

## Mini Core Collections for Enhanced Utilization of Genetic Resources in Crop Improvement

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Plant genetic resources form the raw material for developing high yielding cultivars. About 7.4 million accessions of various economically important crops have been conserved globally. Since the large size of germplasm collections hampers the assessment of their genetic worth, the 'mini core collection concept' was postulated and developed at ICRISAT. The mini core serves as an efficient and convenient option for assessment of genetic diversity, population structure, association mapping and targeted allele mining for agronomically important traits and acts as a gateway to the germplasm. Using the mini core collection approach, scientists at ICRISAT and in national programs have identified diverse sources of resistance/tolerance for many biotic and abiotic stresses, and for agronomic and quality traits in chickpea, groundnut, pigeonpea, sorghum, pearl millet, foxtail millet and finger millet. This is expected to enhance the use of germplasm in crop improvement. Molecular characterization of the mini core will further enhance its use in plant breeding programs.

**Key Words: Plant Genetic resources, Core collection, Mini core collection, Biotic and abiotic stresses, Nutritional quality traits**

### Introduction

Plant genetic resources (PGR) that harbor a large genetic variation are the most basic and essential raw material in crop improvement programs. Vavilov (1926, 1951) was the first to realize the significance of PGR in plant breeding. He recognized the centers of origin and diversity and organized massive germplasm collection missions. The crop germplasm which includes diverse landraces, exotics and wild relatives, holds a wealth of alleles, including rare alleles, which can help raise the yield ceiling and enhance stress resistance and nutritive quality level of elite cultivars. After the success of early plant breeding efforts and introduction of modern high yielding genetically uniform hybrid and inbred varieties much of the species diversity has been lost due to replacement of traditional varieties and landraces over wide areas all over the world. In addition, change in dietary habits, natural calamities, land conversion (deforestation, developmental activities such as hydroelectric projects, roads, and urbanization) and introduction of exotic and industrial (biofuels) crops have further aggravated the situation. Though they helped raise the world food output, the vulnerability of genetically uniform modern varieties to pests and diseases with occurrence of epidemics, changes in climate and market needs became evident since the 1980's. Thus the need for development of genetically broad based cultivars and concurrently stabilizing their yield potential through

incorporation of resistance to biotic and abiotic stresses was recognized. As this requires incorporation of alleles from highly diverse genetic resources, a network of international centers was initiated in 1960's and 1970's to accelerate the collection, characterization, evaluation, documentation, conservation and distribution of the crop genetic resources. Since then the germplasm collections of major crop plants continued to grow in number and size in the world (Brown, 1989a). The crop germplasm, exposed for millennia to edaphic and climatic variations found among and within different regions, socioeconomic differences among regions, as well as among farmers within these regions resulted in the evolution of specialized landraces (Paterniani, 1990). Diversity of cropping systems also contributed to variation and differentiation among landraces. At the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), India, a multi-disciplinary approach is followed for assessing the genetic worth of these genetic resources for biotic and abiotic stresses, agronomic traits and quality traits to identify donor lines as well as for updating and maintenance of databases.

Before modern plant breeding had its impact on agriculture, farmers were cultivating a large number of landraces of each crop. However, in the contemporary commercial and competitive agriculture, intensive monocropping with a few very successful cultivars that shared

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a narrow genetic base, eroded the ecosystem diversity and made crops vulnerable to many pests, diseases and climate change. The extinction of landraces and erosion of genetic diversity in the last century resulted in an estimated 75% loss of the crop diversity. A report by RAFI (Rural Advancement Foundation International, <http://www.rafiusa.org>) indicates that a large number of germplasm accessions in U.S. Department of Agriculture lists have been lost in the last 80 years. In The Philippines, only two rice varieties account for 98% of the area sown today against thousands of landraces farmers used to grow in earlier decades. Of 8000 traditional rice varieties that were grown in China in 1949, only 50 remained in 1970. Mexico has lost an estimated 80% of its maize landraces (<http://www.primalseeds.org/bioloss.htm>). Similarly in India also only a few modern varieties, with common pedigree are making a sustained presence in the seed chain of major crops.

The genetic resources management has two important aspects – germplasm conservation and its utilization in crop improvement. Germplasm can be conserved *in situ* by establishing ‘reserves’ or *ex situ* by assembling collections in genebanks through exchange or exploration. Maintenance is done by monitoring and protecting the reserves or storing the seed and periodically rejuvenating it, *ex situ*, in controlled conditions along with maintaining passport data. The evaluation involves assessing germplasm for agronomic traits which interact with the environment. Further the germplasm is enhanced by introgressing high value traits from exotic germplasm into adapted varieties (Bretting and Widrechner, 1995) through pre-breeding. To guard against the loss of valuable diversity, intensive collection of different crop species was undertaken by the global community. As a result, over 7.4 million *ex-situ* germplasm accessions are conserved in ~1750 genebanks globally, of which ~ 11% are in the genebanks of various CGIAR institutions. These genetic materials comprise of landraces or traditional varieties,

wild and weedy forms, related wild species, genetic stocks, inbred lines and even modern cultivars. ICRISAT has one of the largest collections in the CGIAR system, conserving 119,739 accessions of its mandate crops and six small millets from 144 countries (Table 1).

### The Need and Use of Germplasm by Plant Breeders

After the initial wave of high yielding cultivars in most crops, further improvement in yield potential has slowed down and is progressing in small increments and current breeding efforts are mainly directed towards stabilizing the yield potential as the vulnerability of genetically uniform modern varieties to pests, diseases, changes in climatic conditions and consumer preferences is well recognized. The diverse landraces, exotics and wild relatives hold a wealth of alleles, which, if included in breeding programs can help raise the yield ceiling as well as enhance stress resistance level and nutritional quality of cultivars. Most breeders concentrate their efforts on yield enhancement using already genetically alike cultivars/superior breeding lines as parents. Usually breeders deploy a small working collection of germplasm they are familiar with, as reliable information on potential donors for important traits on large germplasm collection is not readily available.

