Plant genetic resources management: collection, characterization, conservation and utilization

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Abstract

Genetic resources provide basic material for selection and improvement through breeding to ensure food security needs of the world's rapidly rising population. Conservation and utilization of plant genetic resources are important components of ex-situ collections. Management of ex-situ collections requires creative and adaptive decisions tailored to operating conditions that are specific and continuously changing. The establishment of large, crop-genepool-specific collections at ICRISAT (International Crops Research Institute for the Semi-Arid Tropics) genebank, Patancheru, India was based on donations from existing collections and on targeted collection efforts. Majority of the conserved accessions are of orthodox seed producing nature. The conserved germplasm has been characterized for important morpho-agronomic characters and germplasm seed samples distributed to bonafide researchers for utilization in crop improvement programs all over the world. Exiguous use of germplasm has been observed in breeding programs mainly due to lack of information on economic traits. Core collections (10% of entire collection) and mini-core collections (10% of the core or 1% of entire collection) have been developed to enhance the use of germplasm in breeding programs. Core and mini-core collections have been used to identify genetically diverse trait-specific germplasm with resistance to abiotic and biotic stresses and for agronomic traits. These will be used in breeding programs to develop broad-based cultivars. This article describes the collections, genebank operations and practices from conservation to utilization perspectives.

Introduction

Plant genetic resources (PGR) are the most important components of agro-biodiversity. The PGR include primitive forms of cultivated plant species and landraces, modern cultivars, obsolete cultivars, breeding lines and genetic stocks, weedy types and related wild species (IPGRI 1993). Genetic diversity created in the farmers' fields over millennia, complemented by the diversity present in wild relatives of crops, provides the raw material for improving crop productivity through plant breeding. The PGR are finite and vulnerable to losses due to introduction of new crop varieties in agriculture, growing urbanization, natural hazards, etc. The PGR contribute enormously towards achieving the Millennium Development Goals of food security, poverty alleviation, environmental protection and sustainable development. Over the years, genebanks have been established in a number of countries and the number of accessions conserved in about 1400 genebanks now exceeds six million (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 research and development in the fields of agriculture, forestry, fisheries, policy and environment. Exploration, exchange and conservation of PGR is one of the main objectives of the CGIAR. The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) (one of the 15 CGIAR centers) has responded to this need by establishing a Genetic Resources Unit for assembly, characterization, evaluation, maintenance, conservation, documentation and distribution of germplasm of sorghum (*Sorghum bicolor*), pearl millet (*Pennisetum glaucum*), chickpea (*Cicer arietinum*), pigeonpea (*Cajanus cajan*), groundnut (*Arachis hypogaea*), finger millet (*Eleusine coracana*), foxtail millet (*Setaria italica*), barnyard millet (*Echinocloa crus-galli*), kodo millet (*Paspalum scrobiculatum*), little millet (*Panicum sumatrense*) and proso millet (*Panicum miliaceum*).

Genetic variation, once considered unlimited, is fast eroding as modern cultivars replace traditional cultivars over large areas, and natural habitats of wild relatives of cultivated species are being destroyed. Several landraces of some of the above crops that are conserved in the ICRISAT genebank at Patancheru, India have now disappeared from their natural habitats in Africa and Asia. Crop genetic diversity must be conserved to combat new pests and diseases, and to produce better adapted varieties for the changing environments. Seed conservation has vital role in preservation of genetic variability as it is

simple to handle, cost-effective and capable of maintaining genetic stability over long time periods. Thus, seed conservation is a popular and most efficient tool for germplasm conservation at the global level. There are several components in managing the PGR ex-situ; the most important among them are collection, characterization, conservation, distribution and utilization.

Germplasm assembly at ICRISAT

Assembly of germplasm through donation and exchange is the primary approach of establishing a genebank. When ICRISAT was established in 1972, efforts were made to assemble the germplasm of its mandate crops that existed with various research institutes in India and other countries. The Rockefeller Foundation had assembled over 16,000 sorghum germplasm accessions from major sorghum areas, and ICRISAT acquired 11,961 accessions in 1974 that existed in India and USA. ICRISAT also obtained 2000 accessions of pearl millet collected by the Institut Francais de Recherché Scientifique pour le Développement en Coopération (formerly Office de la Recherché Scientifique et Technique d 'Outremer) (ORSTOM) in francophone West Africa. The germplasm of chickpea and pigeonpea originally collected and assembled by the former Regional Pulses Improvement Project (RPIP), a joint project of the Indian Agricultural Research Institute (IARI), New Delhi; the United States Department of Agriculture (USDA); and Karaj Agricultural University, Iran, formed the initial collection. Sets of this germplasm, which were available in several agricultural research institutes in India and Iran, and at USDA, were donated to ICRISAT in 1973. ICRISAT also acquired over 1,200 chickpea accessions from the Arid Lands Agricultural Development (ALAD) program in Lebanon. Similarly, much of the groundnut germplasm was received from the Indian groundnut research program [now the National Research Centre for Groundnut (NRCG), Junagadh] and USDA. Besides germplasm donations by the All India Coordinated Research Projects on various crops, considerable numbers of germplasm were received from agricultural universities at Pantnagar (Uttarakhand), Rajendranagar (Andhra Pradesh), Ludhiana (Punjab), Coimbatore (Tamil Nadu), Jabalpur (Madhya Pradesh), Rahuri (Maharashtra) and IARI at New Delhi.

Recently, we obtained chickpea germplasm samples: 682 cultivated, 21 wild from the International Center for Agricultural Resarch in Dry Areas (ICARDA), Syria; and 2083 cultivated, 68 wild from Washington State University, Pullman, USA. We also received from our regional genebank in Niamey, 482 accessions of sorghum and 355 of pearl millet collected in Niger; and from Nairobi, Kenya, over 200 pigeonpea accessions collected from Mozambique, Tanzania and Uganda.

