

# **Management of Germplasm Collections and Enhancing Their Use by Mini Core and Molecular Approaches**

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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 raising population. Conservation and utilization of plant genetic resources are important components of *ex situ* collections. The establishment of large, germplasm collections at ICRISAT genebank, Patancheru, India, was based on donations from existing collections. This was augmented by targeted germplasm collection missions. Majority of them are orthodox seed producing accessions. The conserved germplasm accessions have been characterized for important morpho-agronomic characters and germplasm seed samples are 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 and mini core collections have been developed to enhance use of germplasm in breeding programs globally. The core and mini core collections were evaluated to identify genetically diverse trait specific germplasm for resistance to abiotic and biotic stresses and for agronomic traits for use in breeding programs to develop broad based cultivars. Molecular characterization of composite sets of germplasm, that include core or mini core collections, have helped in understanding genetic diversity and population structures in each species. Genotype based reference sets of genetically diverse 300 accessions have been established. Seeds of mini core collection and reference sets are available for use by the global research community.

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## Introduction

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

Over the years, genebanks have been established in a number of countries and the number of accessions conserved now exceeds the six million in about 1400 genebanks (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 plant genetic resources are 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* (L.) Moench.), pearl millet (*Pennisetum glaucum* (L.) R. Br.), chickpea (*Cicer arietinum* L.), pigeonpea (*Cajanus cajan* (L.) Millsp.), groundnut (*Arachis hypogaea* L.), finger millet (*Eleusine coracana* (L.) Gaertn.), foxtail millet (*Setaria italica* (L.) P. Beauv.), barnyard millet (*Echinochloa crus-galli* (L.) P. Beauv.), kodo millet (*Paspalum scrobiculatum* L.), little millet (*Panicum sumatrense* Roth. ex Roem. & Schult.), and proso millet (*Panicum miliaceum* L.).

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 destroyed. Several landraces now conserved in the ICRISAT genebank have disappeared from their natural habitats in Africa and Asia. Genetic variation must be conserved and effectively utilized 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 capability of maintaining genetic stability over long time periods. Seed conservation is a popular tool for

germplasm conservation at the global level. The most important components of managing *ex situ* germplasm include well established procedures for collection/assembly, characterization, conservation and sound scientific approaches for effective utilization of conserved germplasm.

### **Germplasm assembly at the ICRISAT**

When ICRISAT was established in 1972, efforts were made to assemble the germplasm of 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 of collections 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 Pulse 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 in Iran, formed the initial collection. Sets of this germplasm, which were available in several agricultural research institutes in India and Iran, and at the 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 Center for Groundnut (NRCG), Junagadh], and USDA. Besides germplasm donations by the All India Coordinated Research Projects on various crops, considerable number 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, in 2004~05, we obtained chickpea germplasm samples from Washington State University, Pullman, USA (2083 cultivated, 68 wild) and ICARDA, Syria (682 cultivated, 21 wild). Over 400 accessions each of sorghum and pearl millet collected in Niger, about 200 samples of pigeonpea collected from Mozambique, Tanzania and Uganda were received from our regional genebanks in Niamey and Nairobi.

### ***Assembly of cultivars and elite germplasm from research organizations***

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 germplasm lines are also conserved in ICRISAT genebank for future utilization.

### ***Germplasm collections through explorations***

The fundamental objective of collecting plant genetic resources 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 the information on the type of genetic variation in target taxa populations and their distribution in the target geographical region (Allard 1970). However, when information is lacking on the target species and the collecting area it might be prudent to organize an exploration mission to collect such information. In collaboration with national programs, ICRISAT scientists conducted over 200 expeditions to collect several landraces on the verge of extinction, braving difficult terrain, hostile environments, and harsh conditions. Details on collected samples are recorded in the germplasm collection data sheet (Appendix 1) (p.62). The existing collections represent 70%~80% of the available diversity, there is continuing need to rescue endangered germplasm. Analyses of diversity in the existing collections using GIS tools facilitate identification of gaps to launch targeted collections. Germplasm collecting is expensive. 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, Harlan (1973) suggested fresh systematic collecting in such cases.

## **Germplasm collection at ICRISAT**

The Rajendra S Paroda Genebank, ICRISAT, Patancheru, India conserving 118,882 accessions of the five mandate crops and six small millets from 144 countries (Table 1), represents one of the largest collections in the CGIAR system sharing the institute's mission to achieve global food security. The collection includes landraces (94,595), non-domesticated species (2597) advanced and old cultivars (1545) and breeding lines (17,874).

Information on coordinates of sites of collection is documented on 52,704 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.

Table 1. Germplasm holdings in the ICRISAT Genebank, Patancheru, India.