A large number of germplasm lines are distributed by the international genebanks for use in crop improvement programs. ICRISAT genebank distributed ~ 1.4 million seed samples to scientists in 144 countries (Table 1). In several instances, the exotic germplasm lines have been found high yielding and adapted to local conditions. Seventy five of such germplasm accessions (33 sorghums in 17 countries, 13 pigeonpeas in 7 countries, 15 chickpeas in 15 countries, 10 groundnuts in 14 countries, 2 finger millets in 1 country, 1 pearl millet in 3 countries and 1 barnyard millet in 1 country) which have performed significantly better for yield and other traits of economic importance have been directly released as cultivars. In

**Table 1. Status of germplasm collections conserved at ICRISAT, Patancheru, India**

Crop	Number of accessions conserved	Samples distributed		
		India	Other countries	ICRISAT
Sorghum	37,949 (92)*	130,212	128,749 (106)	237,035
Pearl millet	22,211 (50)	61,424	33,624 (79)	54,729
Chickpea	20,267 (60)	72,477	58,078 (88)	188,535
Pigeonpea	13,632 (74)	49,257	21,481 (111)	84,224
Groundnut	15,445 (92)	47,173	51,797 (94)	96,299
Small millets (6)**	10,235 (50)	42,604	20,803 (58)	7,906
Total	119,739 (144)	403,147	314,532 (143)	668,728

\* Figures in parenthesis indicates the number of countries

\*\* Finger millet, Foxtail millet, Proso millet, Barnyard millet, Little millet and Kodo millet

addition, 787 cultivars in 78 countries were released by the NARS partners from breeding materials supplied by ICRISAT that were developed using germplasm lines.

Only a very small proportion (<1%) of the germplasm collections has been used in crop improvement programs. During the period 1986-2008, the ICRISAT groundnut breeding program, for example, developed a total of 10331 advanced breeding lines (ICGV #) from thousands of crosses involving 1270 unique parents – out of these only 171 were germplasm lines, including 10 wild, from an entire collection of 15445 accessions. The most frequently used lines being Robut 33-1 (3110 times), Chico (1180 times), JL 24 (845 times), NCAc 1107 (481 times) and NCAc 2214 (469 times); they being either popular cultivars or breeding/germplasm lines. Like wise in chickpea (1978-2008), out of 20,267 accessions conserved only 94 unique accessions (including 5 wild) were used in developing 3728 advanced breeding lines; L550 (903 times), K850 (854 times) and GW 5/7, Annigeri and H 208 (>500 times) being the most frequently used. The pedigree analysis of the grain legume cultivars released by India's national and regional breeding programs (229 cultivars, up to 2003), showed 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), which clearly indicates their narrow genetic base. Similar situation prevails in other crops as well. Low use of germplasm has also been reported in wheat (Dalrymple, 1986), spring barley (Vellve, 1992) and maize (Cantrell *et al.*, 1996).

The reasons for low use of germplasm by crop breeders are that they continue to make reasonable progress, with their working collections and the apprehension that broadening the adapted genetic base would result in diminished agronomic performance (Kannenberg and Falk, 1995). In fact, elite inbred lines are considered the best genetic resources simply because each line contains a select combination of genetic traits that satisfies the farmer and the marketplace (Troyer, 1990). Yet, new germplasm if used in crop improvement programs can (a) raise the ceiling of genetic yield potential, (b) improve resistance to biotic and abiotic stresses, and (c) add new developmental pathways and ecological adaptations (Kannenberg and Falk, 1995).

Although plant breeders recognize the potential value of the diverse genetic resources, they are often reluctant to use these resources due to lack of reliable knowledge about their genetic worth; the load of unwanted genetic

linkages; lack of time and resources for identifying new superior donor genotypes for yielding ability, stress tolerance or better nutritional quality from a reservoir of germplasm; possibility of introducing toxic, allergenic, or pharmaceutically active plant products into food products, a risk that is virtually absent in crossing elite, widely grown germplasm (Heslop-Harrison, 2002). Thus a wide gap between available genetic resources and their use in breeding programs (Marshall, 1989) continues to exist.

### Strategies to Enhance the Use of Germplasm

Most economically important traits are quantitative, which are highly environment sensitive and display a great deal of genotype  $\times$  environment interaction. Hence, donor lines with very specific and simply inherited traits such as resistance to biotic stresses and occasionally abiotic stresses which can be followed easily through generations are preferred. Moreover, selecting a few lines from the vast pools of germplasm is like searching for a needle in a haystack. Obviously, it is more appropriate to have a small sample of a few hundred accessions, representing the entire diversity exhibited by the crop species, coupled with a multi-environment evaluation data, which would greatly encourage the breeders to opt for induction of more germplasm lines in to their breeding programs. Frankel (1984) proposed 'core collection' approach to meet this objective, which would 'represent with a minimum of repetitiveness, the genetic diversity of a crop species and its relatives'.

### The Core Collection

A representative sample that more or less reflects the diversity in the large entire collection would be cost effective and easy to maintain by individual breeders and facilitate the enhanced use of germplasm in breeding programs. The representative sample, named "core collection" (Frankel, 1984) is a subset, consisting of ~10% of total accessions, which between them capture most of the available diversity in the entire collection (Brown, 1989a). The size of the core collection should be limited to ~10%, using the sampling theory of selectively neutral alleles, with a ceiling of ~3000 per species. This level of sampling is effective in retaining a minimum of 70% of alleles of entire collection (Brown, 1989b). These can be thoroughly evaluated and the information so derived can be utilized for improving the efficiency of breeding programs. The guiding principles to constitute a core collection are that: a) the entire collection is a large taxonomic entity; b) the core collection has a greatly reduced size; c) the core

is a true representative of the entire collection and d) the core too is nearly as diverse as the entire collection.

It is not desirable to opt for absolute maximum possible diversity in the core collection, as it would lead to inclusion of large numbers of wild relatives. So a good core collection need not represent every part of the entire collection equally. Steps involved in constituting the core (Brown, 1989b; Upadhyaya *et al.*, 2009c) are:

1. **Deciding the size of the core:** The size of the core depends on the purpose for which it is being constituted. In general, the core should ultimately be of great use for all types of diverse breeding programs. If the core is assembled with a specific objective such as grain nutrient quality, only relevant accessions that are most diverse can be included, limiting the size of the core. The data on taxonomy, passport and characterization of the entire collection should be assembled and verified. About 10% of accessions from the total collection that retain most (at least 70%) of the alleles present in the entire collection are to be selected, to form the core.