The national research programs in most countries, agricultural universities, regional research organizations and the international agricultural research centers are engaged in developing crop cultivars/elite breeding lines. These collected lines are also conserved in ICRISAT genebank for future utilization.

Germplasm collections through explorations

The fundamental objective of collecting PGR is to capture the maximum amount of genetic variation in the smallest number of samples (Marshall and Brown 1975). The development of efficient strategies depends on the extent of available information on the type of genetic variation in target taxa populations and their distribution in the target geographical region (Allard 1970). However, when there is lack of information on the target species and the collection area it might be prudent to organize an exploration mission to collect such information. In collaboration with national programs, ICRISAT conducted over 200 expeditions to collect landraces, some of which were on the verge of extinction, challenging difficult terrain, hostile environments and harsh conditions. Details on each collection are recorded in the germplasm collection data sheet (Appendix 1). The existing collections represent 70–80% of the available diversity, and there is continuing need to rescue endangered germplasm. Analyses of diversity in the existing collections using GIS (geographic information systems) facilitate identification of gaps to launch targeted collections. Germplasm collection is expensive and a time consuming exercise. Therefore, we should review the past collections of the crop before embarking on a new collection trip. If others have already explored the area under consideration, we should try to secure the germplasm from them. In some crops, for example in pearl millet, which is a highly cross-pollinated crop, the early collections may have lost their genetic identity because of poor maintenance. Hence, fresh systematic collection in such cases can be taken up (Harlan 1973).

Germplasm collection at ICRISAT

The ICRISAT genebank, Patancheru, India currently conserves 118,882 accessions of the five mandate crops and six small millets from 144 countries (Table 1). This is one of the largest collections in the CG system. The collection includes landraces (96,866), non-domesticated species (2,597), advanced and old cultivars (1,545) and breeding lines (17,874). Information on coordinates of sites of collection is documented on 51,417 accessions.

The collection represents both insurance against genetic erosion and as sources of resistance/tolerance to diseases and pests, climatic and other environmental stresses, improved quality and yield traits for crop improvement.

Germplasm collections held in-trust

The earth's natural resources are finite and vulnerable. Several countries have pledged to stem the rapid loss of biodiversity and sustain this vital resource for the present and future generations. The plant genetic diversity created in farmers' fields over the millennia and by the research institutions over the last century is complemented by diversity present in the wild relatives of the crop species. A majority of ICRISAT's collection (95.8%) has been placed in-trust with the Food and Agriculture Organization of the United Nations (FAO) on behalf of the Governing Body of International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) with an agreement that the materials will be maintained at international technical standards. With the enforcement of the ITPGRFA the materials are placed in the multilateral system and available to the world community using Standard Material Transfer Agreement (SMTA). (http://www.icrisat.org/ip_managemen/Intellectual_Property_Management.htm)

Germplasm characterization

Adequate characterization for agronomic and morphological traits is necessary to facilitate utilization of germplasm by breeders. To achieve this, germplasm accessions of all crops are characterized for morphological and agronomic traits in batches over the years. Germplasm screening against biotic and abiotic stresses, and the estimations of grain food quality are conducted jointly with various disciplinary scientists. Germplasm sets were evaluated for agronomic performance over locations jointly with NARS (national agricultural research systems) scientists in India, Nepal, Thailand, Indonesia, Ethiopia and Kenya and more intensively with the National Bureau of Plant Genetic Resources (NBPGR), India. The results of joint evaluations have led to better understanding of the germplasm material conserved at ICRISAT genebank by the NARS scientists.

The conserved germplasm at ICRISAT genebank has been characterized (96.3% of the collection) for important morpho-agronomic characters and germplasm seed samples distributed to bonafide researchers for utilization in crop improvement programs all over the world. The collection represents a wide range of diversity for different morpho-agronomic characters, including some important seed traits such as shape, size and texture and chemical composition. Majority of the accessions in the collection (99.7%), except a few in groundnut, sorghum and pearl millet, are seed-producing and seeds are essentially orthodox in nature.

Germplasm characterization is the recording of distinctly identifiable characteristics, which are heritable. This needs to be distinguished from preliminary evaluation, which is the recording of a limited number of agronomic traits considered to be important in crop improvement. Germplasm characterization is carried out in precision fields by spaced planting under adequate agronomic conditions and plant protection (Figs. 1 and 2). For each accession several morpho-agronomic traits are recorded using the descriptors developed in collaboration with Bioversity International [formerly IPGRI (International Plant Genetic Resources Institute)]. Following these procedures, majority (96.3%) of the germplasm collection at ICRISAT genebank has been characterized. Systematic description of each accession will eventually lead to classification in small and well-organized sectors that will facilitate enhanced utilization of germplasm. The major objectives of germplasm characterization are:

- Describe accessions, establish their diagnostic characteristics and identify duplicates;
- Classify groups of accessions using sound criteria;
- Identify accessions with desired agronomic traits and select entries for more precise evaluation;
- Develop interrelationships between, or among traits and between geographic groups of cultivars; and
- Estimate the extent of variation in the collection.

Through intensive field and laboratory screening and purification, a wide range of sources for desirable traits were identified in the assembled germplasm (Fig. 3).

Regeneration of germplasm

Seeds lose viability even under good storage conditions and it is necessary to regenerate accessions from time to time; the frequency of regeneration depends on the initial viability, the rate of loss of viability and the regeneration standard (ie, the percentage viability at which it is

Figure 1. ICRISAT germplam characterization at Patancheru, India – sorghum (left), pearl millet (middle) and finger millet (right).

Figure 2. ICRISAT germplasm characterization at Patancheru, India – chickpea (top), groundnut middle) and wild species of chickpea (bottom left) and groundnut (bottom right).