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

### ***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 farmer's fields over the millennia and by scientific research institutions over the last century is complemented by diversity present in the wild relatives of the crop species. 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, 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, Rome, Italy. The materials will be maintained at

international standards, and will continue to be made available to the world community with the understanding that no intellectual property protection is to be applied to the material.

### **Germplasm characterization**

Adequate characterization for agronomic and morphological traits is necessary to facilitate the utilization of germplasm by breeders. To achieve this, germplasm accessions of all crops were sown in batches over the years and characterized for morphological and agronomic traits. Germplasm screening against biotic and abiotic stresses, and the estimations of grain food quality were conducted jointly with various disciplinary scientists. Germplasm sets were evaluated for agronomic performance over locations jointly with NARS scientists in Canada, China, Ethiopia, India, Indonesia, Japan, Kenya, Nepal, Thailand, Ukraine, USA, and Vietnam. 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 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%), excepting few in groundnut, sorghum, and pearl millet, are seed producing and seeds are essentially orthodox in nature.

Germplasm characterization refers to recording of distinctly identifiable characteristics, which are heritable. This is distinct from preliminary evaluation, which is the recording of a limited number of agronomic traits important in crop improvement. Germplasm characterization is carried out in precision fields under adequate agronomic conditions and plant protection. For each accession several morpho-agronomic traits are recorded using the descriptors developed in collaboration with Bioversity International (formerly IPGRI). Following these procedures, majority of the germplasm collection at ICRISAT genebank has been characterized. Systematic description of each accession leads 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
- To estimate the extent of variation in the collection

To accomplish these objectives, a multi-disciplinary approach is essential. At ICRISAT, the data generated by various disciplines are fed back to the germplasm database. As a result of intensive field and laboratory screening and purification, a wide range of sources for desirable traits were identified in the assembled germplasm.

### **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 (i.e. the percentage viability at which it is 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 of the accessions. The methods employed for regeneration vary considerably according to the crop species (Figure 1 and 2) (p.63 and p.64), and its reproductive system (inbreeding or out-breeding) (Breese 1989).

Germplasm regeneration (except pigeonpea and small millets) 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 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. The complexity 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- and post-harvest 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 in Hanson (1985). It is important that harvested germplasm material is processed as soon as possible to avoid unnecessary losses or decrease in seed longevity. Widrechner (1998) has described various managerial decisions for germplasm regeneration.

### **Germplasm conservation**

The conservation of germplasm in genebanks in the form of seeds requires that the integrity of the material conserved be maintained to the highest standard over prolonged periods of time. For this to happen, it is necessary to set standards based on current scientific knowledge and available technologies for the proper handling and storage 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 Engels (2003). 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. Therefore, maximum seed quality and vigor are of paramount importance in germplasm management. Several pre- and post-harvest 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.



### ***Types of 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, 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). Ideally, these are maintained in sufficient quantity to be available on request. The active collections of ICRISAT genebank are stored in standard aluminum cans for all crops and in plastic cans for groundnut at 4°C and 30% relative humidity (Figure 3) (p.65). 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%. Base collections ensure long-term viability of material (more than 50 years) as a security to the active collection. The storage conditions maintained for both the collections are the preferred standards for international genebanks. Hamilton *et al.* (2003) have described considerations for improved conservation and utilization concepts and strategies.

### ***Safety backup***

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 out side India. We have initiated efforts conserving 3800 chickpea at ICARDA (Syria) and 5205 pearl millet, 2006 groundnut, and 7622 small millets (six crops) accessions at ICRISAT Regional Genebank at Niamey in Niger.

### ***Svalbard Global Seed Vault (SGSV)***

Located on the Norwegian island of Spitsbergen near the town of Longyearbyen (130 m above seas level) in the remote Arctic Svalbard Archipelago, a Seed Vault has been established to preserve unique duplicate samples of seeds held in genebanks worldwide (Figure 4) (p.65). The Seed Vault provides an insurance against the loss of seeds in

genebanks, as well as a refuge for seeds in the case of large scale regional or global crisis. Seed Vault has capacity to conserve 4.5 million seed samples, with each sample containing on average 500 seeds, a maximum of 2.25 billion seeds can be stored at  $-18^{\circ}\text{C}$ . ICRISAT has committed to place 111,000 FAO-designated germplasm of sorghum, pearl millet, chickpea, pigeonpea, and groundnut, and six small millets, in phased manner over next five years, with first batch of 20,000 accessions deposited in September in 2008.

