

Core and Mini Core Approaches for Enhancing Use of Germplasm in Crop Improvement

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Domestication of plants began long ago when the nomadic human beings turned from gatherers to growers and started growing plants of their choice for food and other needs. Since then man has depended heavily on biodiversity including plants and its parts as source of food. Crop diversity is part of the biological diversity and includes the resources that contribute to people's livelihoods by providing food, feed, medicine, fiber, clothing, shelter and energy. Hence, it contributes towards achieving the global objectives of food security, poverty alleviation, environment protection, and sustainable development. Crop diversity is a major component of crop improvement and is required for both short-term and long-term food security and to increase productivity and reduce malnutrition.

Collection of Germplasm

Widespread cultivation of high-yielding cultivars has posed a great threat to the reservoir of diversity evolved over millennia, particularly, to the landraces cultivated by the farmers. To conserve this diversity, large-scale efforts were made to collect before it is lost forever. This resulted in collection and conservation of more than 6 million accessions in over 1300 genebanks globally. The ICRISAT genebank conserves more than 116, 000 accessions of its five mandate crops, chickpea, pigeonpea, groundnut, sorghum and pearl millet and six small millets from 142 countries.

Utilization of Germplasm in Crop Improvement

The increase in accession numbers in genebanks and lack of corresponding increase in their use by the crop improvement scientists was a clear indication that the collections were not being used to their full potential (Marshall, 1989). A very large gap exists between availability and actual utilization of the materials. This was true both in the International Programs (CGIAR institutes) as well as in the national programs. For example, very few of the >14, 000 groundnut and >19, 000 chickpea accessions conserved in the genebank have been utilized in cultivar development of these two crops at ICRISAT (Upadhyaya et al., 2003, 2006a). Similarly, in the national programs, the germplasm lines used in breeding programs are very limited. In China, the introduced germplasm and wild relatives have seldom been used in groundnut improvement. In USA, the cultivar 'Dixie Giant' was a germplasm source in all pedigrees of runner type groundnut and 'Small White Spanish-1' cultivar in >90% pedigrees. These two lines contributed nearly 50% of the germplasm of runner cultivars of groundnut in the USA. In India, 86 chickpea, 14 lentil, and 47 pigeonpea varieties have been developed through hybridization between 1967 and 2003. Only 10 germplasm lines contributed 35% of genetic base in chickpea, 30% in lentil, 48% in pigeonpea, 69% in urdbean, and 71% in mungbean. Most plant breeders prefer to work with their own lines, rather than exotic materials. Not only the limited use of germplasm is a worrisome issue, the large-scale deployment of single cultivar complicates the whole situation even more. For example in the Netherlands, the three top varieties of nine major crops covered from 81% to 99% of the respective planted area. One cultivar accounted for 94% of spring barley. Sometimes, even if the number of cultivars is more, the degree of genetic diversity

between them is very low. In European barley, the protection against powdery mildew is increasingly dependent on one gene and one fungicide. Extensive use of fewer and closely related parents in crop improvement is contrary to the purpose of collecting large number of germplasm accessions, and could result in vulnerability of cultivars to pests and diseases. The fears of epidemics similar to the southern corn leaf blight in the USA (resulting in huge economic loss) and late blight of potato (that wiped out the potato crop resulting in the famine in Europe) due to narrow genetic base of crop cultivars looms large even today.