Figure 3. Diversity in germplasm assembled at ICRISAT genebank: sorghum and pearl millet (top); chickpea and pigeonpea (middle); groundnut and small millets (bottom).

decided to regenerate the accession) (Roberts 1984). The aim of regeneration is to increase the quantity of seed of any accession where the number of seeds available has been depleted, or to restore maximum viability to a seed lot. Regeneration of germplasm is one of the most crucial processes in genebank management. It is costly in terms of resources and time, and it involves the risk to genetic integrity. The methods employed for regeneration vary considerably according to the crop species (Figs. 4 and 5), and its reproductive system (inbreeding or out-breeding) (Breese 1989).

Germplasm regeneration is mainly carried out in the postrainy season (November–May) at ICRISAT, Patancheru. Due to low ambient relative humidity (RH) and absence of rains, incidences of diseases and pests are low, and consequently the quality of seed produced is high. Regeneration is carried out in precision fields and under good agronomic management for obtaining seeds of good quality and vigor. Optimum plant stand and suitable pollination control measures are required for maintaining genetic integrity in crops like sorghum, pearl millet and pigeonpea (where out-crossing exists). Germplasm collections contain accessions originating from a wide range of environments and the site of regeneration may not be optimal for all accessions. It would be ideal to regenerate germplasm in near-optimum locations, and meet the requirements of specific cultivars. Efficient management of seed germplasm collections therefore entails minimizing the frequency of regeneration. This can be achieved by maximizing the seed longevity.

Seeds are a product of the seed production environment as well as the genetic constitution of the parent plants.

Figure 4. Regeneration in cross-pollinated crops by bagging – sorghum (left) and pearl millet (right).

Figure 5. Regeneration in pigeonpea: bagging (left) and in cage (right).

The complex of environmental conditions, including soil and climate, frequently override the expression of genetic characters. Therefore, to improve seed quality, germplasm regeneration programs should stress improved management and production practices. Several pre-harvest and postharvest and seed drying practices influence initial vigor and subsequent longevity of regenerated seed lots (Kameswara Rao and Sastry 1998). Wild species and critical accessions with low viability/limited seed stocks need to be multiplied in the glasshouse under adequate protection. Detailed cleaning procedures for germplasm seeds are presented by Hanson (1985). It is important that harvested germplasm material is processed as soon as possible to avoid unnecessary losses or decrease in seed longevity. Widrlechner (1998) has described various managerial decisions for germplasm regeneration.

Germplasm conservation

The purpose of conservation of germplasm in genebanks in the form of seeds is to maintain the integrity of the material conserved to the highest standard over prolonged periods of time. It is necessary to set standards based on current scientific knowledge and available technologies for the proper handling and storage of seeds in genebanks that will ensure their conservation over the longest possible time, without the need for frequent costly regeneration. Standards for routine genebank operations and quality assurance were described by Dulloo and Engles (2003). Seeds are stored for short term as required for carry-over seeds, or for considerably longer term as required for germplasm accessions and high value seed stocks. The full benefits of any storage system are realized only when the seeds intended for storage have high initial quality. Therefore, maximum seed quality and vigor are of paramount importance in germplasm management. Several pre- and postharvest factors such as crop management, seed production environment, maturity, harvest, and cleaning and drying practices influence initial seed quality and its subsequent longevity. Maintaining seed quality in the accessions of a large collection requires careful planning and following standard protocols during the process of seed production and storage. Ex-situ seed storage is the most convenient and widely used method of conservation. However, some of the wild species accessions especially of sorghum, pearl millet and groundnut, which do not produce seed are maintained as live plants in the field or in special facilities **(**Fig. 6).

Types of conservation. Active collections refer to collections kept for medium term, which are immediately available for distribution for utilization and multiplication. Active collections are kept in conditions, which ensure that the accession viability remains above 65% for 10–20 years. Different combinations of storage temperature and moisture content can provide this longevity (IPGRI 1996). The active collections of ICRISAT genebank are stored in standard aluminum cans for all crops and in

Table 1. Germplasm holdings in ICRISAT genebank.

Crop	Active collection	Base collection	Accessions held in-trust
Sorghum	37,904	34,313	36,771
Pearl millet	21,594	20,343	21,563
Chickpea	20,140	16,977	17,124
Pigeonpea	13,632	11,794	12,389
Groundnut	15,419	12,640	14,803
Finger millet	5,949	4,620	5,949
Foxtail millet	1,535	1,054	1,535
Proso millet	842	576	835
Little millet	466	384	462
Kodo millet	658	630	656
Barnyard millet	743	487	743
Total	118,882	103,818	113,830

Figure 6. Maintenance of germplasm wild species: pearl millet (top) and groundnut (bottom).

plastic cans for groundnut at 4°C and 30% RH (Fig. 7). Depending on the crop species, the equilibrium moisture content for these samples ranges between 7 and 10%.

Base collections refer to collections kept for long term, solely for 'posterity', and are not drawn upon except for viability testing and subsequent regeneration. The accessions in base collection should be distinct, and in terms of genetic integrity, as close as possible to the sample provided originally. The base collections of ICRISAT germplasm are maintained at –20°C in vacuum packed standard aluminum foil pouches at 3–7% seed moisture content, depending on the crop species and with initial seed viability above 85% (Fig. 8). The storage conditions maintained for both the collections are preferred standards for international genebanks.

Base collections are stored under conditions (–20°C) ensuring long-term viability of material (more than 50 years) as a security to the active collection. Active collections are stored under conditions (4°C and 20–30% RH) that ensure medium-term viability (10–20 years). Ideally, these are maintained in sufficient quantity to be available on request. Hamilton et al. (2003) have described considerations for improved conservation and utilization concepts and strategies.

Figure 7. A view of medium-term storage of germplasm at ICRISAT genebank.

Table 2. Seed viability of active collection of cultivated germplasm conserved at ICRISAT genebank.

