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Efficient use of crop germplasm resources: identifying useful germplasm for crop improvement through core and mini-core collections and molecular marker approaches

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Abstract

Conservation of crop germplasm diversity involves the establishment of *in situ* and *ex situ* genebanks. The major activities for *ex situ* genebanks include assembling, conserving, characterizing and providing easy access to germplasm for scientists. More than six million accessions are currently assembled in over 1300 genebanks worldwide. ICRISAT is one of the 15 CGIAR centres, with headquarters at Patancheru, India, and conserves genetic resources of sorghum, pearl millet, chickpea, pigeonpea, groundnut, and six small millets. The ICRISAT genebank holds 114,870 accessions from 130 countries, including both archival materials from various organizations throughout the world, and from fresh collections resulting from 213 missions in 62 countries. The ICRISAT genebank supplies annually over 40,000 germplasm samples to scientists worldwide. Sixty-six varieties selected from the basic germplasm have been released for cultivation in 44 countries, and ICRISAT has restored/repatriated crop germplasm to eight countries. The research focus is on germplasm diversity assessment, developing core and mini-core collections, and using a molecular characterization approach to both enhance the utilization of germplasm in research and improve the efficiency of germplasm management. Following these approaches, we have been able to identify a significant number of accessions with traits potentially relevant for crop improvement.

Keywords: chickpea; core collection; crop germplasm; groundnut; mini-core collection; molecular characterization; pearl millet; pigeonpea; sorghum

Introduction

Most of the natural resources on the earth are finite and vulnerable. This realization led to the Convention on Biological Diversity at the Earth Summit in Rio in 1992. Biological diversity refers to the totality of life forms, and plants, particularly food crops, play an important role in nourishing and sustaining humankind and other animals. Plant genetic resources contribute significantly towards achieving the Millennium Development Goals on food security, poverty alleviation, environmental protection and sustainable development. Specifically, they represent a critical component of crop improvement efforts aimed at increasing food security—both for short-term gain and for long-term increase in productivity.

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Although germplasm exchange and plant introduction have occurred sporadically for centuries, purposeful efforts started only in the 1920s. Over the years, genebanks have been established in a number of countries and the number of accessions conserved in genebanks now exceeds six million (Food and Agriculture Organization (FAO), 1998). The mission of the Consultative Group on International Agricultural Research (CGIAR) is to achieve sustainable food security and reduce poverty in developing countries through scientific research and research-related activities in the fields of agriculture, livestock, forestry, fisheries, policy and environment. Supporting exploration, exchange and conservation of plant genetic resources is one of the main objectives of the CGIAR. CGIAR germplasm collections are readily accessible to all, and this has helped the recovery of agriculture in countries emerging from conflict (such as Afghanistan, Angola, Mozambique and Somalia), and rebuilding following natural disasters such as hurricanes and flooding (e.g. Hurricane Mitch, which affected Honduras and Nicaragua).

Eleven CGIAR centres together maintain in the public domain about 600,000 accessions of crop, forage and agroforestry species. Of these, almost 533,000 are designated and are held in trust for the world community under agreements with the FAO. The terms of the agreements between the FAO and the CGIAR centres stipulate that this germplasm is available without restriction to researchers around the world, on the understanding that no intellectual property protection is applied to the material. The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) has responded to this need by establishing a Genetic Resources Unit for the assembly, characterization, evaluation, maintenance, conservation, documentation and distribution of germplasm of its mandate crops (sorghum, pearl millet, chickpea, pigeonpea and groundnut) and their wild relatives, and six small millets (finger millet, foxtail millet, barnyard millet, kodo millet, little millet and proso millet). In this paper we present a brief description of (i) germplasm collections; (ii) patterns of diversity in germplasm; and (iii) means to enhance the use of germplasm in crop improvement.

Germplasm assembly in the ICRISAT Rajendra S. Paroda genebank

Since the inception of ICRISAT in 1972, efforts have been initiated to assemble the pre-existing germplasm accessions of the mandate crops. The Rockefeller Foundation in India had assembled over 16,000 sorghum germplasm accessions from major sorghum areas in the 1960s and ICRISAT acquired 11,961 accessions of these in 1974, along with 2000 pearl millet accessions. A further 2000 pearl millet accessions collected in Francophone West Africa were obtained from ORSTOM.

The initial chickpea and pigeonpea collection was inherited in 1973 from the former Regional Pulse Improvement Project (RPIP), a joint project of the Indian Agricultural Research Institute (IARI), the United States Department of Agriculture (USDA) and the Karaj Agricultural University in Iran. ICRISAT also acquired over 1200 chickpea accessions from the Arid Lands Agricultural Development (ALAD) programme in Lebanon. Similarly, much of the groundnut germplasm was received from the Indian groundnut research programme (now the National Research Center for Groundnut, NRCG-Junagadh) and USDA.

