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Lead Paper

Mini core germplasm collections for infusing genetic diversity in plant breeding programs

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Abstract
Plant genetic resources are essential components to meet future food security needs of world. Crop germplasm diversity contributes to developing improved crop cultivars aimed at increasing crop productivity. The large size of germplasm collections, coupled with unavailability of detailed data and information, has resulted in low use (<1%) of germplasm leading to a narrow genetic base in many crops. The miniaturization of crop collections with almost full representation of genetic diversity in the form of mini core (~1% of the entire collection) approach is an effective methodology to enrich and enhance crop improvement programs. The concept and process of developing mini core at The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) has been recognized worldwide as an "International Public Good" (IPG). The mini core provides a means for accessing the larger collections for further exploration and also helps in proper assessment of genetic diversity and population structure and for association mapping and targeted gene mining. Use of mini core approach will lead to greater utilization of diverse germplasm for developing broad-based cultivars, especially in the context of climate change. Many national programs have shown immense interest in evaluating mini core as reflected by the supply of 114 sets of mini core of chickpea, groundnut, pigeonpea, sorghum, pearl millet, foxtail millet and finger millet to researchers in 14 countries. Scientists have been able to identify new and diverse sources of variation for morpho-agronomic, quality, biotic, and abiotic stress resistance traits in various crops. The molecular characterization of the mini core will further enhance its use in plant breeding programs.

Key words:
Plant Genetic resources, mini core

Introduction

Germplasm is the total gene pool of a species consisting of landraces, advanced breeding lines, popular cultivars, wild and weedy relatives. It forms the raw material for any crop improvement program. Edaphic and climatic variations found among and within different regions, socioeconomic differences among regions, as well as among farmers within these regions result in the evolution of specialized landraces (Paterniani, 1990). Diversity of cropping systems also contributes to variation and differentiation among landraces. Nikolai Ivanovich Vavilov (1951), was the first to recognize the importance of genetic diversity for crop improvement and organized extensive germplasm collections of various crops from their 'centers of origin' and distribution for conservation. Since then the germplasm collections of major crop plants continued to grow in number and size in the world (Brown, 1989a).

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Loss of germplasm

Before modern plant breeding had its impact on agriculture, a large number of landraces of each crop were cultivated by the farmers. However, with the advent of modern plant breeding much of the species diversity has been lost due to replacement of traditional varieties and landraces with high yielding cultivars over wide areas. In addition, change in dietary habits, natural calamities, land and crop conversion (deforestation, developmental activities such as hydroelectric projects, road laying, urbanization), introduction of exotic crops, environmental pollution (loss of pollinators) etc. have further aggravated the situation. According to an estimate, 75% of the genetic diversity of crop plants was lost in the last century. A survey by RAFI (Rural Advancement Foundation International) found that approximately 97% of U.S. Department of Agriculture lists have been lost in the last 80 years.

Filipino farmers once grew thousands of kinds of rice, where as today only two varieties account for 98% of the area sown. Mexico has lost an estimated 80% of its varieties of maize. Of 8000 traditional rice varieties that were being grown in China in 1949, only 50 remained in 1970. The main reason for the loss of traditional varieties is their replacement by modern varieties (http://www.primalseeds.org/bioloss.htm). The same situation prevails in India also, with only a few modern varieties making a sustained presence in the seed chain of major crops. The vulnerability of genetically uniform modern varieties, which are planted to large areas, to new pests, diseases, climatic conditions and changes in the market needs is widely acknowledged. The diverse landraces, exotics and wild relatives hold a wealth of genes/ alleles, which, if included in breeding programs can help raise the yield ceiling as well as enhance stress resistance level of agronomically superior cultivars.

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 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, etc. The *evaluation* involves assaying 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 Widrlechner, 1995) through pre-breeding. To guard against the loss of valuable diversity, intensive collection of different crop species were 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, traditional or heirloom varieties, wild and weedy forms, related wild species, genetic stocks, inbred lines and even our modern cultivars. ICRISAT has one of the largest collections bank in the CGIAR system, holding 119,739 accessions of its mandate crops from 144 countries (Table 1).