Crop	No. of accessions tested	Mean viability (%)	No. of accessions in viability range	
			≤75%	76–100%
Sorghum	36,591	95.0	92	36,499
Pearl millet	20,770	93.4	167	20,603
Chickpea	16,974	96.1	73	16,901
Pigeonpea	12,786	95.0	3	12,783
Groundnut	13,489	97.7	258	13,231
Finger millet	5,010	96.2	59	4,951
Foxtail millet	1,535	87.5	195	1,340
Proso millet	833	96.2	17	816
Little millet	464	95.3	11	453
Kodo millet	658	95.1	7	651
Barnyard millet	739	93.4	56	683
Total	109,849	94.6	938	108,911

Safety back-up. ICRISAT's agreement with FAO places the germplasm collections under the auspices of FAO, and requires safety back-up for long-term conservation in countries outside India. We have initiated efforts conserving 3,800 chickpea accessions at ICARDA (Syria) and 5,205 pearl millet, 2,006 groundnut, 479 barnyard millet, 4,580 finger millet, 1,039 foxtail millet, 628 kodo millet, 375 little millet and 521 proso millet accessions at ICRISAT Regional Genebank at Niamey, Niger.

MOU between Nordic Gene Bank and ICRISAT. The Nordic Gene Bank has invited ICRISAT to deposit its seed collections at the Svalbard Global Seed Vault (Fig. 9). ICRISAT accepted this responsibility and signed the Standard Deposit Agreement with the Royal Norwegian Ministry of Agriculture and Food on 20 September 2007. Further to this Agreement, ICRISAT proposed a 5-year schedule to deposit about 111,000 germplasm seed samples of its five mandate crops and six small millets. Formal agreements from the Governments of Norway and India have been obtained for the safe transfer of the germplasm seed samples.

Seed moisture content during conservation. Seed moisture content is the amount of water in the seed and is usually expressed as a percentage. Under all storage conditions, the moisture content of seeds comes to equilibrium with the RH of the surrounding atmosphere.

Figure 8. A view of long-term storage of germplasm at ICRISAT genebank.

Even small changes in moisture content have a large effect on storage life of seeds. For genebank purposes, seed moisture content is usually expressed on a wet weight basis. Estimation of seed moisture content is one of the most important aspects of seed processing as several management decisions are based on seed moisture level. Methods prescribed by the International Seed Testing Association (ISTA) are used for determining the seed moisture content in the genebank – a low-constant temperature oven-drying method (103°C for 16 hours) for groundnut seeds and a high-constant temperature oven-drying method (130°C for 1–2 hours) for sorghum, millets, chickpea and pigeonpea. Estimates of moisture content of seed determine the need for drying depending on where the seed is stored. Seed moisture content for long-term conservation ranges between 3 and 7% for different crops, while under medium-term conditions it is 6–8% for groundnut and 8–10% for other crops.

Seed drying. The longevity of seeds can be improved to a large extent with comparatively little investment. Seed longevity depends upon crop species, the initial quality of the seed, the moisture content to which seeds have been dried and the storage temperature. Sun drying and/or forced ventilation drying with heated air are generally used to reduce seed moisture content. For long-term conservation of germplasm seeds, it is recommended to dry at low temperature (15°C) and low RH (15%) (Fig. 10). Such an environment avoids any adverse effects of drying on the initial quality and subsequent longevity (Cromarty et al. 1982). Clean muslin cloth bags permitting free flow of air during drying are used in this process. Following this process, seeds of different crops with initial moisture contents between 8.6 and 11.9% are safely dried to 3.4–5.9% within four weeks for packaging (Fig. 11) and transfer to long-term conservation (Sastry et al. 2003). Ellis et al. (1990) recommended that the moisture content of many oilseeds could be reduced to values between 2 and 4% with considerable benefit. Ultra dry storage of oily seeds would be worth not only in refrigerated conditions but also under ambient storage conditions when funds do not allow refrigerated facilities.

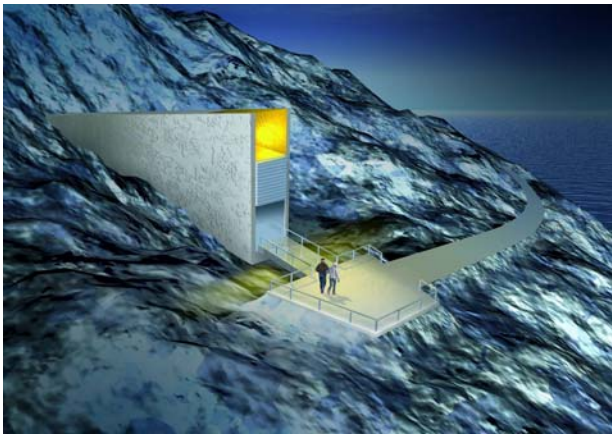

Figure 9. The Global Seed Vault at Svalbard, Norway for safety back-up.

Figure 10. Controlled environment facility for germplasm seed drying.

Seed viability testing. The standard germination test is the most widely accepted and direct measure of seed viability (Fig. 12). Seedlings are evaluated and classified as normal, which are capable of developing into plants given favorable conditions; and abnormal, which are incapable of further development, suffer deficiency, decay or weakness (Fig. 13). General guidelines and specific advice on the conduct of germination and appropriate dormancy breaking procedures are available in the Hand Book of Seed Technology for Genebanks (Ellis et al. 1985).

Figure 11. Seed processing for long-term storage of germplasm.

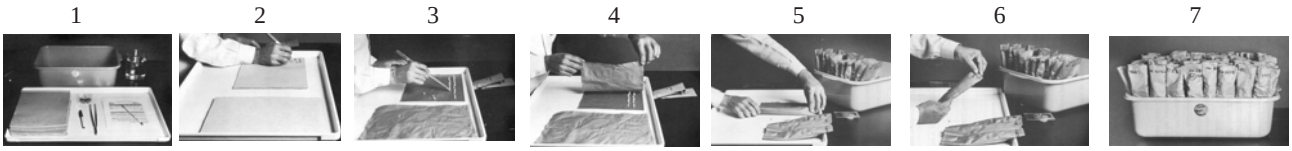

Figure 12. Testing germination of seeds between moist paper towels (1–7).