Securing new germplasm of mandate crops from the priority areas was also given priority. Between 1975 and 2003, 213 joint missions were carried out in 62 countries, resulting in the collection of over 33,000 accessions of five mandate crops and six small millets. Besides these genebank activities, ICRISAT also works on the genetic improvement of the mandate crops. In this process, a large number of improved breeding lines, stress-resistant types and cultivars have been developed, and these have been registered in the genebank for future utilization. Currently, these number 3905 (sorghum 549; pearl millet 1336; chickpea 286; pigeonpea 1619; groundnut 114; and finger millet 1). Overall, the collection includes 114,870 accessions of sorghum, pearl millet, chickpea, pigeonpea, groundnut, and six small millets originating from 130 countries (Table 1). Nearly 96% of the collection is designated and held in trust on behalf

Table 1. Germplasm holdings in the ICRISAT genebank, December 2004

Crop	Active collection[a]	Base collection[b]	Accessions held in trust[c]
Sorghum	36,774	31,669	35,780
Pearl millet	21,594	15,150	21,250
Chickpea	17,258	15,984	16,961
Pigeonpea	13,632	10,266	12,698
Groundnut	15,419	6,820	14,357
Finger millet	5,949	4,620	4,931
Foxtail millet	1,535	1,054	1,534
Proso millet	842	576	835
Little millet	466	384	460
Kodo millet	658	630	547
Barnyard millet	743	487	743
Total	114,870	87,640	110,096

[a] Germplasm seeds stored in medium-term storage facility and available for current utilization.
[b] Germplasm seeds stored in long-term storage facility for utilization in posterity.
[c] FAO-designated germplasm freely available for use by researchers.

of the FAO. Many institutes and organizations have assisted in building up this resource, and the 15 most significant of these are listed in Table 2.

Germplasm management in the ICRISAT genebank

Phenotypic characterization and evaluation

Adequate characterization with respect to agronomic and morphological traits is necessary to facilitate the utilization of germplasm. To this end, each year accessions of all the crops have been sown and characterized for both morphological and agronomic traits. These characterizations have included the observation of reactions to various biotic and abiotic stresses (in collaborative experiments), and the measurement of nutritional value of grain (starch, protein and oil contents, cooking time, etc.). Agronomic evaluations have been conducted jointly with national agricultural research system (NARS) scientists in India, Nepal, Thailand, Indonesia, Ethiopia and Kenya, and more intensively with the National Bureau of Plant Genetic Resources (NBPGR), India. These joint evaluations have led to a better understanding of the germplasm material.

Regeneration

The need for regeneration of an accession is triggered by one of the three criteria: (i) accessions that had reached a minimum level of seed stock or viability; (ii) accessions required for medium-term storage (MTS) and/or long-term storage (LTS); and (iii) germplasm repatriation. Some of the germplasm accessions that do not produce seeds in ICRISAT-Patancheru climatic conditions (such as some wild *Arachis* species) are vegetatively propagated in the greenhouse. Some other accessions (such as wild *Cicer* species) need long day-length and cool weather to grow and produce seeds, so these species are also regenerated in greenhouse facilities.

Conservation

Seed must be cleaned and dried to minimal seed moisture content, before storing in cool and dry conditions with regular monitoring of seed health. In the ICRISAT genebank, the seeds of the entire collection are stored in aluminium cans at 4°C, 20–30% RH for MTS. ICRISAT germplasm accessions are also conserved in LTS (− 20°C) after packing in vacuum-sealed aluminium foil pouches. Before packing, the seeds are dried to about 5% moisture content in a walk-in drying room (100 m^3 size; 15°C and 15% RH) facility. By September 2005, we had 79.6% of the FAO-designated germplasm in the LTS facility.

Recently conducted seed health monitoring of seeds conserved for 10–25 years (MTS) indicated greater than 75% seed viability for the majority of the accessions. Accessions with declining seed viability (less than 75% seed germination) are regenerated as a priority and old stock is replaced with fresh seeds. Most of the accessions found to have low germination had been held in storage for more than 25 years (see Table 3). Seed is also evaluated for diseases and treated with appropriate agrochemicals where possible before regeneration.