Low utilization of germplasm in crop improvement

A large number of germplasm lines are distributed by the genebank for use in crop improvement programs. ICRISAT genebank distributed > 700,000 samples of accessions to scientists in India and 143 other countries (Table 1). Many germplasm accessions have performed significantly better for yield when evaluated in different environments and have been released directly as varieties. Globally, 75 germplasm accessions (33 sorghum accessions in 17 countries, 13 pigeonpea accessions in 7 countries, 15 chickpea accessions in 15 countries, 10 groundnut accessions in 14 countries, 2 finger millet accessions in 1 country, 1 pearl millet accession in 3 countries and 1 barnyard millet in 1 country) distributed from ICRISAT genebank have been directly released as cultivars. In addition, 657 cultivars in 78 countries have been released by the NARS partners from the breeding materials supplied by ICRISAT that were developed using germplasm lines (Upadhyaya and Gowda, 2009).

Of the germplasm supplied by the genebanks a very small proportion has been used in crop improvement programs. For example, at ICRISAT, between 1986 and 2008, a total of 10331 advanced groundnut breeding lines (ICGV #) were developed from thousands of crosses involving 1270 unique parents – out of these only 171 were germplasm lines, including 10 wild out 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 superior breeding lines. Like wise in chickpea (1978-2004), out of 20,267 accessions only 91 were used in the development of 3548 advanced breeding lines (Upadhyaya et al, 2006a). India has one of the largest breeding programs in legumes, and the pedigree analysis of the released 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 points out to their narrow genetic base. The 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). Breeders prefer to use parental lines from their working collections only, because they make reasonable progress in most crop species and broadening the activated genetic base generally will dilute agronomic performance (Kannenberg and Falk, 1995). Actually elite inbred lines are considered the

best genetic resources simply because each line contains a combination of genetic traits that satisfies the marketplace (Troyer, 1990). Yet new germplasm if used in crop improvement programs can (1) raise the genetic ceiling on improvement, (2) decrease vulnerability to biotic and abiotic stresses, and (3) add new developmental pathways and ecological adaptations (Kannenberg and Falk, 1995).

Reasons for low use

Although plant breeders recognize the limitation of their working germplasm and the potential value of wild and landrace resources, they are often reluctant to use these resources for following reasons:

a) lack of reliable knowledge about the genetic worth of the large germplasm collections;
b) a linkage load of many undesirable genes inherent to these genetic resources;
c) the search for a few superior donor genotypes for yielding ability, stress tolerance or better nutritional quality from a vast reservoir of germplasm is difficult and expensive;
d) unlike crossing of elites, complete program failures are possible; timescales may be too long; or the value of the new varieties may never allow costs to be recouped. Importantly, there is the 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 breeders tend to concentrate on adapted and improved materials avoiding wild parents, landraces and exotics available in germplasm banks (Nass and Paterniani, 2000) and thereby widening the gap between available genetic resources and use in breeding program (Marshall, 1989).

Strategies to enhance the use of germplasm

Crop breeders are reluctant to select parental lines from thousands of available germplasm line without knowing their performance especially for quantitative characters, which are highly environment sensitive and display a great deal of genotype × environment interaction. They generally opt for donor lines with very specific and simply inherited characters such as resistance to biotic stresses and occasionally abiotic stresses which can be followed easily through generations. Selecting a few lines from these 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 germplasm lines, 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'.

Core collection

Selecting a representative sample of all the diversity in the large collection would facilitate the enhanced use of germplasm in the breeding programs. Such samples would be cost effective and easy to maintain by individual breeders. A 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 entries to a 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 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:

- the entire collection is a large taxonomic entity
- the core collection has a greatly reduced size
- the core is a true representative of the entire collection and
- the core too is nearly as diverse as the entire collection

The core does not require the absolute maximum possible diversity, in which case it would be biased towards 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 (Upadhyaya et al., 2009c) are:

1. **Defining the collection to be represented and deciding the size of the core**: The core should ultimately be of great use for all types of diverse breeding programs. The data on taxonomy, passport and characterization of the entire collection should be assembled and verified. From this

total collection, ~10% of accessions are to be selected to form the core and retaining most (at least 70%) of the alleles present in the entire collection.