Seed viability monitoring. The viability of seeds stored in a genebank decreases gradually during storage and genebank accessions should be monitored regularly for viability to avoid excessive deterioration. The monitoring intervals depend on the species, viability at the beginning of storage and the conditions of storage. A recent monitoring of the health of seed conserved for 10–25 years (ie, medium-term conservation) indicated greater than 75% seed viability for majority of the accessions (Table 2). Accessions with declining seed viability (less than 75% seed germination) are regenerated on priority and the old stock is replaced with fresh seeds, after proper seed health testing.

Seed health testing. Germplasm seed health testing is carried out on all accessions regenerated for storage as active and base collections. Seed health tests are conducted on a minimum of 50 seeds by following a blotter test for all samples and an agar test for a specific pathogen. The procedure includes seed plating; incubation at 22°C under near ultra violet (NUV) light for 7 days and microscopic examination (Fig. 14) of individual seed for detecting seed associated microorganisms (Girish et al. 2004). Germplasm accessions with significant infestation levels are identified for inclusion in the next regeneration plans.

The Plant Quarantine Laboratory at ICRISAT, Patancheru, India caters to the plant quarantine requirements of the ICRISAT scientific community with respect to the germplasm exchange of ICRISAT's mandate crops and the small millets. The NBPGR, New Delhi, India is the plant quarantine authority responsible for ICRISAT's germplasm exchange. Plant quarantine guidelines and procedures for germplasm exchange of ICRISAT mandate crops have been documented (Chakrabarty et al. 2005).

Documentation

Documentation is essential for a genebank management to allow efficient and effective use of germplasm. Characterization and evaluation data are of little use if they are not adequately documented and incorporated into an information system that can facilitate access to data. Information plays a significant role in biodiversity conservation. Accurate information about conserved materials is essential for greater use. Computerized documentation systems enable rapid dissemination of information to users as well as assist curators to manage the collections more efficiently. Tools like GIS and satellite imagery help in searching for germplasm with specific characteristics, monitoring changes in crops and varieties, or deciding where to locate an in situ reserve. The Genebank Information Management System (GIMS) of ICRISAT is a user-friendly module designed to integrate various documentation activities and provides information on accessions due for regeneration/viability monitoring at any given point of time.

The vast germplasm data collected on chickpea and pigeonpea germplasm has been summarized and presented to the users in the form of catalogs (Pundir et al. 1988, Remanandan et al. 1988). Details on germplasm exploration and collection missions were summarized as progress reports. During the past 15 years, we had a very purposeful collaboration with the NBPGR, India on germplasm exploration, and evaluation at a number of locations, and the outcome was reviewed and discussed in the workshop "Collaboration on Genetic Resources" held in 1988 at ICRISAT, Patancheru (ICRISAT 1989). Core collection (10% of entire collection) and mini-core collection (10% of core collection or 1% of entire

Table 3. Global distribution of ICRISAT germplasm samples to scientists, 1974–2007.

Crop	1973–1983	1984–1993	1994–2007	Total
Sorghum	58,627	158,762	33,700	251,089
Pearl millet	15,302	62,769	12,837	90,908
Chickpea	52,015	45,413	28,904	126,332
Pigeonpea	19,546	30,593	17,986	68,125
Groundnut	20,908	44,034	30,417	95,359
Small millets	20,067	17,352	16,905	54,324
Total	186,465	358,923	140,749	686,137

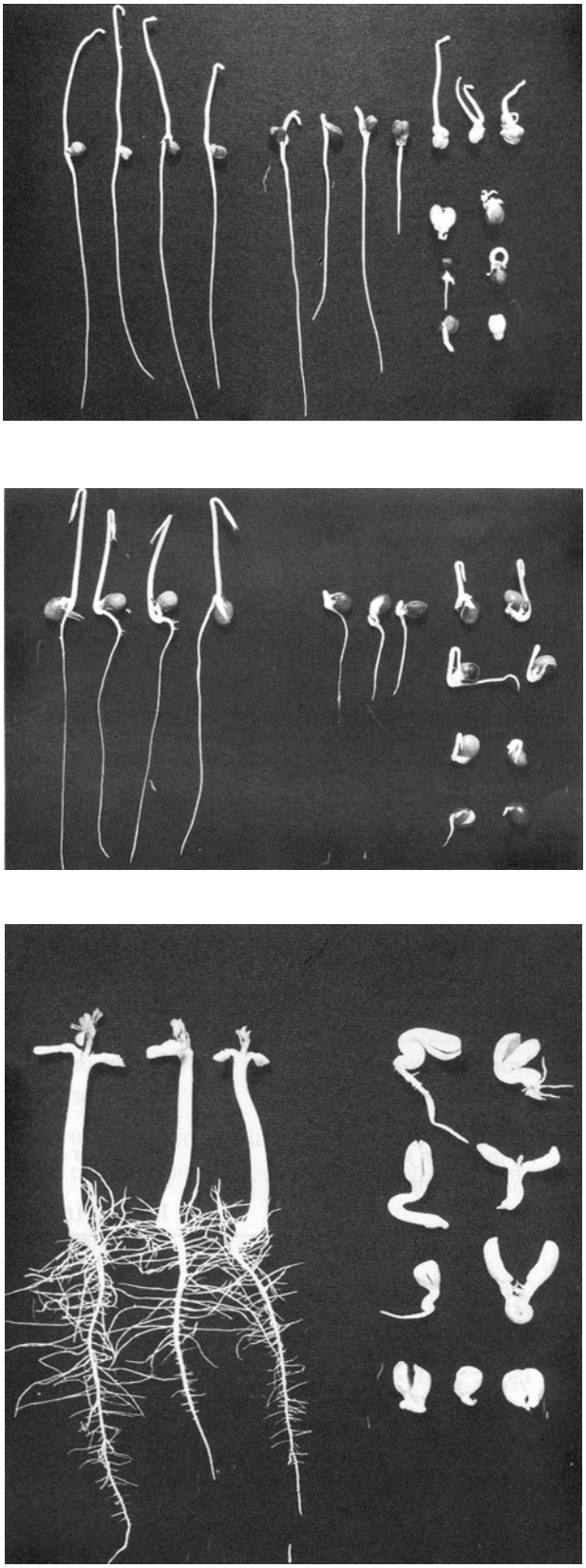

Figure 13. Seed germination test – evaluation of seedlings – normal (left) and abnormal (right) in different crops: chickpea (top), pigeonpea (middle) and groundnut (bottom).

