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## Mini Core Collection: An International Public Good to Enhance Use of Germplasm in Crop Improvement

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### Abstract

Crop genetic resources provide the basic raw material for breeding new cultivars of crops. A large number of germplasm accessions of various crops have been conserved in a number of genebanks in different countries. However, only a very small proportion is being used by the scientists in the crop improvement programs which is mainly due to the lack of information on traits of economic importance that show genotype x environment (G x E) interactions. The mini core collection (10% of core collection or 1% of entire collection), representing over 80% diversity in the entire collection provides an opportunity to the scientists for efficient evaluation and identification of trait specific, genetically diverse, and agronomically desirable parental lines for use in improvement programs. The concept and process of developing mini core has been recognized as an International Public Good (IPG) that provides a gateway to access genetic diversity by global community. At ICRISAT, mini core collections of chickpea, sorghum, pigeonpea, groundnut, pearl millet and finger millet have been developed and evaluated. New sources of genetic variation for resistance/tolerance to various biotic and abiotic stresses and for agronomic traits have been identified. The utilization of these new sources of variation in crop improvement programs would have a great impact in developing improved high yielding cultivars with a broad genetic base.

### Introduction

The crop genetic diversity evolved over 10,000 years has given opportunity to the farmers to develop their crops and select new varieties. At present, global community is facing one of the greatest environmental and economic threats due to climate change. The crop genetic diversity would play a significant role to overcome adverse effects of climate change as it gives species the ability to adapt to new climatic conditions including against new pests and diseases. Crop genetic resources comprising local land races, obsolete varieties and wild species serve as a reservoir of many useful genes of economic importance and contribute enormously towards achieving the global objectives of food security, poverty alleviation, environmental protection and sustainable development.

During the 1960s, the Green Revolution had transformed agriculture around the globe. The local varieties and land races were replaced with the high yielding and fertilizer responsive varieties and the world grain production was increased by over 250%. However, the adoption of genetically uniform high yielding varieties resulted in the irreversible loss of innumerable heterogeneous traditional farmer's varieties and local landraces that contained useful



variation/genes. Since Green revolution a plateau has occurred in crop production and productivity and the problems related to food insecurity, malnutrition and environmental degradation are still unsolved.

To meet the food demand of ever increasing world population is the greatest challenge to agricultural scientists worldwide. The genetically uniform modern cultivars could become vulnerable to new pests and diseases resulting into epidemics. This necessitated the use of diverse sources in plant breeding program with a view to broaden the genetic base of cultivars. In addition, several new niches may have to be found for important crop species. To overcome these situations, it is essential to extend fruits of green revolution by sustainable crop production through efficient conservation and judicious use of diverse genetic resources for the present and future generations.

#### **Conservation of crop genetic resources**

The systematic efforts to explore, collect and conserve germplasm resources started in the 1960s. Today, there are about 1400 genebanks holding over seven million germplasm accessions across the world. The ICRISAT genebank has the global mandate for the germplasm collection, characterization, conservation and distribution of sorghum, pearl millet, chickpea, pigeonpea and groundnut and six small millets and holds about 1,19,691 accessions from 144 countries (Table 1). These accessions have been collected directly from the centre of origin and other high priority areas through the explorations as well as assembled through donations from the other institutes. The crop cultivars/elite breeding lines developed by breeding programs at ICRISAT have also been conserved in ICRISAT genebank for future utilization. The existing collections represent 70% - 80% of the available diversity and represent both insurance against genetic erosion and sources of resistance/tolerance to insect-pests and diseases, improved quality, yield related traits and changing climatic conditions for future crop improvement programs.

#### **Utilization of crop genetic resources for crop improvement**

The role of crop genetic resources in the improvement of cultivated plants has been long recognized (Frankel and Hawkes, 1975). At ICRISAT, over 98% of the accessions have been characterized for morphological and agronomic traits. Many germplasm accessions have performed significantly better and produced higher grain yield when evaluated in different environments across multilocations by NARS 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, two finger millet accessions in one country, one pearl millet accession in three countries, and one barnyard millet accession in one country), distributed to users 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 and developed using germplasm lines. These cultivars have greatly benefited the farmers by contributing to increase in production and productivity in those countries.