2. **Classifying the accessions into groups**: This is done hierarchically using the available data, into taxonomic groups (subspecies and races), followed by geographic groups (country, state), climatic (agro-ecological) groups and by characterization data into specialized groups. Grouping the collection into smaller subgroups within groups is 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 and when there is no base 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**: Having divided the whole collection in to groups, the next step is deciding number and choice of accessions from each group, which should be 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 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., 2001a), groundnut (1704 accessions; Upadhyaya et al., 2003), pigeonpea (1290 accessions; Reddy et al., 2005), finger millet (622 accessions, Upadhyaya et al, 2006b) and foxtail millet (155 accessions, Upadhyaya et al., 2008c), 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. All the other germplasm that is not included in the core is retained and maintained as 'reserve collection'.

Mini core collection

The germplasm collections held by most International Agricultural Research Centers (IARCs) genebanks are very large in size. For example the IRRI genebank holds more than 108,000 rice accessions; hence the size of core collection (~10%) will be about 11000 accessions, which again restricts its proper evaluation and use by breeders. To overcome this Upadhyaya and Ortiz (2001) to postulated the mini core collection concept, and developed the two stage strategy.

1. The first stage in constituting the minicore involves development of a core collection from the entire collection
2. The second stage involves evaluation of the core for various morphological, agronomic 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 (Fig. 1) to select the core/ mini core entries. Following this strategy scientists in different countries such USA (Holbrook and Dong, 2005), Japan (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., 2006c), sorghum (242 accessions; Upadhyaya et al., 2009b), pearl millet (238 accessions; Upadhyaya et al., 2010c), finger millet (80 accessions; Upadhyaya et al., 2010b) and foxtail millet (35 accessions; Upadhyaya et al., 2010a). The reduced size of mini core collections has provided ample opportunities to the breeders for their efficient and economic multi-environment evaluation, which has lead to the identification of several new

sources of variation for different traits for utilization in crop improvement programs.

Identification of promising donors

The use of genetic resources in the breeding programs have been mainly as sources of resistance to pests and diseases (Knauft and Gorbet, 1989), or as sources of male sterility, short stature or any such character with simple inheritance. In fact there have been fewer efforts for identifying germplasm lines for increasing yield potential than for pest resistance and nutritional quality (Halward and Wynne, 1991), because such traits are highly environment interactive and require multi-environment testing to accurately characterize them. Thus identification of promising resources for the environment sensitive quantitative characters is a difficult task. Important germplasm lines identified in various crops at ICRISAT for tolerance to abiotic and biotic stresses and for agronomic and nutritional characters are presented here.

Tolerance to abiotic stresses:

Drought: Drought affects the crop production adversely. 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. Kashiwagi et al. (2005) evaluated the chickpea mini core collection for root traits and identified two accessions with high root length density (RLD), ten accessions with long deep roots and six accessions with large shoot to root length density ratio (S/RLD) in comparison to a known drought tolerant accession, ICC 4958. ICC 8261, a landrace from Turkey, had a unique character of large RLD with long deep roots and large biomass allocation into the root system, which could be of high importance under severe drought conditions. Similarly two large-seeded Kabuli accessions were identified for high RLD (Kashiwagi et al., 2007)

Water use efficiency: When water availability is limited, productivity of the crops can be increased by culturing genotypes with high water use efficiency. 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 water use efficiency and identified 18 (5 *vulgaris* and 13 *hypogaea*) highly diverse drought tolerant accessions with high SCMR and low SLA. Evaluation of groundnut mini core also led to the identification of 10 accessions (1.58- 3.64 g biomass kg^{-1}) for transpiration efficiency, 10 accessions for root length density, and 10 accessions for total dry mass. Kashiwagi et al. (2006a) evaluated chickpea mini core collection and identified ICC 16374 for high SCMR (66.4). Similarly, lines for water use efficiency and high SCMR were identified (ICC 1422, ICC 4958, ICC 10945, ICC 16374, ICC 16903) (Kashiwagi et al., 2006 b, 2010) and for high $\delta 13^C$ (-26.0%) and high TE under stress (3.9 g kg^{-1}) and under well-watered (2.8 g kg^{-1}) conditions (ICC 5337) (Kashiwagi et al., 2006b). Further, ICC 14799 had largest area occupied by relatively cool canopy temperature (Kashiwagi et al.,2008).

Salinity: Vadez et al. (2007) evaluated chickpea mini core under saline condition (80mM NaCl; pot screening) and observed large variation for seed yield under salinity. 16 salinity tolerant accessions yielding more than the tolerant control CSG 8962 were identified. Likewise, in pigeonpea mini core also, 16 salinity (1.9 L of 80mM NaCl per 7.5 kg vertisol) tolerant lines were identified (Srivastava et al., 2006). Additionally, 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 (ICRISAT Archival Report, 2009).

