Current Status of Genetic Resources Conservation, Characterization and Utilization

HD Upadhyaya, CLL Gowda, MA Mgonja, BIG Haussmann

Introduction

Plant Genetic resources (PGR) are the most important components of biodiversity. PGR include primitive forms of cultivated plants and landraces, modern cultivars, obsolete cultivars, breeding lines and genetic stocks, weedy types and related wild species (IPGRI, 1993). Genetic variation 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. The most important components of *ex situ* collections are conservation, characterization and utilization of conserved germplasm.

Germplasm collection at ICRISAT

The Rajendra S Paroda Genebank, ICRISAT, Patancheru, India conserving 116,799 accessions of the five mandate crops and six small millets from 141 countries (Table 1) represents one of the largest collections in the CG system sharing the institute's mission to achieve global food security. The collection includes landraces (89,531), non-domesticated species (2,310), advanced and old cultivars and breeding lines. Information on coordinates of sites of collection is documented on 49,603 accessions. The collection represents both insurance against genetic erosion and a source of tolerances to diseases and pests, climatic and other environmental stresses, improved quality and yield traits for crop improvement. Three regional genebanks at Niamey in Niger, Nairobi in Kenya and Bulawayo in Zimbabwe are holding substantial germplasm collections of the mandate crops. These included, 34,485 accessions at Niamey, 5,930 accessions at Nairobi, and 7,831 accessions at Bulawayo.

Germplasm collections held in-trust

The collections held at the genebank have been assembled over the last three decades through donations from other genebanks, through collaborative collection expeditions with national programs or advanced research institutes, from breeders around the world and from within the crop improvement programs of the Centre. A majority of this collection (97.6%) was placed in-trust with the Food and Agriculture Organization (FAO) of the United Nations for access to the world community for utilization in crop improvement research. At present, after coming in to force of the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) the collections are available under multi-lateral systems. The number of accessions in-trust with the regional genebanks is 6,870 at Niamey, 138 at Nairobi and 4,886 at Bulawayo.

Ex situ germplasm conservation

Ex situ seed storage is the most convenient and widely used method of conservation. Seeds are stored 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.

Types of ex-situ conservation

Active collections refer to collections kept for medium-term, which are immediately available for distribution for utilization and for multiplication. Active collections are kept in conditions to ensure that the viability of accessions 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 (except plastic cans for groundnut) at 4°C and 30% relative humidity. 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. Base collections are stored under conditions (-20ºC) ensuring long-term viability of material (more than 50 years) as a security to the active collection. 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 per cent. The storage conditions maintained for both collections are the preferred standards for international genebanks.

The viability of seeds stored in a genebank decreases gradually during storage and genebank accessions should be monitored regularly for viability to avoid deterioration. The monitoring intervals depend on the species, viability at the beginning of storage, and the conditions of storage. Our work (up to year 2002) on monitoring viability of seed conserved for 10–25 years (MTC) 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. The physical facilities of the three regional genebanks have been substantially improved for efficient processing, regeneration and conservation of germplasm samples for utilization.

Seed moisture content during conservation

Seed moisture content for long-term conservation ranges between 3-7% for different crops, while under medium-term conditions it is 6-8% for groundnut and 8-10% for other crops. For long-term conservation of germplasm, it is recommended to dry seeds at low temperature (15°C) and relative humidity (15%) to avoid any adverse effects of drying on the initial quality and subsequent longevity (Cromatry *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 to 5.9% within four weeks for long-term conservation (Sastry *et al*. 2003).

Regeneration of germplasm

Regeneration of germplasm is one of the most crucial processes in genebank management. Germplasm regeneration is mainly carried out in the post-rainy season (Nov-May) at ICRISAT, Patancheru. Due to low ambient relative humidity 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 followed for maintaining genetic integrity in crops like sorghum, pearl millet, and pigeonpea (where out-crossing exists). Wild species and critical accessions with low viability/limited seed stocks need to be multiplied in the glasshouse under adequate protection. The assembled germplasm at the regional genebanks in Africa are regenerated for meeting the regional demands.

Germplasm characterization

Germplasm characterization refers to 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 thought to be important in crop improvement. 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

- To describe accessions, establish their diagnostic characteristics and identify duplicates,
- To classify groups of accessions using sound criteria,
- To identify accessions with desired agronomic traits and select entries for more precise evaluation,
- To develop interrelationships between, or among traits and between geographic groups of cultivars, and
- To estimate the extent of variation in the collection.

At ICRISAT, the data generated by multi-disciplinary teams are fed back to the germplasm database. As a result of intensive field and laboratory screening, a wide range of sources for desirable traits were identified in the assembled germplasm.

Germplasm characterization is carried out in precision fields by spaced planting under adequate agronomic conditions and plant protection. For each accession several morpho-agronomic traits are recorded using the standard ICRISAT-IPGRI descriptors. Following these procedures, majority (96.3%) of the germplasm collection at ICRISAT genebank has been characterized. The germplasm 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.