2. **Sorting accessions into groups:** Using the available data, the accessions are grouped hierarchically into taxonomic groups (subspecies and races), geographic groups (country, state), climatic (agro-ecological) groups and by characterization data into specialized groups. Grouping the collection into smaller subgroups within groups is also done in such a way that the within group or subgroup variance is very low and between group variance is high. This type of stratification will increase the efficiency of sampling with the right choice of sample size for each group. When there is no basis for stratification, simple random sampling can be used (Brown, 1989a). The accessions that constitute a subgroup would be more or less uniform and therefore ~10% of accessions are retained from each subgroup generally.

3. **Selecting accessions for core:** After dividing the entire collection into groups, the number and choice of accessions from each group that enter into core is decided, based on considerations such as group size, within group genetic diversity, or the accessions with special merit and utility. The magnitude of diversity in the core is then compared statistically with that of entire collection to confirm that the core is representative and has captured most of the diversity in the entire collection.

4. **Managing the core collection:** The final stage is managing the core accessions themselves. They may be regenerated, held separately from the parent collection and further evaluated in multiple environments for agronomic,

quantitative traits or screened for specific purposes.

Following the above strategies, ICRISAT has developed core collections capturing over 80% of variability in the entire collections of sorghum (3575 accessions, Prasada Rao and Ramanatha Rao, 1995; 2247 accessions, Grenier *et al.*, 2001), pearl millet (1600 accessions, Bhattacharjee *et al.*, 2007; 2094 accessions, Upadhyaya *et al.*, 2009a), chickpea (1956 accessions, Upadhyaya *et al.*, 2001), groundnut (1704 accessions; Upadhyaya *et al.*, 2003), pigeonpea (1290 accessions; Reddy *et al.*, 2005), finger millet (622 accessions, Upadhyaya *et al.*, 2006a) foxtail millet (155 accessions, Upadhyaya *et al.*, 2008c), and Proso millet (Upadhyaya *et al.*, 2011b) using passport information and characterization data generated over a period of time (Table 2). The core collection could differ on scale and can be global, regional or even trait specific. However, against trait specific core, the arguments are: a) if the information is available for a trait on entire germplasm which is required, there is no need to develop core, as scientists can select the desirable genotypes from entire collection, and b) there would be multiple core collections (as many as traits) from a single entire collection, thus diluting the purpose of constituting the core collection. All the other germplasm that is not included in the core is retained and maintained as 'reserve collection'.

### The Mini Core Collection

The germplasm collections held by genebanks at most International Agricultural Research Centers (IARCs) are very large in size. For example the IRRI genebank holds ~ 120,000 rice accessions; hence the size of core collection (~10%) will be about 12000 accessions, which again restricts its proper evaluation and use by breeders. To overcome this constraint of large sized core collections, Upadhyaya and Ortiz (2001) postulated the "mini core" collection concept, and developed a two stage strategy to constitute it. The first stage in constituting the mini

**Table 2. Core and mini core collections developed at ICRISAT, India**

Crop	Number of accessions			
	Entire Collection	Used for core development	Core collection	Mini core collection
Sorghum	37,949	22,474	2,247	242
Pearl millet	22,211	20,844	2,094	238
Chickpea	20,267	16,991	1,956	211
Pigeonpea	13,632	12,153	1,290	146
Groundnut	15,445	14,310	1,704	184
Finger millet	5,949	5,940	622	80
Foxtail millet	1,535	1,474	155	35
Proso millet	842	833	106	–

core involves development of a core collection from the entire collection and the second stage involves evaluation of the core for various morphological, agronomic, stress tolerance and quality traits or need specific characters and selecting a further subset of about 10% accessions from the core. At both stages, standard clustering procedures are used to create groups of similar accessions to select entries to represent the group in the core/ mini core (Figure 1, Upadhyaya *et al.*, 2009c).

Following the strategy suggested by Upadhyaya and Ortiz (2001), scientists in different countries such as USA (groundnut, 112 accessions, Holbrook and Dong, 2005), Japan (rice, 50 accessions, Ebana *et al.*, 2008) and at ICRISAT have developed mini core collections (Table 2) of chickpea (211 accessions; Upadhyaya and Ortiz, 2001), groundnut (184 accessions, Upadhyaya *et al.*, 2002), pigeonpea (146 accessions; Upadhyaya *et al.*, 2006b), sorghum (242 accessions; Upadhyaya *et al.*, 2009b), pearl millet (238 accessions; Upadhyaya *et al.*, 2011d), finger millet (80 accessions; Upadhyaya *et al.*, 2010a) and foxtail millet (35 accessions; Upadhyaya *et al.*, 2011a). The reduced size of mini core collections has facilitated the efficient and economic multi-environment evaluation of germplasm lines by scientists resulting in identification of several new sources of variation for different traits for utilization in breeding programs.

#### Identification of Promising Donor Lines

Knauff and Gorbet (1989) observed that in general the use of germplasm in breeding programs has been limited to sources of - resistance to pests and diseases, male sterility, short stature or traits that have a simple inheritance. Efforts to identify germplasm lines for increasing yield potential are rare compared to stress resistance or nutritional quality traits (Halward and Wynne, 1991). Thus identification of promising sources for quantitative traits is a difficult task. Important accessions for tolerance to abiotic and biotic stresses and for agronomic and nutritional traits identified in ICRISAT mandate crops (chickpea, pigeonpea, groundnut, sorghum and pearl millet) and small millets using mini core collections are presented here.

**Drought:** Deep and extensive root system has been recognized as one of the most important traits for improving the productivity of the crop plants under limited soil moisture. From the chickpea mini core collection, Kashiwagi *et al.* (2005, 2007) identified 10 accessions each in desi and kabuli types of chickpea with high root length density (RLD), 10 accessions with long deep

roots and 6 accessions with slightly large shoot to root length density ratio (S/RLD) in comparison to a known drought tolerant accession, ICC 4958. A landrace from Turkey (ICC 8261), had a unique character of large RLD with long deep roots and large biomass allocation to the root system, which could be of high importance under severe drought conditions. Similarly in groundnut 18 lines with better drought avoidance traits were identified (Upadhyaya, 2005).