collection) of ICRISAT mandate crops were established and the information was published through journal articles (Grenier et al. 2001, Upadhyaya and Ortiz 2001, Upadhyaya et al. 2001a, 2002, 2003, 2006a, Bhattacharjee et al. 2007) for the benefit of fellow researchers. A Manual of Genebank Operations and Procedures has also been published (Rao and Bramel 2000) documenting the history of the collections, procedures for germplasm acquisition, maintenance, documentation, conservation and distribution.

Germplasm utilization

Germplasm conserved at ICRISAT genebank has become an important source of diversity available to researchers in both public and private sectors throughout the world. For example, between 1975 and 2007, ICRISAT genebank has distributed over 686,000 samples of its mandate crops and small millets to users in 144 countries (Table 3).

The global collections held at ICRISAT serve the purpose of restoration of germplasm to the source countries when national collections are lost due to natural calamities, civil strife, etc. 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; 71 pigeonpea accessions to Sri Lanka and 44,822 accessions of ICRISAT mandate crops to NBPGR, India. Thus the national programs of several countries have regained their precious plant germplasm heritage which could have been lost if this was not conserved in the ICRISAT genebank.

Impact of germplasm utilization

Besides distribution and restoration of native germplasm to several countries, ICRISAT genebank has promoted testing and release of several of its germplasm accessions directly as superior varieties in different countries. Sixty-six germplasm accessions of different crops (conserved in the genebank) have been released directly as cultivars in 44 countries contributing to food security (Fig. 15) and a vast number of germplasm accessions have been used as building blocks for numerous varieties and hybrids that are cultivated in many parts of the world. A few examples of ICRISAT germplasm that have contributed significantly towards food security are described here.

Pigeonpea germplasm accession ICP 8863 collected from a farmer's field in India was found very promising against Fusarium wilt and was purified for the trait. The purified line was found high yielding and subsequently it was released for cultivation in 1986 as Maruthi in

Figure 14. Seed health testing under microscope.

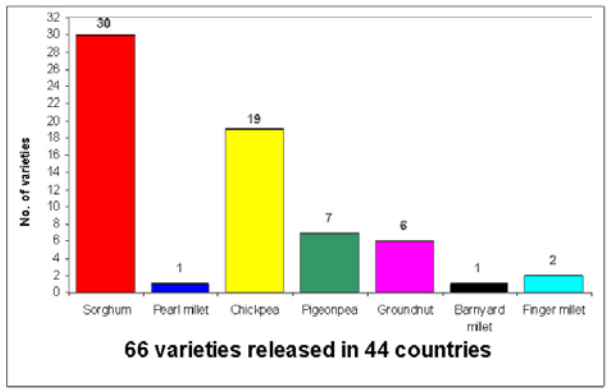

Figure 15. Number of varieties/cultivars released worldwide from basic germplasm supplied by ICRISAT, 1976–2007.

Karnataka state, India. This variety is also grown on large areas in the adjacent states of Maharashtra and Andhra Pradesh (Bantilan and Joshi 1996).

A sorghum variety, Parbhani Moti, was released in Maharashtra in 2002. This variety is an excellent Maldandi-type [predominant postrainy (*rabi*) sorghum landrace in Maharashtra and Karnataka states of India] with large, lustrous grains and high yield. This was selected from a germplasm collection from Ghane Gaon, Sholapur district of Maharashtra, by ICRISAT genebank staff during 1989.

Iniari is a large-seeded, early maturing and high tillering pearl millet landrace found in Benin, Burkina Faso, Ghana and Togo. This landrace was selected and a variety ICTP 8203 was released as MP 124 in Maharashtra and Andhra Pradesh and as PCB 138 in Punjab, India in 1988. The same was released as Okashana 1 in Namibia and as Nyakhombe in Malawi. Direct selection from the same landrace led to the development of the large-seeded, downy mildew resistant male-sterile line ICMA 88004 (Rai 1995).

Another example is the release of barnyard millet variety PRJ 1 in Uttarakhand state, India during 2003. This variety yielded 45.4% higher grain yield than the check variety VL 29. It provides substantial fodder yield as well. This variety is a selection from ICRISAT germplasm collection IEC 542 that originated in Japan.

Enhancing utilization of germplasm in crop improvement for food security

Large collections of germplasm, which are difficult to handle, can be made more accessible through development of core collections. “Core collections” are subsets of germplasm consisting about 10% of entire collection and represent species diversity. However, core collections based on basic passport and characterization data for major morphological characters, and developed primarily to make genetic diversity available to researchers have limited value unless this is evaluated extensively for traits of economic importance. This will make the core collection and eventually entire collection more useful to plant breeders and other crop improvement scientists.

Developing core collections. One of the reasons why plant breeders are using basic germplasm to a limited extent is the lack of information on traits of economic importance, which often show high genotype × environment interactions and require replicated multilocational evaluation. Multilocational evaluation 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. From the germplasm collection in the ICRISAT genebank, we have already developed core collection of all five mandate crops and of finger millet and foxtail millet (Table 4).