Safety back-up

ICRISAT's agreement with FAO places the germplasm collections under the auspices of FAO, and requires safety

Table 2. Fifteen organizations that donated a large amount of germplasm to the ICRISAT genebank

Donor organization	Number of accessions
Rockefeller Foundation, New Delhi, India	14,639
United States Department of Agriculture, USA	4,996
Andhra Pradesh Agricultural University, Andhra Pradesh, India	4,940
Institut Francais de Recherché Scientifique pour le Development en Cooperation (ORSTOM), France	4,055
The Arid Lands Agricultural Development Program, Beirut, Lebanon	3,474
Indian Agricultural Research Institute, New Delhi, India	3,235
North Carolina State University, North Carolina, USA	2,650
Ethiopian Sorghum Improvement Project, Ethiopia	2,338
National Plant Gene Bank, Iran	2,038
University of Arizona, USA	1,698
Punjab Agricultural University, Punjab, India	1,635
Royal Botanical Gardens, Kew, UK	1,583
International Plant Genetic Resources Institute, Italy	1,567
Tamil Nadu Agricultural University, Tamil Nadu, India	1,363
Gujarat Agricultural University, Gujarat, India	1,277

Table 3. Germinability (%) of germplasm accessions conserved in the medium-term storage of the ICRISAT genebank, 2002

Crop	Accessions tested	Germination (%)			
		0–25	26–50	51–75	76–100
Sorghum	36,960	38	58	109	36,755
Pearl millet	20,628	29	104	504	19,991
Chickpea	17,057	7	18	75	16,957
Pigeonpea	12,865	3	16	169	12,677
Groundnut	12,390	20	52	140	12,178
Finger millet	5,008	21	5	36	4,946
Foxtail millet	1,533	58	36	101	1,338
Total	106,439	176	289	1,134	104,862

duplication preferably at −18°C in countries outside India. A Memorandum of Understanding exists with the International Center for Agricultural Research in the Dry Areas (ICARDA), Syria for safety duplication of chickpea germplasm and we have already deposited 2000 accessions with them. For other crops, we are exploring various options to find a cost-effective, secure and long-term strategy for safety back-up of the germplasm.

Documentation

Germplasm data has been systematically collated for chickpea and pigeonpea (Pundir *et al.*, 1988; Remanandan *et al.*, 1988), forage sorghum germplasm (Mathur *et al.*, 1991, 1992), pearl millet (Mathur *et al.*, 1993b, c) and chickpea (Mathur *et al.*, 1993a). Similarly, evaluation data for core and mini-core collections of ICRISAT mandate crops has been published (Upadhyaya *et al.*, 2001b, 2005b; Serraj *et al.*, 2004; Kashiwagi *et al.*, 2005). A *Manual of Genebank Operations and Procedures* has also been published (Rao and Bramel, 2000).

Genebank information system

The genebank operations span from collection and conservation of germplasm to its distribution and use. The need to adhere to international standards of germplasm conservation requires that these operations be computerized. This will help greatly in automating the routine operations of the genebank in a workflow system and in an efficient and timely dissemination of the information. The online genebank management system, developed in Visual Basic™ 6.0 with SQL Server™ 7.0 as the back-end, is structured to query the available information.

System-wide Information Network for Genetic Resources (SINGER)

The germplasm databases contain information generated during the day-to-day management of the genebank: passport, characterization, evaluation, inventory and distribution data. The passport information was recently updated with geographic coordinates for source location (for over 30,000 sorghum and millet accessions) using Microsoft Interactive World Atlas 2000™. An online core selector program was developed based on van Hintum (1999) and a common but stand-alone seed dispatch system for Africa with MS Access™ as the back-end and Visual Basic™ as the front-end has been completed.

Germplasm supply to users

ICRISAT genebank supplies healthy, viable and genetically pure seeds of genetic resources to research workers. During 1973 to 2004, we have supplied over 670,000 seed samples to scientists outside ICRISAT (Table 4).

Germplasm repatriation

The global collections held at ICRISAT serve an important purpose for the restoration of germplasm to the source countries when national collections are lost. For example, we supplied 362 sorghum accessions to Botswana, 1827 sorghum and 922 pearl millet to Cameroon, 1723 sorghum and 931 chickpea to Ethiopia, 838 sorghum and 332 pigeonpea to Kenya, 1436 and 445 sorghum accessions to Nigeria and Somalia, respectively, and 71 pigeonpea accessions to Sri Lanka. The germplasm collections maintained in the ICRISAT genebank include 44,822 accessions received from or jointly collected with the Indian National

Table 4. Distribution of germplasm samples from the ICRISAT genebank to scientists outside ICRISAT, 1974–2004