Establishing Core and Mini Core Collection

The main reason for low use of germplasm in crop improvement programs is the lack of information on large number of accessions, particularly, for traits of economic importance which display a great deal of genotype \times environment interaction and require multilocation evaluation. To overcome the size related problem of collection, developing a “core collection”, consisting about 10% of entire collection, representing the genetic variability of the entire collection, has been proposed. In developing core collection, available passport and characterization/evaluation data was used. It is always better to have a complete set of data on entire collection. Grouping of accessions from geographically similar countries or regions of a big country helps in making regional groups. The data on accessions in the regional groups is then subjected to multivariate analysis to classify the accessions in to different clusters using a suitable clustering method. From each cluster 10% accessions are randomly selected to identify a core collection. Core and entire collections are compared for various parameters to determine whether core collection is representative of entire collection. Scientists at ICRISAT have developed core collections of all the five mandate crops and finger millet (Table 1). However, it soon became evident that developing core collections will not solve the problem of low use of germplasm, as even the size of core collection would be unwieldy for convenient exploitation by the breeders and other crop improvement scientists. This was particularly true in the crops where entire collection is too large (several thousands). To overcome this, Upadhyaya and Ortiz (2001) proposed “mini core collection” concept and suggested a seminal two-stage strategy in chickpea. The first stage involves developing a representative core collection (about 10%) from the entire collection using all the available information on origin, geographical distribution, and characterization and evaluation data of accessions. The second stage involves evaluation of the core collection for various morphological, agronomic, and quality traits, and selecting a further subset of about 10% accessions from the core collection. Thus the mini core collection contains 10% of the core or ~ 1% of entire collection and represents the diversity of the entire collection. At ICRISAT, mini core collections have been established for chickpea, pigeonpea, and groundnut germplasm (Table 1).

Table 1. Core and mini core collections established at ICRISAT, Patancheru, India.

Crop	Number of accessions used	Number of traits involved (core)	Number of accessions (core)	Reference (core)	Number of traits involved (mini core)	Number of accessions (mini core)	Reference (mini core)
Chickpea	16991	13	1956	Upadhyaya et al., 2001a	16	211	Upadhyaya and Ortiz, 2001
Pigeonpea	12153	14	1290	Reddy et al., 2005	34	146	Upadhyaya et al., 2006d
Groundnut	14310	14	1704	Upadhyaya et al., 2003	47	184	Upadhyaya et al., 2002
Groundnut (for Asia Region)	4738	15	504	Upadhyaya et al., 2001b			
Sorghum	22473	20	2247	Grenier et al., 2001			
Pearl millet	16063	11	1600	Bhattacharjee et al., 2007			

Using Core and Mini Core Collections to Identify Trait-Specific Germplasm for Use

Due to its greatly reduced size, mini core collections provide an easy access to the germplasm collections. Breeders can evaluate the mini core collection easily and economically for traits of economic importance to identify trait-specific germplasm for use. The Asia and global core and mini core collections of groundnut developed at ICRISAT has been used in identifying sources for high oil and protein contents, large seed size, shelling turn over, and yield potential (Upadhyaya et al., 2005), early maturity (Upadhyaya et al., 2006c) and drought related traits (Upadhyaya, 2005). Similarly, in chickpea, sources for high yield (Upadhyaya et al., 2007a), early maturity (Upadhyaya et al., 2007b), large seed size, drought resistance traits (Kashiwagi et al., 2005; 2006), salinity tolerance (Serraj et al., 2004) and diseases resistance (Pande et al., 2006) have been identified (Table 2). In pigeonpea, sources for early maturity and high yield have been identified in core collection (Upadhyaya et al., 2006b) and for resistance to sterility mosaic disease and salinity tolerance (Srivastava et al 2006) in the mini core collection. Mini core collection of groundnut is being evaluated for resistance to *A. flavus* seed colonization and aflatoxin production.

Utilization of Mini Core Collections by National Programs

Mini core collections of groundnut, chickpea and pigeonpea are becoming popular with the national programs scientists in identifying trait-specific germplasm for use in their breeding programs. Twenty-six sets of chickpea mini core have been sent on request to scientists in India, Japan, USA, Canada, and Mexico. Similarly, 20 sets of groundnut mini core in India, China, Japan, Malawi, Nigeria, Thailand, and Vietnam, and eight sets of pigeonpea mini core in India and UAE, Dubai are being evaluated. Scientists have identified several useful sources of different traits for use in their crop improvement programs (Table 2).