### ***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 relative humidity of the surrounding atmosphere. 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 ( $103^{\circ}\text{C}$  for 16 hours) for groundnut seeds and a high-constant temperature oven-drying method ( $130^{\circ}\text{C}$  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%~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 larger 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 temperature at which they were stored. 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 ( $15^{\circ}\text{C}$ ) and relative humidity (15%) to avoid any adverse effects of drying on the initial quality and subsequent longevity (Figure 5) (p.66) (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%–5.9% within four weeks for long-term conservation (Sastry *et al.* 2003). Ellis *et al.* (1990) recommended that the moisture content of many oil seeds 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 don't allow refrigerated facilities.

### ***Seed viability testing***

The standard germination test is widely accepted and direct measure of seed viability (Figure 6) (p.67). 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 (Figure 7) (p.68). 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).

### ***Seed viability monitoring***

The viability of seeds stored in a genebank decrease 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 (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.

## **Germplasm documentation**

Documentation is essential in good 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 to enhance better use of germplasm. Computerized documentation systems enable rapid dissemination of information to users as well as assist curators manages the collections more efficiently. Tools like GIS and satellite imagery help in searching for germplasm with specific characteristics, monitor 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, provide information on accessions due for regeneration /viability monitoring at any given point of time.

Table 2. Seed viability of active collection of cultivated germplasm conserved at ICRISAT Genebank, Patancheru, India.

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

The vast germplasm data collected on chickpea and pigeonpea germplasm has been summarized and presented to 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. Core collection (10% of entire collection) and mini core collection (10% of core collection or 1% of entire collection) of ICRISAT mandate crops were established and the information was published through journal articles (Greiner *et al.* 2001); Bhattacharjee 2000; Upadhyaya and Ortiz 2001; Upadhyaya *et al.* 2001a; Upadhyaya *et al.* 2002; Upadhyaya *et al.* 2003; Upadhyaya *et al.* 2006d; Upadhyaya *et al.* 2008b, Reddy *et al.* 2005) for the benefit of fellow research workers. 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 distribution**

Distribution of germplasm and related information is fundamental to ICRISAT's mission of increasing crop productivity and food security. As per the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) germplasm is supplied under the Standard Material Transfer Agreement (SMTA). 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 the years 1975 and 2008, ICRISAT genebank has distributed over 692,000 samples of its mandate crops and small millets to users in 144 countries. 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 44,701 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 supply***

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 cultivars in different countries. In total 66 germplasm accessions of different crops conserved in the genebank have been released directly as cultivars in 44 countries contributing to food security. As detailed above, and a vast number of germplasm accessions distributed have been used as building blocks for hybrids that are cultivated in many parts of the world. As of December 2007, seventy seven national programs have released 548 cultivars using breeding materials supplied by ICRISAT. 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 hectares 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 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, India made by ICRISAT genebank staff during 1989. Iniari 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 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.

### **Utilization of germplasm in crop improvement**

The increase in accession numbers in genebanks and lack of corresponding increase in their use by the crop improvement scientists was a clear indication that the collections were not being used to their full potential (Marshall, 1989). A very large gap exists between availability and actual utilization of the materials. This was true both in the International Programs (CGIAR institutes) as well as in the national programs. For example, very few of the >14, 000 groundnut and >19, 000 chickpea accessions conserved in the genebank have been utilized in cultivar development of these two crops at ICRISAT (Upadhyaya *et al.*, 2003, 2006a). Similarly, in the national programs, the germplasm lines used in breeding programs are very limited. In China, the introduced germplasm and wild relatives have seldom been used in groundnut improvement. In USA, the cultivar 'Dixie Giant' was a germplasm source in all pedigrees of runner type groundnut and 'Small White Spanish-1' cultivar in >90% pedigrees. These two lines contributed nearly 50% of the germplasm of runner cultivars of groundnut in the USA. In India, 86 chickpea, 14 lentil, and 47 pigeonpea varieties have been developed through hybridization between 1967 and 2003. Only 10 germplasm lines contributed 35% of genetic base in chickpea, 30% in lentil, 48% in pigeonpea, 69% in

urdbean, and 71% in mungbean. Most plant breeders prefer to work with their own breeding lines, rather than exotic materials. Not only the limited use of germplasm is a worrisome issue, the large-scale deployment of single cultivar complicates the whole situation even more. For example in the Netherlands, the three top varieties of nine major crops covered from 81% to 99% of the respective planted area. One cultivar accounted for 94% of spring barley. Sometimes, even if the number of cultivars is more, the degree of genetic diversity between them is very low. In European barley, the protection against powdery mildew is increasingly dependent on one gene and one fungicide. Extensive use of fewer and closely related parents in crop improvement is contrary to the purpose of collecting large number of germplasm accessions, and could result in vulnerability of cultivars to pests and diseases. The fears of epidemics similar to the southern corn leaf blight in the USA (resulting in huge economic loss) and late blight of potato (that wiped out the potato crop resulting in the famine in Europe) due to narrow genetic base of crop cultivars looms large even today.