Crop	1974–1979	1980–1985	1986–1991	1992–1997	1998–2004	Total
Sorghum	23,120	61,988	107,358	42,103	13,929	248,498
Pearl millet	7,829	13,425	48,387	13,470	6,129	89,240
Chickpea	17,214	46,197	29,477	14,184	13,744	120,816
Pigeonpea	10,834	12,442	19,664	14,501	8,899	66,340
Groundnut	7,114	17,684	30,750	25,489	12,937	93,974
Finger millet	2,762	5,487	5,688	8,460	6,222	28,619
Foxtail millet	3,353	2,281	2,906	680	1,464	10,684
Proso millet	676	2,183	1,980	234	244	5,317
Little millet	186	739	409	116	636	2,086
Kodo millet	657	547	708	64	33	2,009
Barnyard millet	679	880	604	61	250	2,474
Total	74,424	163,853	247,931	119,362	64,487	670,057

Programs. The NBPGR, India requested ICRISAT to restore this germplasm. As part of ICAR/ICRISAT Partnership Projects, the genebank had already repatriated most of the accessions by July 2004 (Table 5). Thus the NARS of several countries have regained their precious heritage which could have been lost had it not been conserved in the ICRISAT genebank.

Insight into the germplasm supplied to users

One of the main areas of research is to assess the patterns of demand for germplasm accessions to guide future strategies for germplasm regeneration and management. The germplasm distribution data of sorghum, pearl millet, chickpea, groundnut and pigeonpea till 2004 have been analysed, and the following patterns have emerged.

Sorghum

Of the 36,774 germplasm accessions held in genebank, 248,498 germplasm samples were supplied to users outside ICRISAT in response to 2015 requests. This involved 31,866 (87% of the entire collection) unique accessions distributed at least once. Of this, the majority were landraces. The three accessions that were distributed most frequently were: IS 18758 (E 35-1) from Ethiopia, distributed 195 times; and IS 1059 and IS 5604, both Durra-Bicolor from India, distributed 189 and 123 times, respectively.

Pearl millet

A total of 89,240 germplasm samples have been supplied to the users outside ICRISAT. This involved 16,614 (77% of the entire collection) unique accessions. The diversity in the distributed material was similar to the entire collection. IP 4021, a very early flowering accession from Gujarat, India was distributed 106 times followed by IP 6271 (Sogue landrace from Mali) and IP 3122 (Jakhrana landrace from India), both 87 times.

Chickpea

A total of 120,816 seed samples (from 17,258 unique accessions) have been supplied to scientists in response

Table 5. Restoration of basic germplasm from the ICRISAT genebank to different countries

	Number of accessions						
Country	Sorghum	Pearl millet	Chickpea	Pigeonpea	Groundnut	Small millets	Total
Botswana	362						362
Cameroon	1,827	922					2,749
Ethiopia	1,723		931				2,654
Kenya	838			332			1,170
Nigeria	1,436						1,436
Somalia	445						445
Sri Lanka				71			71
India	14,637	7,189	7,488	5,988	6,060	3,460	44,822

to 1462 requests. This involved 16,439 (95% of the entire collection) unique accessions. A maximum of 322 requests were received for ICC 4973 (L 550), a kabuli cultivar from India, followed by ICC 4918 (Annigeri), a landrace/cultivar from India, 319 requests and ICC 5003 (K-850), an improved cultivar from India, 268 requests.

Groundnut

A total of 93,974 seed samples (from 15,419 unique accessions) have been supplied to scientists in response to 1326 requests. This involved 14,399 (94% of the entire collection) unique accessions. ICG 799 (Kadiri 3), a hypogaea cultivar from India, was supplied most frequently (297 times) followed by ICG 221 (TMV 2) and ICG 156 (M 13), both cultivars from India, and supplied 282 and 225 times, respectively.

Pigeonpea

Between 1974 and 2004 66,340 seed samples have been supplied to users. These samples represented 10,687 unique accessions (78% of the entire collection). Scientists from India were the major recipients of these seeds (69% of the total). The pigeonpea accessions requested most frequently were: ICP 7035 (DSLR-55), from Madhya Pradesh, distributed 307 times, followed by ICP 26 (T 21) and ICP 7182 (BDN 1) distributed 269 and 255 times, respectively.