Table 2. Number of chickpea, pigeonpea, and groundnut mini core sets evaluated and trait-specific germplasm accessions identified in different countries

Crop	Number of sets evaluated	Countries	Trait-specific germplasm Identified
Chickpea	26	5 (India, Japan, USA, Canada, and Mexico)	Early maturity (28), large-seeded Kabuli (16), high yield (39), resistant/tolerant to: ascochyta blight (3), botrytis gray mold (55), wilt (67), dry root rot (5), helicoverta (7), drought (18), salinity (29) and multiple resistance (31).
Pigeonpea	8	2 (India and UAE (Dubai))	High yield combined with other agronomic traits (54), early maturity (20), sterility mosaic (11), wilt (4), phytophthora blight (78), salinity (16) and multiple resistant (2).
Groundnut	20	7 (India, China, Japan, Malawi, Nigeria, Thailand, and Vietnam)	Early maturity (21), high yield combined with other traits (60), salinity (6), drought (18), aflatoxin (5), bacterial wilt (14), high oleic/linoleic acid ratio (4), high oil content (7), large seed size (5 each in china and Thailand), high shelling turn over (5 in Thailand), tolerant to low temperature (24).

Molecular Characterization of Mini Core Collection

Molecular characterization of core and mini core collections is important to discern the diversity at DNA level and identify genetically diverse parents for mapping and use in breeding programs. Mini core collections of chickpea, groundnut, pigeonpea, and core collections of sorghum, pearl millet, and finger millet have been genotyped as part of the composite collections (3000 each in chickpea and sorghum, and 1000 in other crops) under the Generation Challenge Program using 50 or 20 SSR markers. The analysis of genotyping data revealed population structure in these crops and diversity among the lines identified for a particular trait in the core or mini core collection. This helped us to clarify the reasons for low polymorphism in the mapping

populations made on phenotypic data beside providing breeders with the avenue of using genetically diverse parents to enhance trait (s) and developing broad based cultivars.

Conclusions and Future Outlook

Germplasm is basic to crop improvement programs for sustainable agriculture. Trait- specific genetically diverse parents for trait enhancement are the primary need of the plant breeder. Agronomically superior or similar lines are preferred by breeders to maintain the agronomic performance of breeding lines while improving the trait. Our strategic research on core and mini core collections, and identification of new diverse sources will enhance the use of germplasm in breeding programs, aimed at producing agronomically superior cultivars with broad genetic base. Molecular characterization of mini core and trait-specific subsets will further reveal genetic usefulness of the germplasm accessions in allele mining. Another dimension of breeders' requirements is agronomic desirability of the germplasm lines. This helps them maintaining or even improving the agronomic performance of breeding lines while enhancing the traits expression. Thus our aim is to identify the trait-specific genetically diverse and agronomically similar or better germplasm lines for use in the crop improvement programs to develop high yielding cultivars with a broad genetic base. The easy and convenient evaluation of mini core even for agronomic traits would help identifying such lines.

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References

- Bhattacharjee, R., Khairwal, I.S., Bramel, P.J. and Reddy, K.N. 2007. Establishment of a pearl millet [*Pennisetum glaucum* (L.) Br] core collection based on geographical distribution and quantitative traits. *Euphytica* (in press).
- Grenier, C., Bramel P.J. and Hamon, P. 2001. Core collection of sorghum:1. Stratification based on eco-geographical data. *Crop Science* 41:234-240.
- Kashiwagi, J., Krishnamurthy, L., Upadhyaya, H.D., Krishna, H., Chandra, S., Vincent Vadez and Serraj, R. 2005. Genetic variability of drought avoidance root traits in the mini-core germplasm collection of chickpea (*Cicer arietinum* L.). *Euphytica* 146: 213-222.
- Kashiwagi, J., Krishnamurthy, L., Singh, S. and Upadhyaya, H.D. 2006. Variation of SPAD chlorophyll meter readings (SCMR) in the mini core germplasm collection of chickpea. *International Chickpea and Pigeonpea Newsletter* 13: 16-18.
- Marshall, D.R. 1989. Limitations to the use of germplasm collections. In: Brown AHD, Frankel OH, Marshall DR and Williams JT (eds). *The use of plant genetic resources*. New York: Cambridge University Press, pp. 105-120.
- Pande, S., Kishore, G.K., Upadhyaya, H.D. and Narayan Rao, J. 2006. Identification of sources of multiple disease resistance in mini-core collection of chickpea. *Plant Disease* 90:1214-1218.

