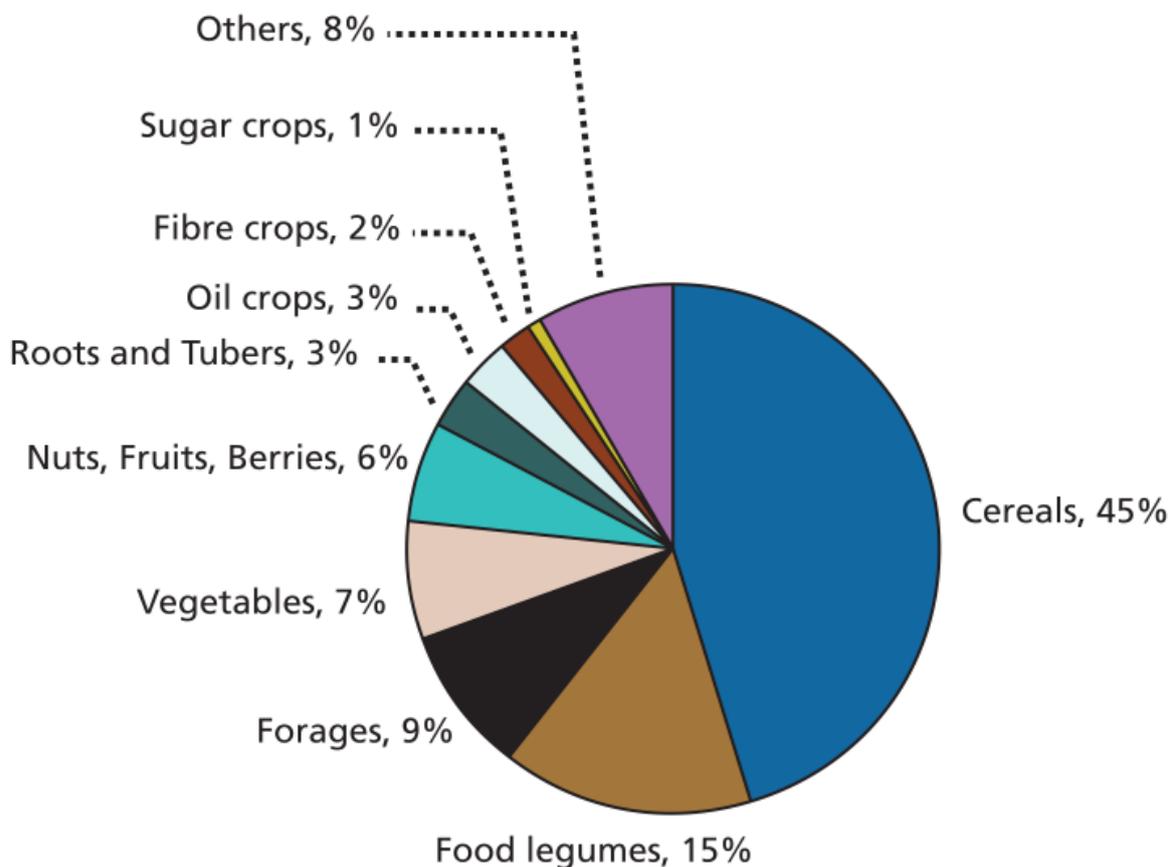


Figure 1. Plant genetic resources conserved over 1750 genebanks globally (FAO 2010; <http://www.fao.org/docrep/013/i1500e/i1500e.pdf>).