Documentation

The Genebank Information Management System (GIMS) of ICRISAT-Patancheru is a user-friendly module designed to integrate various documentation activities, also provides information on accessions due for regeneration/viability monitoring at any given point of time. We are in process of installing this system at the regional genebanks.

Germplasm utilization

Distribution of germplasm and related information is fundamental to ICRISAT's mission of increasing crop productivity and food security. In fact, the use of PGR in crop improvement, followed by adoption, cultivation and consumption or marketing of the improved cultivars by farmers, is one of the most sustainable methods to conserve valuable genetic resources for the future, and to simultaneously increase agricultural production and food security (Haussmann *et al*., 2004). Germplasm conserved at ICRISAT genebank has become an important source of diversity available to researchers in both public and private sectors through out the world. For example, between 1974 and 2006, ICRISAT genebank has distributed 683,717 samples of its mandate crops and small millets to users in 143 countries (Table 3). In addition, we have provided 636,063 samples to ICRISAT scientists for screening, evaluation, breeding, and other studies.

The global collections held at ICRISAT serve the purpose of restoration 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 respectively to Nigeria and Somalia; 71 pigeonpea accessions to Sri Lanka and 44,822 accessions of ICRISAT mandate crops to the National Bureau of Plant Genetic Resources (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. A large number of germplasm accessions distributed have been used as building blocks for numerous varieties and hybrids that are cultivated in many parts of the world. Few examples of ICRISAT germplasm that have contributed significantly towards food security are described here.

Pigeonpea germplasm accession ICP 8863 collected from 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 it was released for cultivation in 1986 as Maruthi in Karnataka state, India. This variety is also grown on large hetaerae in adjacent states, namely, Maharashtra and Andhra Pradesh (Bantilan and Joshi 1996).

A sorghum variety, Parbhani Moti was released in Maharashtra, India, in 2002. This variety is an excellent Maldandi-type [predominant post rainy (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, made by ICRISAT genebank staff during 1989.

Iniadi is 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, India and 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 lead to the development of large seeded, downy mildew resistant male sterile line ICMA 88004 (Rai 1995).

Another example is the release of barnyard variety (PRJ 1) in Uttaranchal state, India during 2003. This variety yielded 45.4% higher grain yield compared to 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.

Core and mini core collections for enhanced 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 but representing the 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 evaluated extensively for traits of economic importance. This will make the core collection and eventually the entire collection more useful to plant breeders and other plant scientists. ICRISAT scientists have developed core collections of all five mandate crops and finger millet. 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 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. ICRISAT scientists have developed mini core collections of chickpea (211 accessions), groundnut (184 accessions) and pigeonpea (146 accessions). For more details refer to paper on “Core and mini core approaches for enhancing use of germplasm in crop improvement”.

The global core collection of pearl millet and the finger millet core collection developed at ICRISAT were evaluated at regional genebanks in Niger and Kenya respectively. Promising sources of germplasm were identified for utilization in their respective breeding programs and by NARS in these countries.

In situ conservation

Germplasm conserved *in situ* continues to adapt to changes in the environment including those caused by biotic and abiotic stresses. However, diversity maintained *in situ* is often much less accessible than that in ex situ collections and its long-term conservation less secure. *In situ* conservation mostly concerns germplasm present in farmers' fields (Engles, 2001). Community-based management of genetic resources (including seed systems and in-situ conservation) are increasingly studied and exploited within ICRISAT's farmer-participatory breeding programs, especially in West Africa. The other area of *in situ* conservation are the gene sanctuaries that are specific pockets of genetic diversity of crop plants and their closely related wild species.

Safeguarding the future

ICRISAT ensures 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 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 International Treaty on Plant Genetic Resources for Food and Agriculture.

For additional information/clarification, please contact Dr H D Upadhyaya (h.upadhyaya@cgiar.org)

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Table 1. Germplasm holdings in the Rajendra S Paroda Genebank, ICRISAT, Patancheru, December 2006.

Crop	Active collection	Base collection	Accessions held in-trust
Sorghum	36,774	33,039	36,771
Pearl millet	21,594	18,870	21,563
Chickpea	19,187	16,977	17,117
Pigeonpea	13,632	11,547	12,389
Groundnut	15,419	10,709	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	116,799	98,893	113,823

Table 2. Seed viability of active collection (medium-term storage) of cultivated germplasm conserved at Rajendra S Paroda Genebank, ICRISAT

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

Table 3. Global distribution of germplasm samples to scientists, 1974 – 2006.

Crop	1974-83	1984-93	1994-2006	Total
Sorghum	58,627	158,762	33,186	250,575
Pearl millet	15,302	62,769	12,767	90,838
Chickpea	52,015	45,413	27,740	125,168
Pigeonpea	19,546	30,593	17,586	67,725
Groundnut	20,908	44,034	30,476	95,418
Small millets	20,067	17,352	16,574	53,993
Total	186,465	358,923	138,329	683,717