Genotypes with high water use efficiency (WUE) sustain their productivity even when water availability is limited. Upadhyaya (2005) evaluated groundnut mini core collection for traits such as SPAD Chlorophyll Meter Reading (SCMR) and Specific Leaf Area (SLA), which are surrogate traits and highly correlated with WUE and identified 18 (5 vulgaris and 13 hypogaea) highly diverse accessions with high SCMR and low SLA. About 10 accessions for transpiration efficiency, 10 accessions for root length density, and 10 accessions for total dry biomass were also identified from the groundnut mini core. Kashiwagi *et al.* (2006a) evaluated chickpea mini core collection and identified ICC 16374 for high SCMR (66.4). Similarly, lines for WUE and high SCMR (ICC 1422, 4958, 10945, 16374, 16903); for high carbon isotope discrimination ( $\delta^{13}\text{C}$ ) (-26.0%) and high TE under stress (3.9 g kg<sup>-1</sup>) and under well-watered (2.8 g kg<sup>-1</sup>) conditions (ICC 5337) were identified (Kashiwagi *et al.*, 2006b). Further, ICC 14799 had largest canopy area with relatively cool canopy temperature (Kashiwagi *et al.*, 2008). In groundnut, 30 (11 from mini core) consistently drought tolerant lines were identified from the reference set. (Hamidou, *et al.*, 2011).

**Water-logging:** Krishnamurthy *et al.* (2011a) identified 24 highly tolerant and 39 tolerant germplasm lines for water-logging from the pigeonpea mini core consisting of 146 accessions.

**Salinity:** Thirty salinity tolerant accessions yielding more than the tolerant control CSG 8962 were identified (Krishnamurthy *et al.*, 2011b) from the chickpea mini core collection screened under saline condition (80mM NaCl; using pot culture screening method) and large variation for seed yield under salinity was observed. Likewise, in pigeonpea mini core, 16 salinity (1.9 L of 80mM NaCl per 7.5 kg vertisol) tolerant lines were identified (Srivastava *et al.*, 2006). Similarly, 10 accessions in sorghum, 13 in pearl millet, 14 in groundnut, 10 in finger millet and 10 accessions in foxtail millet were identified as tolerant to salinity (Updhyaya *et al.*, 2009c, 2010b).

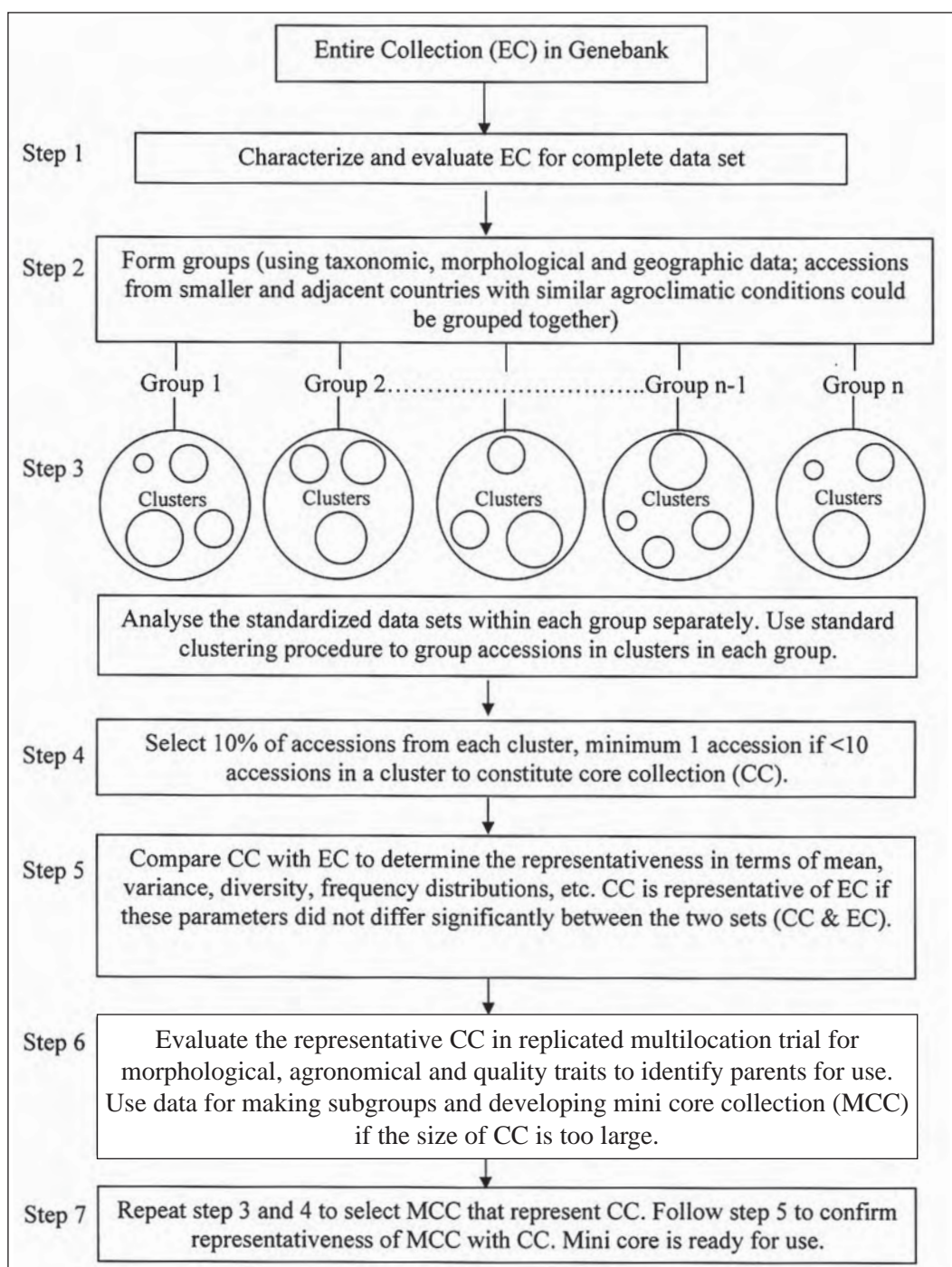


Fig. 1: Flow diagram to establish core and mini core collections in a crop species (adapted from Upadhyaya *et al.*, 2009c)

**Low and high temperature:** In groundnut, tolerance to low temperature at germination (12 °C) is an important trait. Several accessions of the groundnut mini core with capacity to germinate at lower temperature have been identified, many of them maturing and/or yielding similar to or better than the best control (Upadhyaya *et al.*, 2009d). Some of

the best performing low temperature tolerant accessions for pod yield include ICGs 12625, 13284, 2039, 13513, and 1824 in rainy season, ICGs 12553, 12625, 7898, 10595, 6148, 6022, 7013, 7884, 7905, and 4992 in post rainy season, and ICGs 12625, 7898, 11130, 6148, 7013, 6022, 7905, 7884, and 4992 for both the seasons.