Developing mini-core collection. When the size of the entire germplasm collection of a crop is very large, even a core collection size becomes unwieldy for evaluation by breeders. To overcome this, ICRISAT scientists developed a seminal two-stage strategy to develop a mini-core collection, which consists of 10% accessions in 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. At ICRISAT, we have already developed mini-core collections of chickpea (211 accessions) (Upadhyaya and Ortiz 2001), groundnut (184 accessions) (Upadhyaya et al. 2002), pigeonpea (146 accessions) (Upadhyaya et al. 2006b) and sorghum (242 accessions) (Table 4).

Table 4. Core and mini-core collections of ICRISAT mandate crops.

Crop	Number of accessions used	Number of traits involved	Number of accessions	Reference
Core				
Sorghum	22,474	20	2,247	Grenier et al. 2001
Pearl millet	16,063	11	1,600	Bhattacharjee et al. 2007
Pearl millet (augmented)	20,844	12	2,094	
Chickpea	16,991	13	1,956	Upadhyaya et al. 2001a
Pigeonpea	12,153	14	1,290	
Groundnut	14,310	14	1,704	Upadhyaya et al. 2003
Groundnut (for Asia)	4,738	15	504	Upadhyaya et al. 2001b
Finger millet	5,940	14	622	Upadhyaya et al. 2006a
Foxtail millet	1,474	23	155	
Mini-core				
Sorghum	2,247	21	242	
Pearl millet	2,094	18	238	
Chickpea	1,956	16	211	Upadhyaya and Ortiz 2001
Pigeonpea	1,290	16	146	Upadhyaya et al. 2006b
Groundnut	1,704	34	184	Upadhyaya et al. 2002
Groundnut (for Asia)	504	30	60	

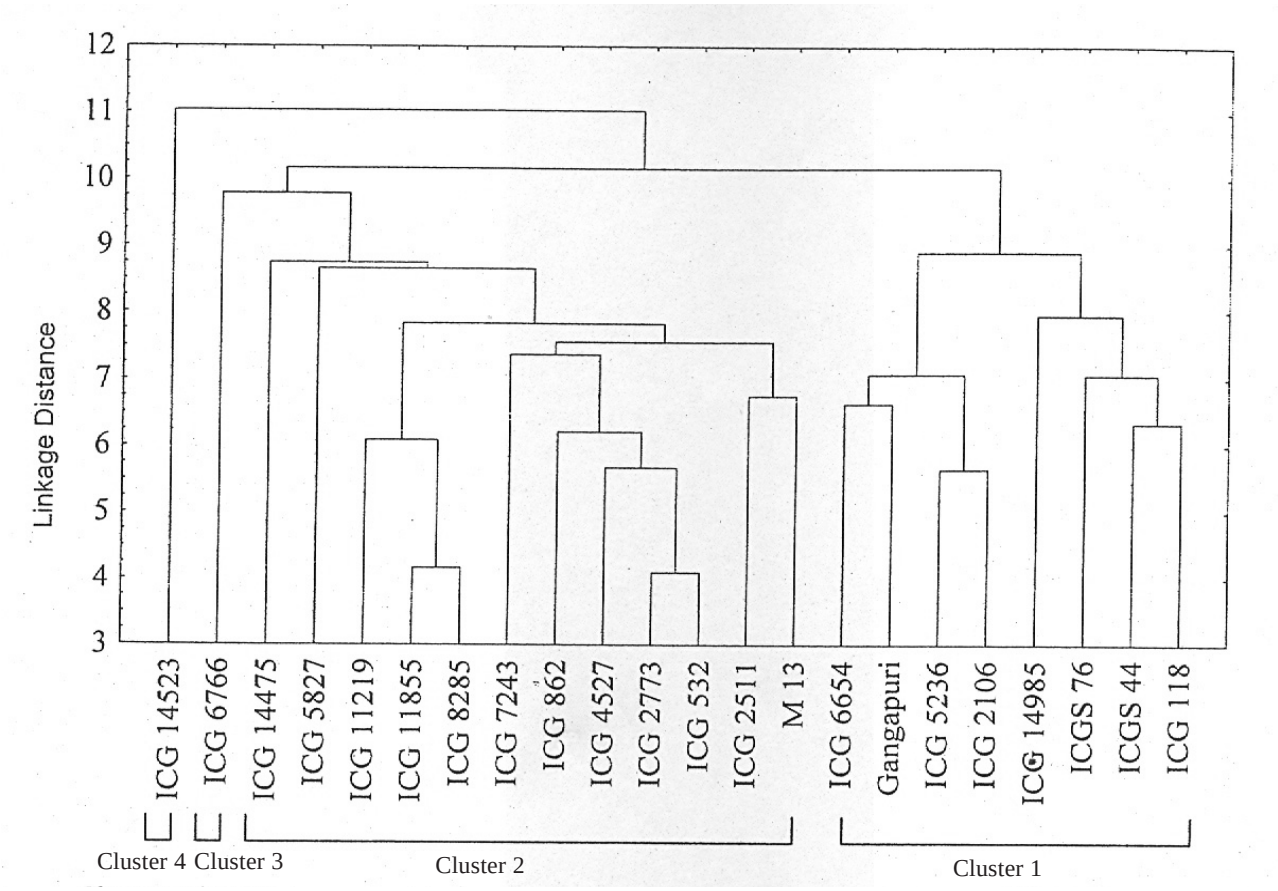

Figure 16. Dendrogram of 18 selected germplasm lines and four control cultivars of groundnut based on scores of first 15 principal components.