Low and high temperature: The groundnut core collection was tested for tolerance to low temperature at germination (12^{o}C). Several accessions with capacity to germinate at lower temperature have been identified, with many of them maturing and/or yielding similar 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 postrainy season, and ICGs 12625, 7898, 11130, 6148, 7013, 6022, 7905, 7884, and 4992 for both season. Similarly 10 chickpea germplasm accessions were identified tolerant to high temperature upon evaluation of chickpea mini core collection under field conditions. ICC 15510, ICC 8318, ICC 8384, ICC 5639, and ICC 4991(2.7 – 2.97 t ha^{-1}) among desi types and ICC 15434, ICC 3410, ICC 9137, ICC 10885, and ICC 15802 (2.90 – 3.27 t ha^{-1}) among kabuli types were the best high yielding lines under high temperature conditions at Patancheru (ICRISAT Archival Report, 2009).

Water logging: Pigeonpea mini core collection was evaluated for tolerance to water logging. In a preliminary analysis, 16 water logging tolerant

accessions were identified from pigeonpea mini core (ICRISAT Archival Report, 2009).

Tolerance to biotic stresses:
Diseases: Sources of moderate (3, accessions with 3.1-5.0, on 1-9 scale) resistance to ascochyta blight (AB), 54 to botrytis gray mold (BGM), 6 for dry root rot (DRR); 21 asymptotic and 24 resistance sources for fusarium wilt (FW) and ICC 11284(AB, BGM); ICC 11763 and ICC12328 (BGM,DRR); ICC 1710, 2242, 2277 and 13441 (DRR, FW); and ICC 2990, 4533, 6279, 7554, 7819, 9848, 12028, 12155, 13219, 13599 and 13816 (BGM,FW) for multiple resistance have been identified in chickpea mini core collection (Pandey et al., 2006). Similarly in pigeonpea mini core collection, 22 accessions resistance to wilt, 11 to sterility mosaic (SM) and 3 accessions to both wilt and SM were identified (ICRISAT Archival Report, 2009).

In groundnut, six mini core accessions were identified as having combined resistance to late leaf spot (LLS) and rust (R), four accessions for early leaf spot (ELS) and three for all the three diseases. Three accessions resistant to the bud necrosis disease, five 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 five accessions with high multiple resistance to *Sclerotinia* blight, pepper spot and web blotch.

Forty nine grain mold resistant, 6 downy mildew resistant and one line with multiple resistances have been identified from sorghum mini core by Sharma et al. (2009). Fifty one lines were resistant (≤3.0 score) to downy mildew; 12 were resistant (≤3.0 score) to anthracnose. Three were found resistant to both anthracnose and grain mold whereas 17 accessions were moderately resistant (3.1 – 5.0) to both diseases; ten accessions highly tolerant to salinity. One accession IS 23992 showed resistance to all the five diseases (Downy mildew, Anthracnose, Leaf blight, Rust and Grain mold) (ICRISAT Archieval Report 2009). Scientists at the Texas A & M University, USA, have identified sorghum mini core lines resistant to anthracnose (123), head smut (58) and downy mildew.

Pearl millet mini core collection was evaluated for downy mildew and identified IPs 8418, 9934, 10263, 11405, 11428, 11930, 17775, 20715) as DM free for use in DM resistance breeding program. In finger millet, three accessions resistant (<10% incidence) to neck blast whereas, >100 highly resistant to finger blast (0% incidence) and three resistant to both neck and finger blast compared to >80% incidence in susceptible controls (VL 149 and VR 708) were identified (ICRISAT Archieval Report 2009).

Blast disease of foxtail millet [*Setaria italica (L.*) P. Beauv.] caused by *Pyricularia grisea* (Cooke) Sacc. (teleomorph- *Magnaporthe grisea*) is a major problem in India and Africa causing substantial yield loss. Foxtail millet core collection was evaluated and neck blast resistant foxtail millet accessions ISe 375, ISe 480, ISe 748, ISe 751, ISe 769, ISe 1037, ISe 1067, ISe 1204, ISe 1320, ISe 1335, ISe 1387, ISe 1419, ISe 1547, ISe 1593, ISe 1685, ISe 376 and ISe 1541) were identified (ICRISAT Archieval Report 2009).