In chickpea, ICC 14346 (BG 274, India) showed high tolerance to heat stress with least reduction in yield. A few accessions (ICC 14284, 6121, 7410, 13124, 14653, 11916, 5597, 14368, and 5829) that were on-par or high yielding than the control under heat stress and responsive to stress mitigation measures such as irrigation and nitrogen management were identified (Upadhyaya *et al.*, 2011c). Similarly from the chickpea reference collection, Krishnamurthy *et al.* (2011c) identified 18 (16 from mini core) stable heat tolerant lines.

**Diseases:** Pande *et al.* (2006) identified sources of moderate (with 3.1-5.0 score, on 1-9 scale) resistance to ascochyta blight (AB, 3 accessions), botrytis gray mold (BGM, 55 accessions) and dry root rot (DRR, 6 accessions). Twenty-one asymptomatic and 24 resistant sources for fusarium wilt (FW) and several multiple resistant lines such as ICC 11284 (for AB and BGM); ICC 11763 and 12328 (for BGM and DRR); ICC 1710, 2242, 2277 and 13441 (for DRR and FW); and ICC 2990, 4533, 6279, 7554, 7819, 9848, 12028, 12155, 13219, 13599 and 13816 (for BGM and FW) were also identified from the chickpea mini core collection (Pande *et al.*, 2006). Similarly in pigeonpea mini core, many accessions resistant to wilt (22), sterility mosaic (11) and both wilt and sterility mosaic (3) were identified.

In groundnut, 6 accessions were identified as having combined resistance to late leaf spot (LLS) and rust (R), 4 accessions for early leaf spot (ELS) and 3 for all the three diseases. Three accessions resistant to the bud necrosis disease, 5 to *A. flavus* colonisation and aflatoxin contamination were identified. In China, 14 accessions resistant to the bacterial wilt were identified. Similarly, Damicone *et al.* (2009) identified 5 accessions with high multiple resistance to *Sclerotinia* blight, pepper spot and web blotch.

Forty-nine grain mold resistant, 50 downy mildew resistant ( $\leq 3.0$  score) accessions and one with multiple resistances have been identified from sorghum mini core collection by Sharma *et al.* (2010). In further evaluation 13 mini core accessions were found resistant ( $\leq 3.0$  score) to anthracnose, 27 to leaf blight and 6 to both diseases (Sharma *et al.*, 2011). One accession, IS 473 showed resistance to all the four diseases (Anthracnose, Leaf blight, Rust and Grain mold) in the mini core collection. Scientists at the Texas A & M University, USA, have identified sorghum mini core accessions resistant to anthracnose (123), head smut (58) and downy mildew.

From the pearl millet mini core collection IPs 8418, 9934, 10263, 11405, 11428, 11930, 17775, and 20715 were identified as downy mildew free, for use in DM resistance breeding program.

In the finger millet core, 11 accessions highly resistant ( $< 1$  score on 1-5 scale) to neck blast, 57 accessions highly resistant to finger blast and 3 accessions resistant to both neck and finger blast, compared to  $> 80\%$  incidence in susceptible controls (VL 149 and VR 708) were identified (Kiran Babu *et al.*, 2011).

Blast disease of foxtail millet [*Setaria italica* (L.) P. Beauv.] caused by *Pyricularia grisea* (Cooke) Sacc. (teleomorph- *Magnaporthe grisea*) is a major problem both in India and Africa, causing substantial yield loss. Foxtail millet core collection was evaluated and neck blast resistant accessions ISe 375, 480, 748, 751, 769, 1037, 1067, 1204, 1320, 1335, 1387, 1419, 1547, 1593, 1685, 376 and 1541 were identified (Upadhyaya *et al.*, 2010b).

**Insect-pests:** Chickpea mini core was evaluated for helicoverpa pod borer resistance using detached leaf assay screening (Sharma *et al.*, 2005). ICC 5878, 6877, 11764, 16903, and 18983(1.0-2.3) had very low leaf-feeding score as compared resistant control cultivar ICC 506-EB (3.1). ICC 12537, 9590, 7819, 2482, and 4533 (37-47%) had least larval survival rate. Larvae fed on ICC 16903, 6877, 3946, 11746, and 18983 (1.2-2.1 mg larva<sup>-1</sup>) gained lowest larvae weight compared to ICC 506-EB (2.3 mg). Similarly, in pigeonpea, ICP 7, 655, 772, 1071, 3046, 4575, 6128, 8860, 12142, 14471, and 14701 exhibited promising levels of tolerance (damage rating 5.0 as compared to 9.0 in ICPL 87) to the helicoverpa pod borer. These lines also showed good yield potential ( $> 0.85$  to  $1.54$  t ha<sup>-1</sup>) under unprotected conditions, and had no wilt incidence as compared to 38.2% wilt in the control cultivar, ICP 8266. Twenty lines from groundnut reference set (including mini core) were promising for insect tolerance (defoliation  $< 5\%$ ) and with resistance to bud necrosis disease ( $< 1$ ) and high pod yield (2.25-4.25 t ha<sup>-1</sup>) compared to control cultivars M 13, Gangapuri, ICGS 44 and ICGS 76 (0.78-1.11 t ha<sup>-1</sup>) based on three years performance (Upadhyaya *et al.*, 2010b).

**Early maturity:** Appropriate time to maturity is a major component of crop adaptation, particularly in the environments where the growing season is restricted by terminal drought and high temperature and most breeding programs target early-maturing cultivars whose maturity

period matches with the available cropping duration. Twenty-eight early maturing chickpea accessions which were similar or earlier than the control cultivar Harigantars and ICCV2 and produced on an average of 22.8% higher seed yield than the control cultivars (Upadhyaya *et al.*, 2007c) were identified. Twenty-one early-maturing groundnut accessions which were similar in maturity to earliest maturing control cultivar Chico and produced 12.6% higher pod yield at 75 days and 8.4% more pod yield at 90 days compared to the average of control cultivars Chico, Gangapuri, and JL 24 were identified, (Upadhyaya *et al.*, 2006c). In pigeonpea, 20 accessions were early in maturity and produced more seed yield than the early maturing control cultivar ICPL 87. ICP 14471, 14903, 16309, 15068, 14832 and 9336 were the most promising accessions for extra early flowering (Upadhyaya *et al.*, 2010b). Khairwal *et al.* (2007) identified 25 pearl millet accessions for early flowering. IEs 501, 2093, 2957, 3543, and 4374 (40-50 days) in finger millet, ISe 1575 and ISe 1647 (<23 days) in foxtail millet were the most promising early flowering accessions. Similarly, six accessions (<50 days) were identified in sorghum for early flowering (Upadhyaya *et al.*, 2010b).