Impact of germplasm supplied to the NARS worldwide

Besides the utilization of germplasm in ongoing research at various R&D institutes globally, 66 germplasm accessions (sorghum 30, pigeonpea 7, chickpea 19, groundnut 6, finger millet 2, and 1 each of pearl millet and barnyard millet) supplied from the ICRISAT genebank have been directly released as cultivars in 44 countries (Fig. 1). These cultivars have greatly benefited those countries by increasing both production and productivity. Pigeonpea accession ICP 8863, collected from a farmer's field in India, was found to represent a very promising source of resistance against fusarium wilt disease. The purified line was found to be high yielding and it was released in 1986 as Maruthi for cultivation in Karnataka state, India (ICRISAT, 1993). This variety is grown on a large hectarage in the adjacent states of Maharashtra and Andhra Pradesh. A systematic survey conducted in Karnataka revealed that ICP 8863 adoption increased

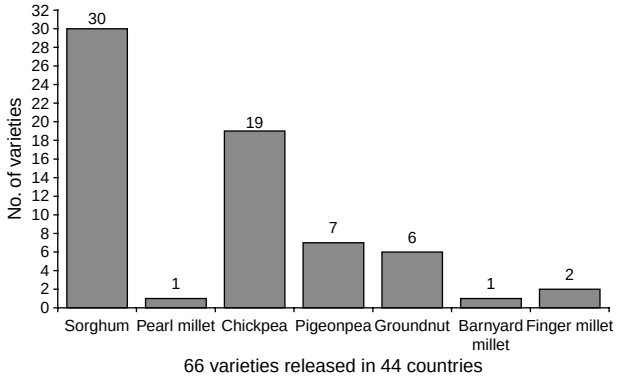

Fig. 1. Number of cultivars released worldwide from the basic germplasm supplied from the ICRISAT genebank, 1976–2003.

from 5% in 1987 to 60% in 1992. The gains from this variety compared to the local variety were 50% for grain, 45% for fodder by-product and 27% for stalk. The cost analysis indicated a unit cost reduction of US$123 per tonne of the grain with use of the improved variety ICP 8863 (Bantilan and Joshi, 1996).

Parbhani Moti, a sorghum variety, was released in Maharashtra, India, in May 2002. This variety is an excellent Maldandi-type (predominant post-rainy season sorghum landrace in Maharashtra and Karnataka states of India) with large lustrous grains and high yield. This has been selected from an entry from Ghane Gaon, Sholapur, Maharashtra, made during 1989. A further example is the release in India during 2003 of barnyard millet variety PRJ 1, a selection from ICRISAT line IEC 542 which originated in Japan. This variety has yielded 45.4% higher grain yield compared to the check variety VL 29, and provides substantial fodder yield as well.

Enhancing plant genetic resources utilization

On average over the period 1974 to 2004, the ICRISAT genebank has supplied over 21,000 samples annually to users outside ICRISAT. According to Marshall (1989), this figure indicates a satisfactory germplasm distribution service of the genebank. However, the use of basic germplasm in breeding programmes is scanty. For example, the summary of parental lines used in the ICRISAT groundnut-breeding programme (1986–2002) revealed that 986 unique parents were used in developing 8279 breeding lines, but this included only 132 unique germplasm accessions of groundnut and 10 of wild *Arachis* species. The two most frequently used cultivars were Robut 33-1 (3096 times) and Chico (1180 times). In the ICRISAT chickpea-breeding programme (1978–2004), 12,887 parents (586 unique parents) were used in

developing 3548 breeding lines, which included only 91 unique germplasm accessions of chickpea and five of wild *Cicer* species. The two most frequently used cultivars were L 550 (903 times) and K 850 (851 times). There are similar reports from China (Jiang and Duan, 1998) and the USA (Knauft and Gorbet, 1989) for groundnut.

Strategies to enhance germplasm utilization

Assessment of diversity in the germplasm collection

The assessment and characterization of diversity in germplasm collections is important to plant breeders for crop improvement and to genebank curators for the efficient and effective management of their collection.

The chickpea germplasm collection (16,820 accessions) has been characterized for seven morphological and 13 agronomic traits and reaction to fusarium wilt to determine phenotypic variation in different geographical regions. The means for different agronomic traits differed significantly between regions. The variances for all the traits among regions were heterogeneous. The South Asia region contained the largest range of variation for all the traits. The Shannon–Weaver (Shannon and Weaver, 1949) diversity index (H') was variable in different regions for different traits, and a cluster analysis delineated two regional clusters consisting of Africa and South and South-East Asia in the first cluster; and the Americas, Europe, West Asia, Mediterranean and East Asia in the second cluster (Upadhyaya, 2003). The analysis revealed the need to secure more entries from Mediterranean countries and Ethiopia.