Insect-pest: Chickpea mini core was evaluated for helicoverpa pod borer resistance. ICC 5878, ICC 6877, ICC 11764, ICC 16903, and ICC 18983(1.0-2.3) had very low leaf-feeding score under detached leaf assay screening as compared resistant control cultivar ICC 506-EB (3.1). ICC 12537, ICC 9590, ICC 7819, ICC 2482, and ICC 4533 (37 – 47%) had least larval survival rate. ICC 16903, ICC 6877, ICC 3946, ICC 11746, and ICC 18983 (1.2 – 2.1 mg larva^{-1}) were the best accessions for lower larvae weight compared to ICC 506-EB (2.3 mg). Similarly, in pigeonpea, ICP 7, ICP 655, ICP 772, ICP 1071, ICP 3046, ICP 4575, ICP 6128, ICP 8860, ICP 12142, ICP 14471, and ICP 14701 exhibited moderate levels of resistance (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^{-1}) under unprotected conditions, and had no wilt incidence as compared to 38.2% wilt in the control cultivar, ICP 8266. Twenty insect tolerant lines (defoliation <5%) with resistance to BND (<1) and high pod yield (2.25-4.25 t ha^{-1}) compared to control cultivars M 13, Gangapuri, ICGS 44, ICGS 76 (0.78-1.11 t ha^{-1}) were identified in groundnut based on three years performance (ICRISAT Archival Report, 2009).

Agronomic traits: The core and mini core collections have provided several new sources of variation for use in crop improvement programs.

Early maturity: Most breeding programs aim at developing early-maturing cultivars whose maturity period matches with the available cropping duration.

Appropriate time to flowering is a major component of crop adaptation, particularly in the environments where the growing season is restricted by terminal drought and high temperature. 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 germplasm 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 compared to the average of control cultivars Chico, Gangapuri, and JL 24 were identified, (Upadhyaya et al., 2006d). In pigeonpea, 20 accessions were early in maturity and produced more seed yield than the early maturing control cultivar ICPL 87. ICP 14471, ICP 14903, ICP 16309, ICP 15068, ICP 14832 and ICP 9336 were the most promising accessions for extra early flowering (ICRISAT Archival Report, 2009). Khairwal et al. (2006) 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 (ICRISAT Archival Report, 2009).

Large seed Size: Seed size and color are important traits in chickpea for trade. Consumers prefer the large seeded types for whole seed consumption, confectionary products, salads and savory meals. Using core collection approach Gowda et al. (2010) identified 49 large seeded (100-seed weight >40g) kabuli chickpea lines for use in crop improvement. ICC 14190, Fusarium wilt highly resistant Kabuli large-seeded (37.4 g) landrace from India also ranked first with a mean yield of 1.43 t ha^{-1} and high productivity (13.64 kg ha^{-1} day^{-1}. ICC 14194 and ICC 7344 were early flowering, extra-large seeded types (>55 g 100 $seeds^{-1}$) with grain yield on par with the best control, L 550. All these three genotypes exhibited high stability with regression value of unity and deviation near zero. Another accession, ICC 17109, is an extra large seeded type (63 g 100^{-1} seed) but with a lower grain yield and low stability (highly significant S^2d_i) (Gowda et al., 2010). The large seeded Kabuli types with high yield and stable performance identified in this study 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, ICP 13359 and ICP 13139 (100-seed weight > 16g) for greater seed size. Similarly, 15 accessions (>5.0g) in sorghum (ICRISAT Archival Report, 2009) showed high 100 seed weight. Khairwal et al. (2006) 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 (ICRISAT Archival Report, 2009).

Yield and components: Evaluation of mini core led to the identification of 39 chickpea accessions for a combination of agronomic traits such as early maturity, seed size and grain yield (Upadhyaya et al., 2007a). These accessions were superior in performance and diverse than the control cultivars. 18 accessions had higher pod number (>50) and two 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 a multi-location evaluation of chickpea mini core, ICCs 637, 1098, 3325, 3362, 4918, 7441, 8384, 8621, 9586, 12307, 14402, 14815 and15868 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 multi-location evaluation of ground core collection for Asia region. Similarly, upon multi-location evaluation of ground 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 (ICRISAT (Archival Report, 2009). 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^{-1}) 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 (29) for greater number of primary branches; 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 greater grain yield and large seed size were identified in pearl millet (Upadhyaya et al. 2007b). Khairwal et al. (2006) identified 15 accessions for green fodder yield and 9 accessions for greater grain yield potential based on multiplication 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 accessions for synchrony 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 (ICRISAT Archival Report, 2009).