**Large Seed Size:** In chickpea consumers prefer the large seeded and white types for whole seed consumption, confectionary products, salads and savory meals. Thus seed size and color are important traits for trade in chickpea. Gowda *et al.* (2011) identified 65 large seeded (100-seed weight >40g) Kabuli chickpea lines for use in crop improvement using the core collection approach. ICC 14190, a highly fusarium wilt resistant, large-seeded (37.4g 100 seeds<sup>-1</sup>) Kabuli landrace from India which also ranked first with a mean yield of 1.43 t ha<sup>-1</sup> and high productivity (13.64 kg ha<sup>-1</sup> day<sup>-1</sup>) was identified. ICC 14194 and ICC 7344 were early flowering, extra-large seeded types (>55 g 100 seeds<sup>-1</sup>) with grain yield on par with the best control, L550. All these 3 genotypes exhibited high stability with a regression value of unity and deviation near zero. Another accession, ICC 17109, is an extra large seeded type (63 g 100 seed<sup>-1</sup>) but with a lower grain yield and low stability (highly significant S<sup>2</sup>d<sub>i</sub>) (Gowda *et al.*, 2011). These large seeded Kabuli types with high yield and stable performance can be used in breeding program to develop large-seeded high yielding Kabuli cultivars or used directly for cultivation. In groundnut, we identified, ICGs 2381, 5016, 5051, 5745, 5662, 6057, 6766, 8760, 11219, 11855, 11862 and 14482 (100-seed weight >60g) and in pigeonpea, ICP 14976, 13359 and 13139 (100-seed weight > 16g) for large seed size.

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Similarly, 15 accessions (>5.0g) in sorghum (Upadhyaya *et al.*, 2010b) showed high 100 seed weight. Khairwal *et al.* (2007) identified 16 large-seeded pearl millet accessions for utilization in crop improvement programs. Evaluation of chickpea mini core in India and groundnut in China, Vietnam, and Thailand resulted in identification of 13 large seeded chickpea accessions (Kaul *et al.*, 2005) in India and five large-seeded groundnut accessions each in China, Vietnam, and Thailand (Upadhyaya *et al.*, 2009c; Upadhyaya *et al.*, 2010b).

**Yield and Components:** Evaluation of chickpea core led to the identification of 39 highly diverse and superior accessions for a combination of agronomic traits such as early maturity, seed size and grain yield (Upadhyaya *et al.*, 2007a). Eighteen accessions had higher pod number (>50) and 2 accessions had higher seed number per pod (>1.5). Twenty three accessions were adapted to irrigated, 11 to non-irrigated, and 14 to both irrigated and non-irrigated environments. In multilocation testing, chickpea mini core accessions, ICCs 637, 1098, 3325, 3362, 4918, 7441, 8384, 8621, 9586, 12307, 14402, 14815 and 15868 produced greater seed yield than the control cultivars. Upadhyaya *et al.* (2005) identified 15 fastigiata, 20 vulgaris, and 25 hypogaea type groundnut accessions for pod yield and its components upon multilocation evaluation of ground core collection for Asia region. Similarly, upon multilocation evaluation of groundnut mini core (Upadhyaya *et al.*, 2002) ICGs 36, 1519, 3992, 5195, 5236, 8083, 9037, 9157, 9809, and 12988 for shelling percentage; ICGs 5745, 6646, 10036, 11088, 13099, and 15419 for pod yield were identified. From the pigeonpea mini core evaluation, several accessions with early maturity, greater harvest index and shelling percentage, and high grain yield were identified. Five accessions with higher grain yield (>2.5 t ha<sup>-1</sup>) compared to the control cultivars ICPL 87 (extra early), UPAS 120 (early), Maruti (medium) and Gwalior 3 (late) were identified. Two accessions ICP 14900 and ICP 1156 flowered in less than 100 days and produced higher seed yield than the extra early control cultivar ICPL 87. The study also identified ICP 8860 for greater number of primary branches (29); ICP 5860, ICP 11230, ICP 4167, ICP 8602 for more pods per plant based on multilocation evaluation of pigeonpea mini core collection (ICRISAT Archival report 2009).

Accessions with high green fodder yield, more productive tillers per plant, high ear head spikelet density, higher grain yield and large seed size were identified in pearl millet (Upadhyaya *et al.*, 2007b). Khairwal *et al.*



(2007) identified 15 accessions for green fodder yield and 9 accessions for higher grain yield potential based on multilocation evaluation of pearl millet core collection. Similarly, pearl millet core collection was evaluated at ICRISAT, Patancheru and we identified 20 accessions for grain yield, 9 for fodder yield, 11 for large seed size, and one accession for synchronous panicle maturity. Several new sources for high grain and/or fodder yield, extra-early flowering, more basal tillers, panicles with variable exertion and head shape were identified in sorghum. Additionally, 12 accessions with higher level of soluble sugar content in stalk (14-20%) were identified in the sorghum mini core collection.