The groundnut germplasm collection of 13,342 accessions was characterized for 16 morphological and 10 agronomic traits in two seasons, and for reaction to early leaf spot and groundnut rosette virus disease, to determine the level of phenotypic variation in different geographical regions. The means for different agronomic traits differed significantly among regions. The variances for all the traits among regions were heterogeneous. South America, which showed 100% range variation for 12 of the 16 morphological traits, also revealed the highest range variation. South America among regions, primary seed colour among morphological traits and leaflet length among agronomic traits showed highest pooled H'. Three of the six botanical varieties, *aequatoriana*, *hirsuta* and *peruviana*, were poorly represented and need to be collected. Principal component analysis (PCA) using 38 traits and clustering on the first seven principal component scores delineated three regional clusters consisting of: North America, Middle East and East Asia in the first cluster; South America in the second cluster; and West Africa, Europe, Central Africa, South Asia, Oceania, Southern Africa, Eastern Africa, South-East Asia, Central and Caribbean in the third cluster (Upadhyaya *et al*., 2002b).

The pigeonpea germplasm collection (11,402 accessions from 54 countries grouped into 11 regions) was analysed for patterns of variation for 14 qualitative and 12 quantitative traits. Semi-spreading growth habit, green stem colour, indeterminate flowering pattern, and yellow flower colour were predominant among qualitative traits. Primary seed colour had maximum variability, and orange colour followed by cream were the two most frequent seed colours in the collection. Variances for all the traits were heterogeneous among regions. The germplasm accessions from Oceania were conspicuous by short growth duration, short height, fewer branches, pods with fewer seeds, smaller seed size and lower seed yields. The accessions from Africa were of longer duration, taller, with multi-seeded pods and large seeds. The germplasm diversity, indicated by H' pooled over all traits, was highest for Africa and lowest for Oceania. The cluster analysis delineated three clusters: cluster 1 includes accessions from Oceania; cluster 2 from India and adjacent countries; and cluster 3 from Indonesia, Thailand, the Philippines, Europe, Africa, America and the Caribbean countries (Fig. 2). Pigeonpea-rich countries such as Myanmar, Uganda, and others like Bahamas, Burundi, Comoros, Haiti and Panama are not adequately represented in the collection, and need priority attention for germplasm exploration (Upadhyaya *et al*., 2005c).

Developing core collections

One of the reasons that plant breeders are using less basic germplasm in research is the lack of information on traits of economic importance, which often show high

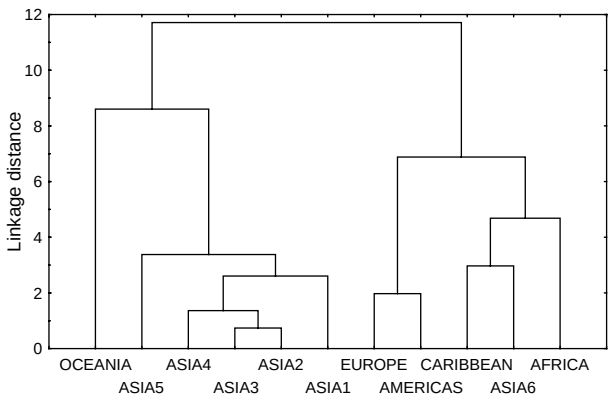

Fig. 2. Dendogram of 11 regions in the entire pigeonpea germplasm based on the first three principal components.

genotype × environment interactions and requires replicated multilocational evaluation. This is a very costly and resource-demanding task owing to the large size of the germplasm collections. To overcome this, our research now focuses on studying the diversity of germplasm collection and developing 'core collections', which are about 10% of the entire collection, but attempt to maximize the diversity represented. From the germplasm collection in the ICRISAT genebank, we have already developed core collections of sorghum (2247 accessions; Grenier *et al.*, 2001); pearl millet (1600 accessions; Bhattacharjee, 2000); 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.*, 2005a); and foxtail millet (155 accessions; H. D. Upadhyaya, unpublished data) (Table 6).

Developing mini-core collections

When the size of the entire collection is very large, even a core collection size becomes unwieldy for evaluation by breeders. To overcome this, ICRISAT scientists have developed a seminal two-stage strategy to develop a mini-core collection, which includes 10% of the accessions of the core collection (and hence only 1% of the entire collection) (Upadhyaya and Ortiz, 2001). This mini-core collection still represents the diversity of the entire core collection. 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. At both stages standard clustering procedures are used to separate groups of similar accessions. At ICRISAT, we have developed mini-core collections of chickpea consisting of 211 accessions (Upadhyaya and Ortiz, 2001), groundnut (184 accessions; Upadhyaya *et al.*, 2002a), and pigeonpea (146 accessions), finger millet (65 accessions), and foxtail millet (46 accessions) (H. D. Upadhyaya, unpublished data) (Table 6).