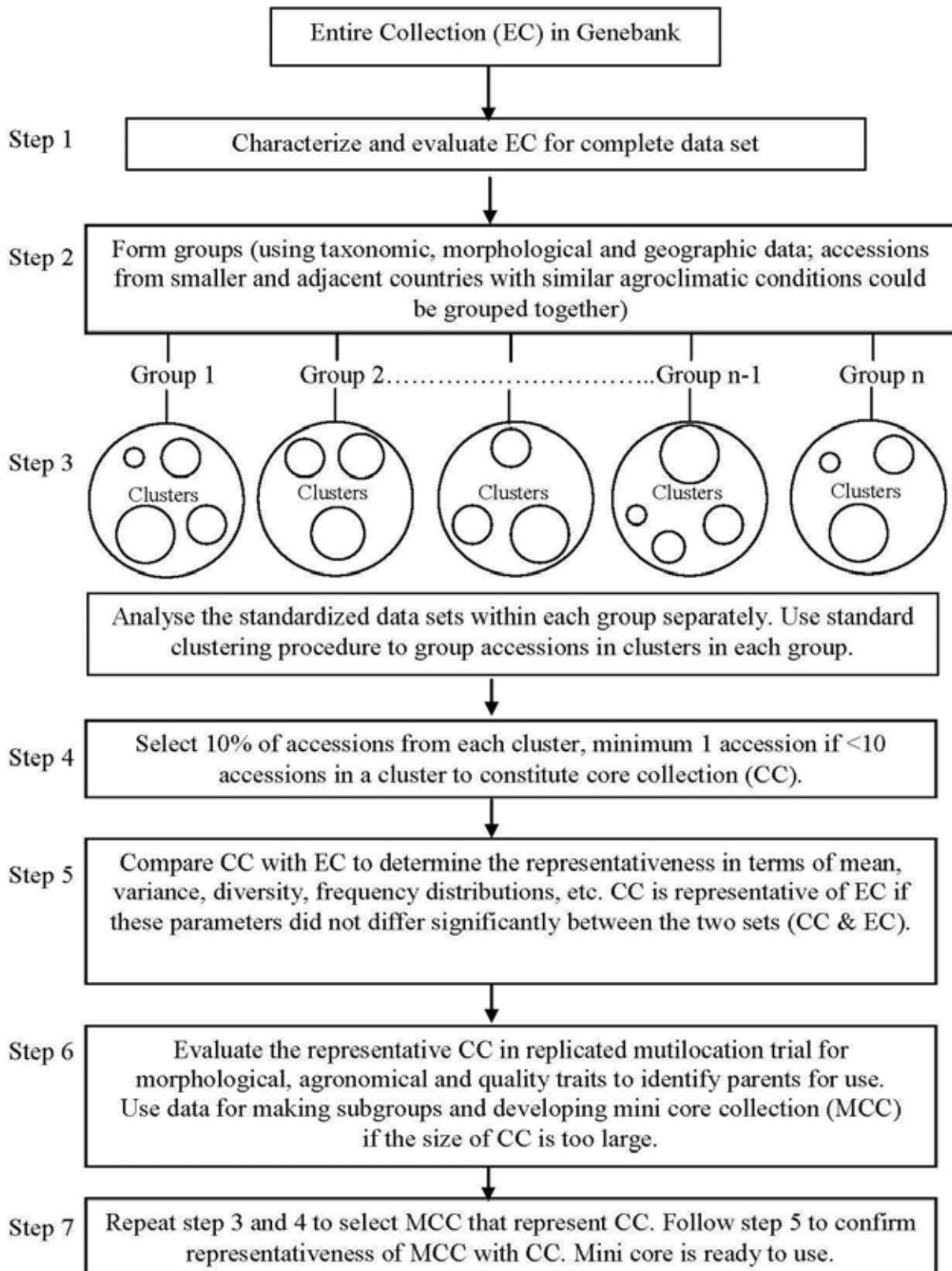


Figure 2. Flow diagram to establish core and mini core collections in a crop (Upadhyaya et al. 2009a).

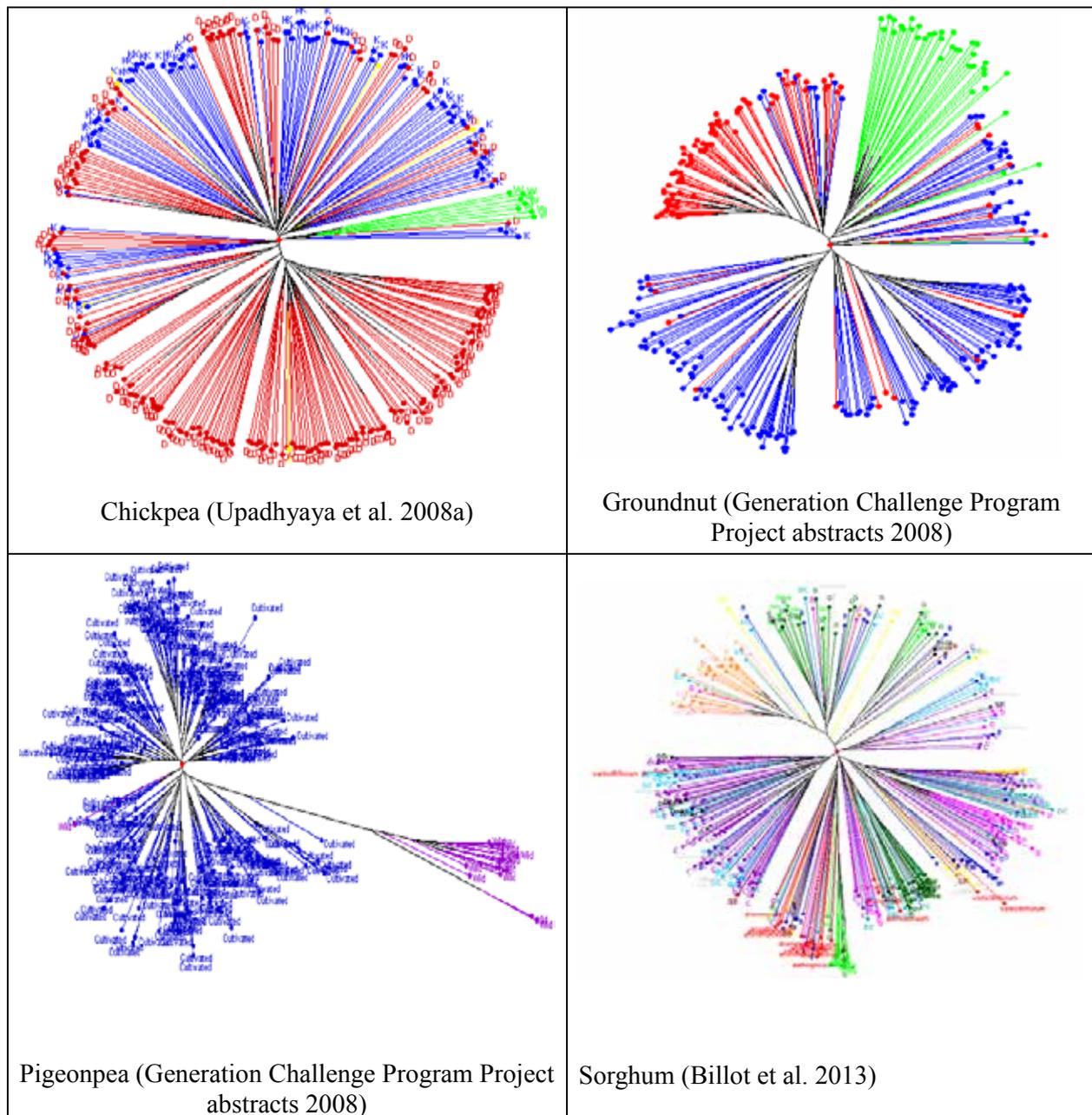


Figure 3: Tree diagram depicting population structure and diversity in reference sets of chickpea, groundnut, pigeonpea and sorghum.

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