Trait-specific accessions identified from the finger millet core collection include those with early flowering, more basal tillers, long inflorescence, high grain and/or fodder yield, more number of fingers per ear (ICRISAT Archival Report, 2009). New sources identified from foxtail millet core collection include ISe 1575 and ISe 1647 for early flowering (<23 days); ISe 792, 1059, 1067, 1258, 1474, 1575, 1581, 1593 and 1647 for high yield (>1.7 t ha<sup>-1</sup>); ISe 1789 and ISe 1851 for longer (>250 mm) and wider (>45 mm) inflorescence (Upadhyaya *et al.*, 2010b)

**Quality traits:** Core and mini core collections were evaluated for nutritional traits and 5 accessions for high seed protein (22.5-23.8%) in chickpea, 14 accessions each for zinc (38.9-41.4 mg kg<sup>-1</sup>) and iron (43.3-45.0 mg kg<sup>-1</sup>) in pigeonpea, 10 accessions each for high iron (58.2-87.0 mg kg<sup>-1</sup>) and zinc (33.4-39.1 mg kg<sup>-1</sup>) in sorghum, 10 accessions each for iron (104.2-123.6 mg kg<sup>-1</sup>) and zinc (83.7-88.3 mg kg<sup>-1</sup>) in pearl millet. Similarly, 15 accessions each for zinc (22.46-25.33 mg kg<sup>-1</sup>), iron (37.66-65.23 mg kg<sup>-1</sup>), protein (8.66-11.09%) and calcium (3860-4890 mg kg<sup>-1</sup>) contents in finger millet (Upadhyaya *et al.*, 2011e) and 21 accessions each for zinc (54.5-74.2 mg kg<sup>-1</sup>), iron (58.2-68.0 mg kg<sup>-1</sup>), protein (15.6-18.5%) and calcium (171.2-288.7 mg kg<sup>-1</sup>) contents in foxtail millet were identified (Upadhyaya *et al.*, 2011a). In a collaborative evaluation at ICRISAT, Patancheru and University of Agricultural Sciences, Dharwad, we identified 18 accessions each with high protein content (>29%), high oil content (>48%), high Oleic acid content (>58%) and high Oleic(O)/Linoleic(L) acid ratio (>2.5) and one accession with very high Oleic acid content (73.3%) and O/L ratio (6.91) (Upadhyaya *et al.*, 2012) and 2 accessions for low lectin content (Kusuma *et al.*, 2006) from the groundnut mini core collection. Similarly in China, 3 accessions with

high O/L ratio were identified. High oil accessions, 5 each in India, China, Thailand, and Vietnam were identified for use in the crop improvement programs (ICRISAT Archival Report 2009).

Trait specific lines for resistance/tolerance to late leaf spot, early leaf spot, rust, bacterial wilt, *A. flavus*, drought, low temperature at germination, and multiple resistance in groundnut; early maturing, large-seeded, high-yielding, high shelling lines with high seed protein, vegetable types, and lines tolerant to salinity, wilt, sterility mosaic, and *Phytophthora* blight in pigeonpea; early maturing, large-seeded, high yielding lines with, high zinc and iron content, and resistance to downy mildew in pearl millet; early maturing, large-seeded, high-yielding lines with high seed calcium and high stalk sugar content, and resistant to grain mold, downy mildew, leaf blight, rust, and multiple resistant in sorghum; early maturing, high-yielding lines with high calcium, iron, zinc and protein content, and resistance/ tolerance to drought, salinity, and blast disease in finger and foxtail millet have been identified.

### Molecular Marker Characterization of Mini Core Collections

Characterization of germplasm with molecular markers provides an opportunity for structural dissection to assess allelic diversity, identification of rare alleles from cultivated and wild species accessions which could be used to select specific accessions for allele mining for crop improvement. ICRISAT in collaboration with generation challenge program (GCP) and partners such as ICARDA, Syria; CIRAD, France; EMBRAPA, Brazil; and CAAS, China has developed the composite collections of sorghum, pearl millet, chickpea, pigeonpea, groundnut, finger millet and foxtail millet. The composite collections include core and mini core collections and were genotyped using 20-50 SSR (Simple Sequence Repeats) markers to study genetic diversity, population structure and to establish reference sets of genetically diverse accessions (200-400 accessions). To cite an example, the genetic structure, diversity and allelic richness in a world composite collection of chickpea (3000 accessions), using 48 SSR markers, was assessed and a reference set of 300 accessions was established at ICRISAT (Upadhyaya *et al.*, 2008b). The 48 SSR markers detected 1683 alleles in 2915 accessions, of which, 935 were considered rare, 720 common and 28 most frequent. The composite collections were also characterized for morpho-agronomic traits at ICRISAT, India. Reference sets based on SSR markers, qualitative traits, quantitative

traits and their combinations were formed and compared for allelic richness and diversity. In chickpea, for example, 48 SSR based reference set captured 78.1% alleles of the composite collection (1683 alleles) compared to 73.5% of alleles in the reference set based on 7 qualitative traits. The reference sets based on SSR and qualitative traits captured 80.5% alleles (1354) of composite collection. Similarly, in groundnut the SSR-based reference set captured 95.1% alleles (466) of composite collection (490) compared to 93.3% of alleles (457) in the reference set based on 14 qualitative traits. The reference sets based on SSR and qualitative traits captured 95.9% (470) alleles of the composite collection (Upadhyaya, 2008). In pigeonpea, a reference set based on SSR data and consisting of 300 most diverse accessions, captured 187 (95%) of the 197 alleles of the composite collection. Another reference set based on qualitative traits captured 87% alleles of the composite set (Upadhyaya *et al.*, 2008a). This demonstrated that both SSR and qualitative traits were equally efficient in capturing the allelic richness in the reference sets. Further, the reference set selected using quantitative agronomic traits performed well for these traits than the reference set based on SSRs.

### Mini Core Collection and Plant Breeders

Mini core collection, an International Public Good, is the gateway to access the genetic diversity by global community in the large germplasm collections of any species. Many national programs have shown interest in the mini core sets of different crops and ICRISAT, on request, has supplied 116 sets (Table 3) of mini core of chickpea, groundnut, pigeonpea, sorghum, pearl millet, finger millet and foxtail millet to NARS researchers in 18 countries. In many other countries the development of core and mini core sets is in progress in various crop species.