Trait-specific accessions identified from core collection include those with early flowering, more basal tillers, long inflorescence, high grain and/or fodder yield, more number of fingers per ear head and high protein, calcium, iron and zinc content in finger millet. (ICRISAT Archival Report, 2008). New sources identified from foxtail millet core collection include ISe 1575 and ISe 1647 for early flowering (<23 days); ISe 792, ISe 1059, ISe 1067, ISe 1258, ISe 1474, ISe 1575, ISe 1581, ISe 1593 and ISe 1647 for high yield (>1.7 t ha-1); ISe 1789 and ISe 1851 for high inflorescence length (>250 mm) and width (>45 mm) (ICRISAT Archival Report, 2008).

Quality traits: Core and mini core collections were evaluated for nutritional traits and 5 accessions for high protein in chickpea seed, 14 accessions for zinc in pigeonpea seed, 10 accessions each for high iron and zinc in sorghum seed, one accessions for iron and two accessions for zinc in pearl millet seed, 10 accessions each for zinc, iron, protein, calcium, and beta carotene contents in both finger millet and foxtail millet were identified (ICRISAT Archival Report, 2009). Our NARS partners in India (UAS Dharwad) identified 11 accessions of groundnut mini core with high Oleic (O)/Linoleic (L) acid ratio and 11 for high lectin content. 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 improvement programs (ICRISAT Archival Report 2009).

The trait specific lines for high seed oil contents and high O/L ratio and resistant/tolerant 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 seed protein, high shelling percentage, vegetable type, and tolerant to salinity, wilt, sterility mosaic, and Phytopthora blight in pigeonpea; early maturing, large-seeded, high-yielding, high seed zinc content, high seed iron content, and resistant to downy mildew in pearl millet; early maturing, large-seeded, high-yielding, high seed calcium high stalk sugar content, and resistant to grain mold, downy mildew, leaf blight, rust, and multiple resistant in sorghum; early maturing, high-yielding, high seed calcium, iron, zinc, protein content, and resistant/tolerant to drought, salinity, and blast diseases in finger and foxtail millet.

Molecular characterization of mini core collections

An extensive characterization of plant genetic resources provides an opportunity for structural dissection to mine the allelic variations, and identify diverse accessions for crop improvement. These mini core collections can be used for molecular characterization to analyze genetic diversity at DNA level and to select distinct parents with maximizing diversity. The molecular characterization provides information related to rare alleles from cultivated and wild species accessions which could be used to select specific accessions for allele mining. 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 (Table 3).

The composite collections include core and mini core collections and have been 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 Center, Patancheru, 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 seven qualitative traits. The reference sets based on SSR and qualitative traits captured 80.5% (1354 alleles) of composite collection (Upadhyaya et al., 2008b). 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.

Mini core collection and plant breeders

Mini core is now an International Public Good and a gateway to access the genetic diversity by global community in any species. Many national programs have shown interest in the mini core sets of different crops and ICRISAT, on request, has supplied 114 sets (Table 4) of mini core of chickpea, groundnut, pigeonpea, sorghum, pearl millet, finger millet and foxtail millet to NARS researchers in 20 countries. In many other countries the development of core and mini core sets is in progress in various crop species. Mini core forms part of project proposals submitted in several countries and is subject of investigation for thesis research in India and the USA.

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 four large-seeded kabuli (ICCs 12033, 14203, 14187 and 14199) and six 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, two 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; five large-seeded groundnut accessions each in China and Thailand; and five groundnut accessions for high shelling percentage each in China, Thailand and Vietnam provided useful variation for use in crop improvement in those countries. (ICRISAT Archival Report, 2008). 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 optimal and convenient size of mini core collections have led to better evaluation and increased use of germplasm by breeders. Intensive screening and evaluation of the mini core sets have led to identification of diverse accessions for use in crop improvement programs. The utilization of such diverse accessions would definitely lead to enrichment of plant breeding by infusing new genetic diversity and in development of broad based cultivars thus paving way towards an ever green revolution.