Table 6. Core and mini-core collections of ICRISAT mandate crops

Crop	Number of accessions used	Number of traits involved	Number of accessions
Core			
Sorghum	22,473	20	2,247
Pearl millet	16,063	11	1,600
Chickpea	16,991	13	1,956
Pigeonpea	12,153	14	1,290
Groundnut	14,310	14	1,704
Finger millet	5,940	14	622
Foxtail millet	1,474	13	155
Asian core			
Groundnut	4,738	15	504
Mini-core			
Groundnut	1,704	31	184
Chickpea	1,956	22	211
Pigeonpea	1,290	16	146
Finger millet	622	14	65
Foxtail millet	155	13	46

Identification of new sources for traits of economic importance for use in crop improvement programmes

Due to the reduced size, the core collection can be evaluated extensively to identify the useful parents for crop improvement. By evaluating the core collection of chickpea, we have identified new sources of important traits, namely early maturity (28 accessions), large-seeded kabuli (16 accessions) and high-yielding (39 accessions) types. The evaluation of the groundnut core collection has resulted in the identification of 21 accessions with early maturity, and 158 accessions having low temperature tolerance at germination (Upadhyaya *et al.*, 2001b). Also we have found 15 Valencia, 20 Spanish and 25 Virginia-type germplasm lines in groundnut with high yield, good shelling percentage and 100-seed weight through multilocational evaluation of the 'Asia region core collection' (Upadhyaya *et al.*, 2005b). These new sources performed as well as or better than the best control cultivars for particular trait(s) but were diverse from them. Holbrook *et al.* (1997) achieved a similar result through examining all accessions in the groundnut core collection (Holbrook *et al.*, 1993) for resistance to the groundnut root-knot nematode (*Meloidogyne arenaria* (Neal) Chitwood race 1) and resistance to pre-harvest aflatoxin contamination (Holbrook *et al.*, 1998) while Franke *et al.* (1999) later extended this to include resistance to rhizoctonia limb rot (*Rhizoctonia solani* Kuhn AG-4).

To gain benefits, the mini-core collections of chickpea and groundnut have been evaluated and diverse sources of useful traits have been identified. From the chickpea mini-core, 10 accessions having traits related to drought tolerance (Kashiwagi *et al.*, 2005) and 29 accessions tolerant to soil salinity (Serraj *et al.*, 2004) have been identified. The evaluation of the groundnut mini-core has resulted in the identification of 18 diverse accessions with high water-use efficiency (Upadhyaya, 2005) (Fig. 3). The evaluation

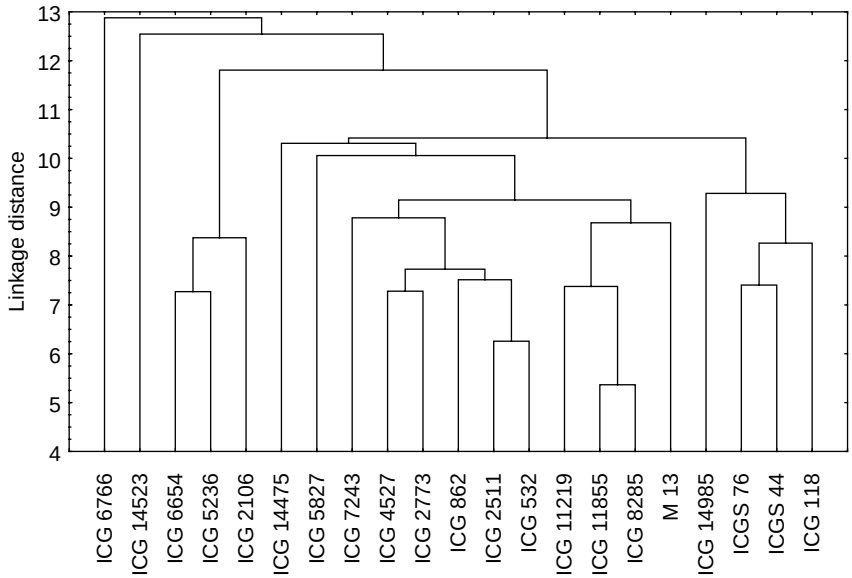

Fig. 3. Dendogram of 18 selected drought-tolerant germplasm accessions and three control cultivars based on scores of the first 12 principal components.

of the chickpea mini-core at the Indian Institute of Pulses Research (IIPR), Kanpur, India during the 2002–2004 seasons identified 12 very promising accessions. Of these, six (ICCs 14194, 14196, 14197, 14199, 12034, and EC 381882) were used as parents for developing large-seeded kabuli cultivars. Similarly, scientists in China, Vietnam and Thailand have identified 8, 10 and 12 lines, respectively, from an evaluation of the groundnut mini-core collection.