Developing composite collections. The revolution in molecular biology, bioinformatics and information technology has provided the scientific community with tremendous opportunities for solving some of the world's most serious agricultural and food security issues, and has led to the formation of Generation Challenge Program (GCP) entitled 'Unlocking Genetic Diversity in Crops for the Resource-Poor' (www.generationcp.org). The GCP is designed to utilize molecular tools and comparative biology to explore and exploit the valuable genetic diversity existing in germplasm collections held at the CGIAR and national genebanks, with particular focus on drought tolerance. ICRISAT and collaborating institutes have constituted composite collections of chickpea, sorghum, groundnut, pigeonpea and finger millet that contain maximum diversity known in the species, accessions with economic traits and some representation of the related wild species. Phenotypic and genotypic characterization of these sets will provide vast scope of identifying useful and unique germplasm resources for utilization in crop improvement. The composite collections are being genotyped using SSR (simple sequence repeat) markers. The data generated will be used to define the genetic structure of the collection for functional and comparative genomics. The analysis of genetic diversity helps to determine population structures that influence the analysis of the associations between molecular markers and the traits. Using genotypic information, a reference set of 200–400 accessions will be selected containing maximum diversity for research use.

New sources identified in germplasm using core/mini-core approach

The core and mini-core subsets of the germplasm were evaluated at diverse locations to identify trait-specific diverse parents. Due to the reduced size, the core and the mini-core sets have been characterized more precisely and some very useful trait specific accessions have been identified (Fig. 16). These accessions are similar to or better than the control cultivar(s) for a particular trait, agronomically good and genetically diverse. Their use in breeding programs would result in broad-based cultivars.

To enhance the use of germplasm in crop improvement programs, core and mini-core collections of chickpea, groundnut and pigeonpea were evaluated at ICRISAT and at locations in the national programs. This has resulted in identification of trait-specific, genetically diverse and agronomically superior germplasm for use in the crop improvement programs. Some of the examples are given below:

- Eighteen new sources of drought tolerance identified each in groundnut (Fig. 16) (Upadhyaya 2005) and chickpea (Kashiwagi et al. 2005). These new sources are similar or better than the known sources for drought resistance related traits and superior or similar for the agronomic traits.
- Sources of salinity tolerance in chickpea and pigeonpea: 12 tolerant sources identified in chickpea (Serraj et al. 2004) and 16 in pigeonpea (Srivastava et al. 2006).
- Sources of diseases resistance in chickpea: 67 accessions resistant to Fusarium wilt; 6 resistant to dry root rot; 3 tolerant to Ascochyta blight; 55 tolerant to Botrytis gray mold; and 18 accessions for multiple disease resistance (Pande et al. 2006).
- New sources of early maturity in groundnut and chickpea: 21 diverse landraces of groundnut, which are similar to the earliest maturing Chico, but have high yield and better pod and seed traits (Upadhyaya et al. 2006c). Similarly, 28 new diverse sources of early maturity in chickpea, which mature as early as earliest maturing germplasm Harigantars (85–90 days) but produce up to 70% more yield.
- Sources of productivity traits in groundnut, chickpea and pigeonpea: A number of high-yielding sources were identified from the Asia region core collection of groundnut in different botanical varieties (20 Spanish, 15 Valencia and 25 Virginia). They yielded similar or more than the best control cultivars, JL 24 (Spanish), Gangapuri (Valencia) and ICGS 76 (Virginia), and were diverse from these cultivars (Upadhyaya et al. 2005). Similarly high-yielding and diverse sources have been identified in chickpea (Upadhyaya et al. 2007) and pigeonpea.
- New sources of large-seeded kabuli chickpea: 16 diverse germplasm lines, which have 100-seed weight up to 55 g compared to 15 g of the popular Indian cultivar L 550 have been identified.
- Sources for agronomic traits in chickpea in India: Scientists at Indian Institute of Pulses Research, Kanpur, India have identified 12 accessions for large-scale evaluation and five accessions for use in breeding large-seeded kabuli cultivars.
- Sources for agronomic traits in groundnut in Thailand and China: Thai scientists have identified five groundnut accessions each for high pod yield, shelling percentage and seed size. Similarly, scientists in China have identified five accessions with large seed size, 14 accessions with resistance to bacterial wilt, and four accessions with high oleic and low linoleic acid content.

Conclusions and future outlook

The Global Plan of Action (GPA) for the Conservation and Sustainable Utilization of Plant Genetic Resources for Food and Agriculture (PGRFA) endorsed by the Conference of the Parties to the Convention on Biological Diversity (CBD) underscore the importance of and the responsibilities on the large ex-situ collections held by the CGIAR Centers within the frame-work of the ITPGRFA. ICRISAT needs to ensure that the assembled germplasm is maintained in a safe, secure and cost-effective manner and distributed to all bonafide users for utilization in crop improvement. The agreement between FAO and ICRISAT requires that the collections are maintained under long-term conditions, monitored regularly, and regenerated with appropriate plant population and pollination controls to ensure genetic integrity.

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 in 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 subset even for agronomic traits would help in identifying such lines.

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Appendix 1. ICRISAT germplasm collections data sheet.

ICRISAT-GENETIC RESOURCES UNIT-COLLECTION DATA SHEET

1. Collection Number ☐ 2. ICRISAT Accession No. ☐

3. Crop Species_____

4. Collectors(s)_____ 5. Date_____

6. Country_____ 7. State/Province_____ 8. District_____

9. Village_____ 10. Precise locality _____

11. Altitude_____ m 12. Latitude_____ 13.Longitude_____

14. Soil & topography _____

15. Precipitation: < Normal ☐ Normal ☐ > Normal ☐

16. Sample source: Field ☐ Threshing Floor ☐ Store ☐ Market ☐ Institution ☐ Other ☐

17. Local name_____ 18. Type/Race etc _____

19. Ethnic group_____ 20. Donor's name_____

20. Donor's source: Own ☐ Local ☐ Market ☐ Others ☐

21. Cultural practices: Rainfed ☐ Irrigated ☐ Flooded ☐ Transplanted ☐

22. Planting date_____ 23. Harvesting date_____

24. Associated Crop: Sole ☐ Mixed ☐ With_____

25. Population variability: Uniform ☐ Low ☐ Medium ☐ High ☐

26. Diseases _____

27. Insects_____

28. Agronomic score: Very poor ☐ Poor ☐ Average ☐ Good ☐ Very good ☐

29. Remarks:_____ ☐