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Table 1.Status of germplasm collections held at ICRISAT genebank as on 01-07-2010 (no. of countries)

Crop	Number of accessions conserved			Number of samples distributed		
				India	Other countries	ICRISAT
	Cultivated	Wild	Total			
Sorghum	37491	458	37949 (92)	129405	127957 (105)	233198
Pearl millet	21461	750	22211 (50)	61034	33535 (79)	53570
Chickpea	19959	308	20267 (60)	71568	57182 (86)	185226
Pigeonpea	13077	555	13632 (74)	48155	21220 (110)	83957
Groundnut	14968	477	15445 (92)	46731	51598 (93)	95772
Small millets	10076	159	10235 (50)	40520	20303 (58)	7575
Total	117,032	2,707	119739 (144)	397413	311795 (143)	659298

Table 2. Core and mini core collections developed for ICRISAT mandate crops

Crop	Accessions	Traits	Collection developed	Accessions in subset	Reference
Chickpea	3350		Core	505	Hannan et al. 1994
	16,991	13	Core	1,956	Upadhyaya et al. 2001a
	1956	22	Mini core	211	Upadhyaya and Ortiz 2001
Groundnut	7,432		Core collection	831	Holbrook et al. 1993
		15	Asian core	504	Upadhyaya et al. 2001b
	14,310	14	Core	1,704	Upadhyaya et al. 2003
			Valencia core	77	Dwivedi et al. 2008
	1704	31	Mini core	184	Upadhyaya et al. 2002
Pigeonpea	12,153	14	Core	1,290	Reddy et al. 2005
	1,290	33	Mini core	146	Upadhyaya et al. 2006c
Sorghum	33,100	7	Core	3,475	Prasada Rao and Ramanatha Rao 1995
	22,473	20	Core	2,247	Grenier et al. 2001
	40,000		Core	3,011	Dahlberg et al. 2004
	2,247	21	Mini core	242	Upadhyaya et al. 2009b
Pearl millet	16,063	11	Core	1,600	Bhattacharjee et al. 2007
	20,766	12	Core (Augumented)	2,094	Upadhyaya et al. 2009a
	2,094	18	Mini core	238	Upadhyaya et al. 2010c
Finger millet	5,940	14	Core	622	Upadhyaya et al. 2006b
			Mini core	80	Upadhyaya et al. 2010b
Foxtail millet	1,474	23	Core	155	Upadhyaya et al. 2008
			Mini core	35	Upadhyaya et al. 2010a

Table 3. Core, mini core and composite collections and germplasm reference sets at ICRISAT

Crop	Number of accessions					
	Entire collection	Used in core development	Core collection	Mini core collection	Composite collection	Reference set
Sorghum	37,949	22,474	2,247	242	3,384	384
Pearl millet	22,211	20,844	2,094	238	1,021	300
Chickpea	20,267	16,991	1,956	211	3,000	300
Pigeonpea	13,632	12,153	1,290	146	1,000	300
Groundnut	15,445	14,310	1,704	184	1,000	300
Finger millet	5,949	5,940	622	80	1,000	300
Foxtail millet	1,535	1,474	155	35	500	200

Table 4. Core, Mini-core, and Reference sets supplied

Crop	**Core**	**Mini core**[$]	**Reference set**	**Total**
Sorghum	-	**11** (ARG, IND, JPN,USA)	**5** (FRA, IND, KEN, MLI)	16
Pearl millet	**4** (IND,KEN,NER)	**1** (IND)	**2** (NER)	7
Chickpea	-	**28** (CAN, IND, JPN, MEX, Sweden, USA)	**2** (IND, SYR)	30
Pigeonpea	-	**11** (IND,UAE)	-	11
Groundnut	-	**25** (CHN,IND,JPN,MLI,MWI, NGA,THA,VNM)	**5** (IND,MLI,NER, NGA,SEN)	30
Finger millet	**10** (DEU,IND,KEN)	**4** (KEN,TZA,UGA,USA)		14
Foxtail millet	**9** (DEU,IND,USA)	2 (CHN, FRA)		11
Total	**23**	**82**	**14**	**119**