- Reddy, L.J., Upadhyaya, H.D., Gowda, C.L.L. and Sube Singh. 2005. Development of core collection in pigeonpea [*Cajanus cajan* (L.) Millsp.] using geographic and qualitative morphological descriptors. *Genetic Resources and Crop evolution* 52: 1049-1056.
- Serraj, R. Krishnamurthy, L. and Upadhyaya, H.D. 2004. Screening of chickpea mini-core germplasm for tolerance to soil salinity. *International Chickpea and Pigeonpea Newsletter* 11: 29-32.
- Srivastava, N., Vadez, V., Upadhyaya, H.D. and Saxena, K.B. 2006. Screening for intra and inter specific variability for salinity tolerance in pigeonpea (*Cajanus cajan*) and its related wild species. *SAT Electronic journal*.
- Upadhyaya, H.D. 2005. Variability for drought resistance related traits in the mini core collection of peanut. *Crop Science* 45:1432-1440.
- Upadhyaya, H.D., Bramel, P.J., Ortiz, R. and Sube Singh. 2002. Developing a mini core of peanut for utilization of genetic resources. *Crop Science* 42:2150-2156.
- Upadhyaya, H.D., Bramel, P.J. and Sube Singh. 2001a. Development of a chickpea core subset using geographic distribution and quantitative traits. *Crop Science* 41: 206-210.
- Upadhyaya, H.D., Dwivedi, S.L., Gowda, C.L.L. and Sube Singh. 2007a. Identification of diverse germplasm lines for agronomic traits in a chickpea (*Cicer arietinum* L.) core collection for use in crop improvement. *Field Crops Res.* 100: 320-326.
- Upadhyaya, H.D., Gowda, C.L.L., Buhariwalla, H.K. and Crouch, J.H. 2006a. Efficient use of crop germplasm resources: identifying useful germplasm for crop improvement through core and min-core collections and molecular marker approaches. *Plant Genetic Resources* 4(1): 25-35.
- Upadhyaya, H.D., Mallikarjuna Swamy, B.P., Kenchana Goudar, P.V., Kullaiswamy, B.Y. and Sube Singh. 2005. Identification of diverse accessions of groundnut through multienvironment evaluation of core collection for Asia. *Field Crops Research* 93:293-299.
- Upadhyaya, H.D. and Ortiz, R. 2001. A mini core subset for capturing diversity and promoting utilization of chickpea genetic resources in crop improvement. *Theor. Appl. Genet.* 102: 1292-1298.
- Upadhyaya, H.D., Ortiz, R. Bramel, P.J. and Singh, S. 2001b. Development of a groundnut core collection from Asia region. In: Kharakwal, M.C., Mehra, R.B. (Eds.), *Proceedings of the Diamond Jubilee Symposium (Abstracts). Hundred years of Post-Mendelian Genetics and Plant Breeding - Retrospect and Prospects.* November 6-9, 2001. Indian Society of Genetics and Plant breeding, New Delhi, India, pp. 43-44.
- Upadhyaya, H.D., Ortiz, R., Bramel P.J. and Sube Singh. 2003. Development of a groundnut core collection using taxonomical, geographical and morphological descriptors. *Genetic Resources and Crop Evolution* 50: 139-148.

Upadhyaya, H.D., Reddy, K.N., Gowda, C.L.L. and Sube Singh. 2006b. Phenotypic diversity in pigeonpea core collection. *Genetic Res. And Crop Evolution* (in press).

Upadhyaya, H.D., Reddy, L.J., Gowda, C.L.L. and Sube Singh. 2006c. Identification of diverse groundnut germplasm: Sources of early maturity in a core collection. *Field Crops Research* 97:261-267.

Upadhyaya, H.D., Reddy, L.J., Gowda, C.L.L., Reddy, K.N. and Sube Singh. 2006d. Development of mini core subset for enhanced and diversified utilization of pigeonpea germplasm resources. *Crop Science* 46:2127-2132.

Upadhyaya, H.D., Salimath, P.M., Gowda, C.L.L. and Sube Singh. 2007b. New early-maturing germplasm lines for utilization in chickpea improvement. *Euphytica* (accepted).