The feedback from NARS researchers revealed that mini core is most convenient for evaluation and identification of donors for various beneficial traits. Many scientists have reported useful variation for grain yield, quality and resistance/tolerance to various biotic and abiotic stresses. For example, 4 large seeded kabuli (ICCs 12033, 14203, 14187 and 14199) and 6 desi and kabuli types (ICCs 5879, 7255, 8350, 10393, 10885 and 13125) are being used in chickpea improvement in India (Kaul *et al.*, 2005, Johnson *et al.*, 2007). Likewise, 2

**Table 3. Core, Mini-core, Reference and Composite sets of germplasm supplied to researchers in various countries by ICRISAT, Patancheru, India**

Country	No. of consignments	Crop / collection
Algeria	1	Chickpea mini core
Argentina	1	Sorghum mini core
Australia	2	1 Sorghum reference, 1 Chickpea reference
Canada	3	3 Chickpea mini core
China	6	4 Groundnut, 1 Sorghum and 1 Foxtail millet mini core
Germany	2	1 Finger millet and 1 Foxtail millet Core
France	2	1 Sorghum and 1 Foxtail millet mini core
India	105	Sorghum (1 Core, 3 mini core, 2 reference); Pearl millet (2 core, 1 mini core); Chickpea (22 mini core, 1 reference); Pigeonpea (17 mini core); Groundnut (13 mini core, 1 reference); Finger millet (1 composite, 8 core, 12 mini core); Foxtail millet (13 core, 1 mini core); Proso millet (2 core) and Barnyard millet (4 core, 1 mini core)
Japan	6	3 Sorghum, 1 Chickpea, 1 Groundnut mini core, and 1 Foxtail millet mini core
Kenya	4	1 Finger millet core, 1 Finger millet mini core, 1 Pigeonpea core and 1 Sorghum reference
Mexico	1	Chickpea mini core
Mali	2	1 Sorghum and 1 Groundnut reference
Malawi	2	2 Groundnut mini core
Niger	4	1 Pigeonpea core, 2 Pigeonpea reference and 1 Groundnut reference
Nigeria	3	2 Groundnut mini core and 1 Groundnut reference
Senegal	1	Groundnut reference
Syria	1	Chickpea reference
Sweden	1	Chickpea mini core
Thailand	2	1 Groundnut and 1 Finger millet mini core
Tanzania	1	Finger millet mini core
United Arab Emirates	1	Pigeonpea mini core
Uganda	1	Finger millet mini core
USA	14	1 Chickpea mini core, 1 Finger millet mini core, 2 Groundnut mini core, 7 Sorghum mini core, 1 Foxtail millet core, 1 Pigeonpea mini core, and 1 Finger millet core
Viet Nam	2	2 Groundnut mini core
Total	168	

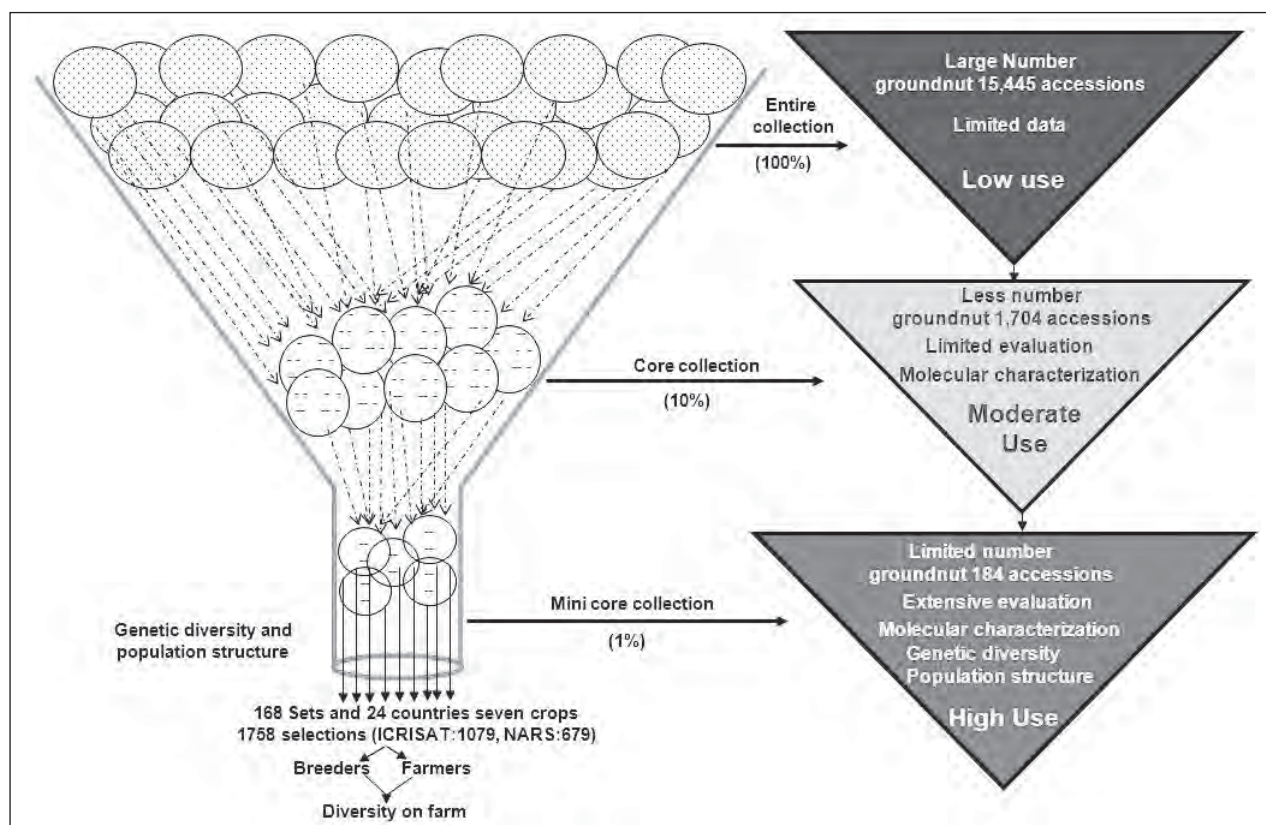


Fig. 2: Utility of the mini core collections for crop improvement

groundnut accessions ICG 8760 and ICG 3787, resistant to rust and late leaf spot in India (Kusuma *et al.*, 2007); 11 groundnut accessions with high quality oil and 14 accessions resistant to bacterial wilt in China; 5 large-seeded groundnut accessions each in China and Thailand; and 5 high shelling groundnut accessions each in China, Thailand and Vietnam provided useful variation for use in crop improvement in those countries (Upadhyaya *et al.*, 2010b). Several pigeonpea mini core accessions exhibited rich diversity for agronomic traits that researchers selected for use in pigeonpea breeding in India (Singh *et al.*, 2007). Preliminary evaluation of pigeonpea mini core further revealed that some of these accessions are adapted to nutrient-poor soil conditions (Rao and Shahid, 2007).

Overall, the mini core collections with their optimal and convenient size have helped in better evaluation, identification and increased use of germplasm by breeders (Fig. 2). This would definitely improve and enrich plant breeding by infusing new genetic diversity for developing broad based cultivars, paving way towards an ever green revolution.

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