### **Developing core and mini core collections**

The main reason for low use of germplasm in crop improvement programs is the lack of information on large number of accessions, particularly, for traits of economic importance which display a great deal of genotype x environment (G x E) interaction and require multilocation evaluation. To overcome the size related problem of collection, developing a "core collection", consisting about 10% of entire collection, representing the genetic variability of the entire collection, has been proposed. In developing a core collection, available passport and characterization/evaluation data was used. Grouping of accessions from geographically similar countries, or regions of a big country, helps in making homogeneous groups. The data on accessions in the regional groups is then subjected to multivariate analysis to classify the accessions in to different clusters using a suitable clustering method. From each cluster 10% accessions are randomly selected to identify a core collection. Core and entire collections are compared for various parameters to determine whether core collection is representative of entire collection. Scientists at ICRISAT have developed core collections of all mandate crops and finger millet and foxtail millet (Table 3).

However, it soon became evident that developing core collections will not solve the problem of low use of germplasm, as even the size of core collection would be unwieldy for convenient exploitation by the breeders and other crop improvement scientists. This was

particularly true in the crops where entire collection is too large (several thousands). To overcome this, Upadhyaya and Ortiz (2001) proposed “mini core collection” concept and suggested a seminal two-stage strategy in chickpea. The first stage involves developing a representative core collection (about 10%) from the entire collection using all the available information on origin, geographical distribution, and characterization and evaluation. The second stage involves evaluation of the core collection for various morphological, agronomic, and quality traits, and selecting a further subset of about 10% accessions from the core collection. Thus the mini core collection contains 10% of the core or ~1% of entire collection, and yet represents the diversity of the entire collection. At ICRISAT, mini core collections have been established for chickpea, groundnut, pigeonpea, and sorghum; and analysis is in progress to establish mini core for pearl millet and finger millet (Table 3).

Table 3. Core and mini-core collections developed at ICRISAT.

Crop	No. of accessions used	No. of traits involved	No. of accessions	Reference
Core				
Sorghum	22,474	20	2,247	Grenier <i>et al.</i> , 2001
Pearl millet	16,063	11	1,600	Bhattacharjee <i>et al.</i> , 2007
Pearl millet (augmented)	20,844	12	2,094	Upadhyaya <i>et al.</i> , 2008b
Chickpea	16,991	13	1,956	Upadhyaya <i>et al.</i> , 2001a
Pigeonpea	12,153	14	1,290	Reddy <i>et al.</i> , 2005
Groundnut	14,310	14	1,704	Upadhyaya <i>et al.</i> , 2003
Groundnut for Asia	4,738	15	504	Upadhyaya <i>et al.</i> , 2001b
Finger millet	5,940	14	622	Upadhyaya <i>et al.</i> , 2006b
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 & Ortiz, 2001
Pigeonpea	1,290	16	146	Upadhyaya <i>et al.</i> , 2006d
Groundnut	1,704	34	184	Upadhyaya <i>et al.</i> , 2002
Groundnut Asia region	504	30	60	
Finger millet	622	20		



## Using core and mini core collections to identify trait-specific germplasm for use

Due to its greatly reduced size, mini core collections provide an easy access to the germplasm collections. Breeders and other crop improvement scientists can evaluate the mini core collection easily and economically for traits of economic importance to identify trait-specific germplasm for use. The global core and mini core collections were evaluated for various biotic and abiotic stresses and for agronomic traits and the following new sources were identified for utilization.

### *Chickpea*

- 28 early maturing accessions with an average 22.8% more seed yield than control cultivars over 5 environments (Upadhyaya *et al.*, 2007b)
- 39 high grain yield accessions (desi 19; kabuli 15, and intermediate 5) based on superior performance in 10 countries (Upadhyaya *et al.*, 2007a)
- 16 large seed sized kabuli type accessions
- 12 salinity tolerance accessions (Serraj *et al.*, 2004, Vadez *et al* 2007)
- 18 accessions for drought related traits (Kashiwagi *et al.*, 2005)
- 5 accessions for high temperature tolerance
- 67 accessions resistant to wilt; 6 resistant to dry root rot; 3 tolerant to ascochyta blight; 55 tolerant to Botrytis gray mold disease and 18 accessions for multiple resistance (Pande *et al.*, 2006)
- 5 accessions tolerant to *Helicoverpa* pod borer