**Table 1. Status of germplasm accessions held at RS Paroda genebank, ICRISAT, Patancheru, India**

| Crop            | Accessions     |              |                | Origin (countries) |
|-----------------|----------------|--------------|----------------|--------------------|
|                 | Cultivated     | Wild         | Total          |                    |
| Sorghum         | 37,485         | 458          | 37,943         | 92                 |
| Pearl millet    | 20,844         | 750          | 21,594         | 50                 |
| Chickpea        | 19,941         | 326          | 20,267         | 60                 |
| Pigeonpea       | 13,077         | 555          | 13,632         | 74                 |
| Groundnut       | 14,968         | 477          | 15,445         | 92                 |
| Finger millet   | 5,844          | 105          | 5,949          | 24                 |
| Foxtail millet  | 1,481          | 54           | 1,535          | 26                 |
| Proso millet    | 842            |              | 842            | 30                 |
| Little millet   | 466            |              | 466            | 5                  |
| Kodo millet     | 658            |              | 658            | 2                  |
| Barnyard millet | 743            |              | 743            | 9                  |
| <b>Total</b>    | <b>116,349</b> | <b>2,725</b> | <b>119,074</b> | <b>144</b>         |

However, studies have shown scanty use of germplasm in crop improvement programs globally. At ICRISAT, the scientists used 986 parents (1986-2002) to develop 8279 advanced varieties in groundnut (ICGV#), but included only 132 unique germplasm and 10 wild *Arachis* species (Upadhyaya et al. 2006a) from about 16,000 germplasm accessions available in its genebank. Likewise, chickpea scientists at ICRISAT (1978-2004) used 12,887 parents (586 unique accessions) to develop 3548 advanced varieties (ICCV#), which included only 91 unique germplasm accessions and five wild *Cicer* species (Upadhyaya et al. 2006a). India has one of the largest breeding programs in legumes, which released 229 cultivars of chickpea, lentil, pigeonpea, blackgram and mungbean through hybridization and selection (data up to 2003). Pedigree analysis of these cultivars revealed that Pb-7 in chickpea, L-9-12 in lentil, T-1 and T-90 in pigeonpea, T-9 in blackgram, and T-1 in mungbean were the most frequently used parents (Kumar et al. 2004). The similar situation prevails in other crops. Low use of germplasm have also been reported in wheat (Dalrymple 1986), spring barley (Vellve 1992), and maize (Cantrell et al. 1996). This may be attributed to the use of working collection mostly consisting of elite breeding lines and some improved trait specific lines by most of the breeders, which results in re-circulation of the same germplasm and hence narrow genetic base of the resulting cultivars. Narrow genetic base of the crops results in genetic vulnerability which has already caused havoc in the past history such as southern corn leaf blight epidemic in USA in 1969-70 due to the use of genetically uniform male sterile lines over a large area. Hence, there is a dire need to identify the genetically diverse trait-specific germplasm accessions and their enhanced and efficient utilization in



crop improvement programs to develop elite and broad based genetic materials to meet the emerging challenges to agricultural production.

### **Strategies to enhance the use of germplasm**

The low use of germplasm accessions is mainly due to the lack of information on traits of economic importance such as yield, resistance to biotic and abiotic stresses, traits related to quality and adaptation, which often show high genotype x environment interactions and require replicated multiloacational evaluations. This is a costly and resource-demanding task owing to the large size of the germplasm collections. Thus, the collection needs to be sampled to get the size of the collections to a manageable level for meaningful evaluation.

#### **Core collection**

Frankel and Brown (1984) suggested that greater use of germplasm in crop improvement is possible if a small collection representing diversity of well characterized accessions is made available to researchers. Frankel (1984) coined the term "core collection" to sample representative variability from the entire collection. A core collection contains 10% accessions from the entire collection that captures most of available diversity in the species (Brown 1989a). Such core collection can be evaluated extensively and the information derived could be used to guide more efficient utilization of the entire collection (Brown 1989b). Using passport information and characterization and evaluation data generated over a period of time, the scientists at ICRISAT have developed global core collections in chickpea, groundnut, pigeonpea, sorghum, pearl millet, finger millet and foxtail millet (Table 2). The study of various parameters found that the adequate variability was sampled, while constituting the core collection in each crop.