Molecular characterization of germplasm

Characterization of germplasm with molecular markers can help improve their utilization. It can form the basis for mining and cloning of genes of agronomically important traits.

Genotyping chickpea accessions

A total of 288 chickpea accessions (including the 211 mini-core, 75% desi type subset; Upadhyaya and Ortiz, 2001), 57 accessions of kabuli chickpea and 20 accessions of wild *Cicer* species from ICARDA have been genotyped with 50 simple sequence repeat (SSR) markers. The results indicated that the chickpea mini-core developed at ICRISAT was allelically more diverse than the germplasm from ICARDA. The accessions from ICARDA consisted of more heterozygous individuals compared with mini-core accessions. The dendrogram based on shared allele distance using the unweighted pair-group method of arithmetical means (UPGMA) indicated the presence of two main groups, one consisting mainly of accessions from the Indian subcontinent and the other group of accessions from the Mediterranean, Middle East and Ethiopia. The wild species accessions (*reticulatum* and *echinospermum*) formed an out-group at each end of the chickpea diversity spectrum (Fig. 4).

Validating the chickpea mini-core collection

Discriminant function analysis (DFA) has been used to determine the level of congruence between the genotypic data set and the 28 phenotypic clusters of the chickpea mini-core (Upadhyaya and Ortiz, 2001) based on morphological and agronomic traits. For DFA analysis, genotypic data from 210 accessions screened with 40 SSR markers were used. Overall, most individuals were assigned with a high degree of confidence to the original (phenotypic) clusters from which the accessions constituting mini-core collection were selected. Only 27% of the individuals were re-assigned into new clusters

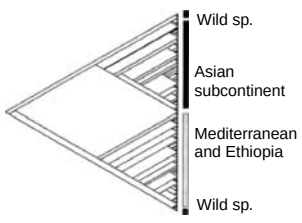

Fig. 4. Phylogenetic tree of 288 *Cicer* accessions constructed based on UPGMA clustering of shared allele distance.

according to genotypic data, which were mainly identified within clusters 4, 6 and 7 of the mini-core (ICRISAT, 2004). This confirmed the quality of the chickpea mini-core set.

Genotyping chickpea accessions of varying maturity duration

Sixty-two accessions (50 early-, six medium- and six late-maturing) were analysed with 37 SSR markers, resulting in the identification of 673 alleles. The number of alleles per locus varied from four to 28, with an average of 18. The polymorphic information content (PIC) values ranged from 0.53 to 0.94, with an average of 0.85. Mean heterozygosity was low (0.0276). The PCA plot of Roger's distance indicated three distinct clusters (ICRISAT, 2004).

Genotyping groundnut accessions

In groundnut, 26 accessions were analysed by randomly amplified polymorphic DNA (RAPD). The genetic similarity (S_{ij}) ranged from 59.0 to 98.8%, with an average of 86.2%. Both multidimensional scaling and UPGMA dendrograms revealed five distinct clusters. Some accessions with diverse DNA profiles (ICGS 1448, 7101 and 1471; ICGVs 99006 and 99014) were identified for mapping and genetic enhancement in groundnut (Dwivedi *et al.*, 2001). Molecular marker-based diversity estimates have been useful to select diverse lines for developing mapping populations to identify DNA markers linked to resistance to rosette disease. Nine amplified fragment length polymorphism (AFLP) primer combinations were applied to nine rosette-resistant and one susceptible accession. Across these 10 accessions 94 polymorphic fragments were identified. The genetic dissimilarity (D_{ij}) values ranged from 3.9 to 50.5%, with an average of 19.6%. Groundnut accessions ICGS 11044, 3436, 9558 and 11968 showed the greatest degree of genetic diversity, and they possess high levels of resistance to rosette disease (mean of $\leq 2\%$ compared to $\geq 90\%$ infection in the susceptible control ICG 7827 over four seasons at Lilongwe, Malawi). These accessions have been intercrossed to produce diversified rosette-resistant breeding populations (Dwivedi *et al.*, 2003).

Future plans

In the future, we seek to focus on germplasm assessment in the context of crop improvement. The core and mini-core subsets of the germplasm will be evaluated at diverse locations to identify trait-specific diverse parents. The molecular characterization of the mini-core collection, and trait-specific germplasm lines, will be enhanced to add value to the germplasm accessions. We have initiated the development of composite sets of ICRISAT mandate crops under the Generation Challenge Program. Phenotypic and genotypic characterization of these sets will provide a great deal of scope to identify useful and unique germplasm resources for utilization in crop improvement.

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