[$]= ARG Argentina, IND India, JPN Japan, USA United States of America, FRA France, KEN Kenya, MLI Mali, NER Niger, CAN Canada, MEX Mexico, SYR Syrian Arab Republic, UAE United Arab Emirates, MWI Malawi, THA Thailand, VNM Vietnam, NGA Nigeria, SEN Senegal, CHN China, TZA Tanzania and UGA Uganda

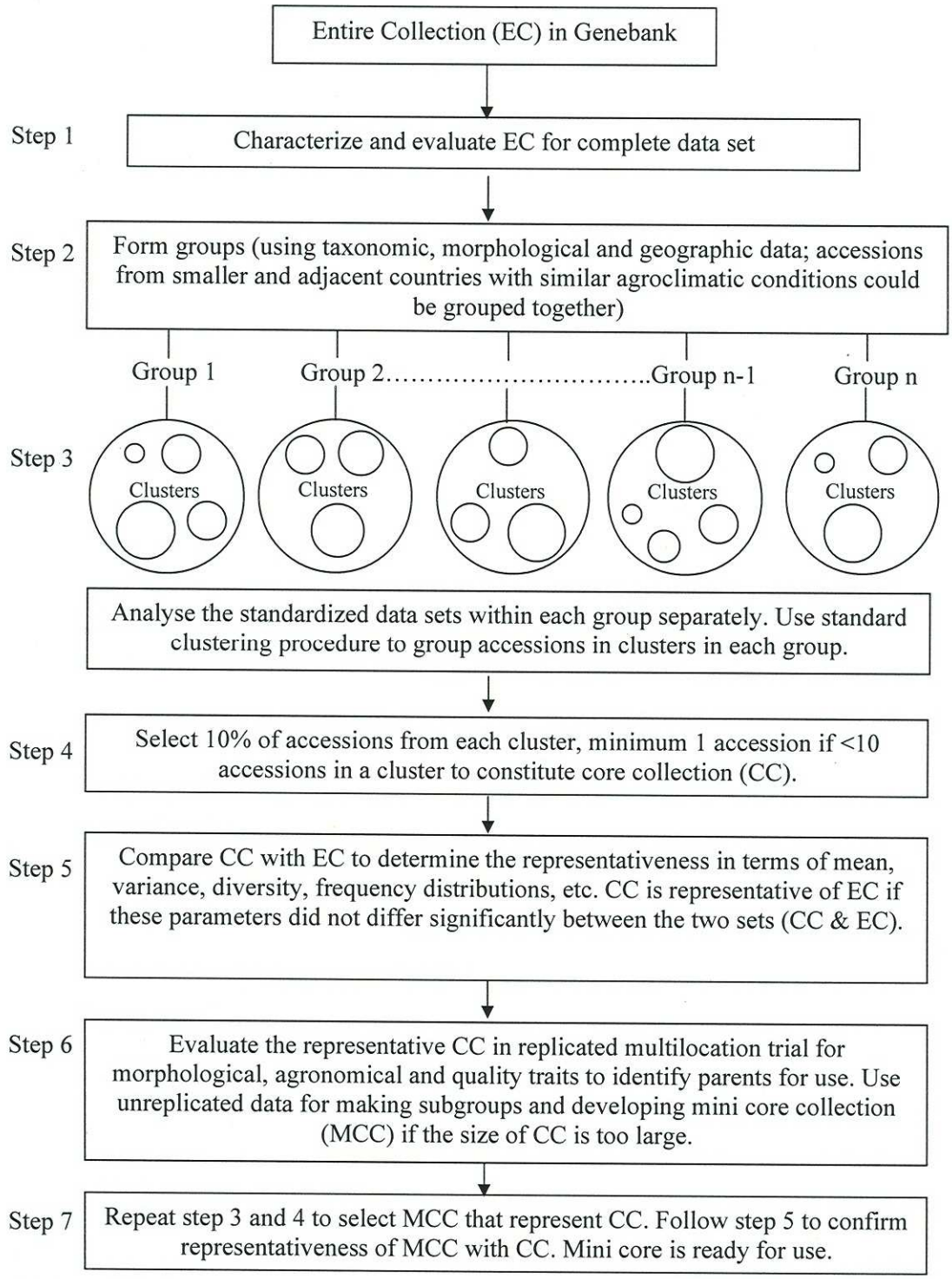

Figure 1. Flow diagram to establish core and mini core collections in a crop species (adapted from Upadhyaya et al., 2009)