#### **Mini core collection**

The germplasm collections at International Agricultural Research Center (IARC) genebanks are very large in size. For example IRRI genebank holds more than 1,08,000 rice accessions, hence the size of core collection will be about 10,000 accessions, which restricts its proper evaluation and use by the breeders. The large size of core collection motivated ICRISAT scientists to think of ways to overcome this obstacle. Upadhyaya and Ortiz (2001) postulated mini core concept and developed a seminal two-stage strategy for selecting mini core collections with minimum loss of variability. They suggested using the core collection as a basis for developing a mini core collection, which consists of ~10% accessions of the core collection (~1% of the entire collection). The first stage in constituting a mini core collection involves developing a representative core collection (about 10%) from the entire collection using the available information on origin, characterization, and evaluation data. The second stage involves evaluation of the core collection for various morphological, agronomic and grain quality traits, and selecting a further set of about 10% accessions from the core collection. At both the stages, standard clustering procedures would be used to create groups of similar accessions. Following this strategy, mini core collections have been constituted in chickpea, groundnut, pigeonpea, sorghum, and pearl millet (Table 2). Validation



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        |                      | Dwivedi et al. 2008                |
| Pigeonpea      | 1704       | 31     | Mini core            | 184                  | Upadhyaya et al. 2002              |
|                | 12,153     | 14     | Core                 | 1,290                | Reddy et al. 2005                  |
| Sorghum        | 1,290      | 33     | Mini core            | 146                  | Upadhyaya et al. 2006c             |
|                | 33,100     | 7      | Core                 | 3,475                | Prasada Rao and Ramanatha Rao 1995 |
| Pearl millet   | 22,473     | 20     | Core                 | 2,247                | Grenier et al. 2001a,b             |
|                | 40,000     |        | Core                 | 3,011                | Dahlberg et al. 2004               |
|                | 2,247      | 21     | Mini core            | 242                  | Upadhyaya et al. 2009b             |
|                | 16,063     | 11     | Core                 | 1,600                | Bhattacharjee et al. 2007          |
| Finger millet  | 20,766     | 12     | Core (Augmented)     | 2,094                | Upadhyaya et al. 2009a             |
|                | 2,094      | 18     | Mini core            | 238                  | ICRISAT unpublished data           |
| Finger millet  | 5,940      | 14     | Core                 | 622                  | Upadhyaya et al 2006b              |
| Foxtail millet | 1,474      | 23     | Core                 | 155                  | Upadhyaya et al. 2008              |

studies of these mini core collections with core collections revealed that these mini core collections represented >80 % diversity of the core or entire collections.

#### Role of mini core collections in crop improvement

The reduced size of mini core collections has provided ample opportunities to the scientists globally for their efficient and economic multilocational evaluation. The evaluation of these collections has identified several new sources of variation for various traits for utilization in crop improvement programs.

#### Tolerance to abiotic stresses

Frequent occurrence of drought is one of the major limiting factors adversely affecting crop production and productivity globally. Extensive and deep root systems have been recognized as one of the most important traits for improving the productivity of the crop plants under



low soil moisture conditions. Keeping these traits in mind, Kashiwagi et al. (2005) evaluated chickpea mini core collection for the root traits using a cylinder culture system and detected large genetic variation for root length density (RLD) and for the ratio of plant dry weight to root length density. Two accessions having the largest root length density, ten accessions having the largest root to total plant dry ratio (R/T), ten accessions having the largest root depth, six accessions having the largest shoot to root length density ratio (S/RLD) in comparison to previously identified drought tolerant accession ICC 4958 have been identified at 35 days after sowing. The line ICC 8261, a landrace collected from Turkey, had the largest RLD among all the mini core accessions. Under limited water conditions, productivity of the crop plants can also be increased by identifying the genotypes having high water-use efficiency. Hence, Upadhyaya (2005) evaluated groundnut mini core collection for the surrogate traits such as SCMR (SPAD Chlorophyll Meter Reading) and SLA (Specific Leaf Area), which are correlated with water-use efficiency and identified 18 drought tolerant accessions (five *vulgaris* and 13 *hypogaea* accessions). The clustering of these selected accessions showed that they are highly diverse and can be used in groundnut improvement programs to develop cultivars with a broad genetic base. Lines with high transpiration efficiency and greater root length have been identified in groundnut mini core collection.

Another ever-increasing factor limiting the crop production and productivity is the salinity. Vadez et al. (2007) evaluated chickpea mini core collection under saline condition (80 mM NaCl; pot screening) and observed a large variation for seed yield under salinity. 16 accessions yielding more than a previously released saline tolerant chickpea cultivar, CSG 8962 have been identified. Screening of pigeonpea mini core collection for salinity tolerance resulted in identification of 16 salinity tolerant genotypes that performed well under saline conditions (1.9 L of 80 mM NaCl per 7.5 kg Vertisol). All these 16 accessions were originated from India and Bangladesh. It was also observed that the level of tolerance was relatively higher in the mini core collection in comparison to those putatively selected from salinity affected areas (Srivastava et al. 2006). New sources of tolerance to early leaf spot and groundnut rosette disease have been identified in groundnut mini core collection (ICRISAT Archival Report 2008).

#### Tolerance to biotic stresses

Breeding disease resistant varieties is one of the most important and economic way to minimize the losses due to diseases in any crop improvement program. When the chickpea mini core collection was evaluated for biotic stresses, Pande et al. (2006) detected high level of resistance to *Fusarium* wilt in 46 accessions while 3 showed moderate resistance to *Ascochyta* blight, 55 to botrytis gray mold, and 6 to dry root rot. They also identified the sources for multiple resistances. Similarly, 49 grain mold resistant, 6 downy mildew resistant and one germplasm line with multiple resistances have been identified from evaluation of sorghum mini core collection at ICRISAT (Sharma et al., 2009). In the USA, scientists have identified sorghum mini core lines resistant to anthracnose (123 accessions), head smut (58) and downy mildew (31) at the college Station, Texas.



### **Agronomic and quality traits**

The mini core collections have also been evaluated to identify accessions with beneficial traits at ICRISAT and by NARS partners. Evaluation of chickpea mini core collection by the Indian scientists has identified 13 kabuli accessions with large seed size. Using the groundnut mini core, Chinese scientists identified four accessions with high oleic/linoleic acid (O/L) ratio, five accessions each with high shelling percentage and large seeds. Similarly, researchers from Thailand and Vietnam identified five accessions each with large seed size and high shelling percentage. Scientists at Dharwad, India have identified six groundnut lines with high O/L ratio.

### **Molecular characterization of mini core collection**

The recent advances in the field of molecular biology and bioinformatics have provided tremendous opportunities to the scientists worldwide to study the structure and variations among crop species and accessions at genotypic level. The molecular characterization of mini core collections will help in analyzing diversity at DNA level and would result in identifying genetically diverse parents and efficient gene mining. Their inclusion in working collections will add value to the working collections and use in crop improvement will result in enhancement of the trait and broaden the genetic base of cultivars. The core and mini core collections of chickpea, groundnut, pigeonpea, sorghum, pearl millet, finger millet and foxtail millet have been genotypes as part of the composite collections under the Generation Challenge program. These include genotyping of about 3000 chickpea accessions using 50 SSR markers; 3000 sorghum accessions using 41 SSR markers; 1000 groundnut accessions using 21 SSR markers; 1000 pigeonpea accessions using 20 SSR markers; 1000 pearl millet accessions using 20 SSR markers; 1000 finger millet accessions using 20 SSR markers; and 500 foxtail millet accessions using 20 SSR markers. Analysis of genotyping data revealed population structure in these crops and identified diverse trait specific lines in core or mini core collections and assisted in the establishment of the reference sets. The molecular characterization of the collections have provided information related to rare and unique alleles for cultivated and wild species accessions as well as accessions of geographic origin and will further reveal the usefulness of germplasm accessions in allele mining. The identification of genetically diverse lines have provided avenues to the breeders for using these diverse trait specific lines in the crop breeding programs for developing broad based cultivars.

### **Mini core collection and plant breeders**

ICRISAT's research on development of mini core collections and their evaluation for finding new sources of variation have been recognized worldwide because of the usefulness of the concept. Mini core is now an International Public Good and a gateway to access the genetic diversity by global community. There is a huge demand from the national and international programs for mini core collection for evaluation and use in crop improvement programs. ICRISAT has supplied 84 sets of mini core collections to researchers in 20 countries (Canada, China, India, Japan, Malawi, Mexico, Nigeria, Thailand, UAE, USA, France, Uganda, Tanzania, Senegal, Mali, Germany, Niger, Syria, Kenya and Vietnam) and scientists have reported





finding useful variation for grain yield, grain quality and resistance/tolerance to 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, groundnut and pigeonpea mini core collections provided useful variation for use in crop improvement in many countries. For example, 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 (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 increased demand for germplasm use by researchers. The small size of mini core collection have provided ample opportunities to the scientists across the globe to undertake intensive screening and evaluation which led to identifying new sources of variation for various traits. The need of plant breeder for trait-specific, genetically diverse, and agronomically desirable germplasm lines for use as parents in their breeding programs is being met by the mini core collection. The utilization of such diverse accessions in crop improvement programs should be enhanced to develop improved cultivars with broad genetic base and would definitely be one step towards sustainable agriculture paving way to Ever Green Revolution.

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