### *Groundnut*

- 21 early maturity accessions similar to controls and 12.6% more yield at 75 days and 8.4% more yield at 90 days after sowing (Upadhyaya *et al.*, 2006c)
- 60 accessions (*fastigiata* 15; *vulgaris* 20; and *hypogaea* 25) agronomically superior and genetically diverse for high yield, large seed size, and high shelling percentage over six environments in India (Upadhyaya *et al.*, 2005)
- 158 accessions with wide range of geographic and genetic diversity for low temperature tolerance and excellent combination of pod-yield, shelling percentage, 100-seed weight, and oil and protein content (Upadhyaya *et al.*, 2008c)

- 12 accessions tolerant to salinity with agronomic traits
- 18 accessions genetically diverse and agronomically similar or superior the control cultivars for drought related traits (Figure 8, Upadhyaya 2005) (p.69)
- five accessions resistant to aflatoxin

### ***Pigeonpea***

- 53 genetically diverse accessions for high yield, large seed size high harvest index
- 20 genetically diverse accessions for early maturity
- 16 genetically diverse and agronomically similar or superior the control cultivars accessions for salinity tolerance (Srivastava *et al.*, 2006)
- 28 accessions resistant to sterility mosaic; 8 accessions for fusarium wilt; 78 accessions for phytophthora blight and 2 accessions for multiple diseases resistance
- 10 accessions for harvest index and 20 accessions for shelling percentage

### ***Small millets***

- 25 accessions of finger millet for high yield, large seed size and early maturity
- 25 accessions of foxtail millet for high yield, large seed size and early maturity

## **Utilization of mini core collections by national programs**

Mini core collections of groundnut, chickpea and pigeonpea are becoming popular with the national programs scientists to identify trait-specific germplasm for use in breeding programs. Eighty one sets of mini core of chickpea, groundnut and pigeonpea have been sent to NARS in Canada, China, India, Japan, Malawi, Mexico Nigeria, Thailand, UAE, USA and Vietnam for evaluation. Scientists have identified several the following useful sources of different traits for use in crop improvement programs:

**Groundnut:** High oleic/linoleic acid ratio: 4 accessions (China); high oil content: 7 accessions (China); large seed size: 5 accessions (each in China and Thailand); high shelling percentage: 5 accessions (each in China, Thailand, and Vietnam); resistant to bacterial wilt: 14 accessions (China).

**Chickpea:** Large seeded kabuli: 13 accessions (Kanpur, India); high yield: 16 accessions and early maturity: 23 accessions (Ludhiana, India)

**Finger millet:** Seed yield: two accessions; fodder yield: seven; early maturity: two; basal tillers: one; and inflorescence length and width: seven (Akola, India)

### **Molecular characterization of germplasm collection**

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. The Generation Challenge Program (GCP) on "Unlocking Genetic Diversity in Crops for the Resource-Poor ([www.generationcp.org](http://www.generationcp.org))" is helping in molecular characterization of core and mini core collections to discern the diversity at DNA level and identify genetically diverse parents for mapping and use in breeding programs.

Core or mini core collections of chickpea, groundnut, pigeonpea, sorghum, pearl millet, finger millet and foxtail millet have been genotyped as part of the composite collections under the GCP. These include chickpea: 3000 accessions, 50 SSR markers; sorghum: 3000 accessions, 41 SSR markers; groundnut: 1000 accessions, 21 SSR markers; pigeonpea: 1000 accessions, 20 SSR markers; pearl millet: 1000 accessions, 20 SSR markers; finger millet: 1000 accessions, 20 SSR markers; and foxtail millet: 500 accessions, 20 SSR markers. Tree diagrams of genotyping composite sets of chickpea, groundnut and pigeonpea are presented in Figure 9 (p.70). Analysis of genotyping data revealed population structure in these crops and diversity among the lines identified for a particular trait in the core or mini core collection and in the establishment of reference sets. This helped us to clarify the reasons for low polymorphism in the mapping populations made on phenotypic data beside providing breeders with the avenue of using genetically diverse parents to enhance trait (s) and developing broad based cultivars.

Molecular characterization of 2915 chickpea accessions using 48 SSR markers resulted in allele information on rare and unique alleles for seed types (desi, kabuli, pea-shaped), wild species and accessions of geographic origin – Africa, West Asia, South and South Asia, South America, North America, Europe, Oceania, Mediterranean and CIS (Table 4 and 5). The gene diversity in chickpea composite collection was 0.869. The gene diversity was similar in desi and kabuli types, low in pea-shaped and wild accessions. The range of gene diversity was more in kabuli types than desi types and gene diversity was maximum in the accessions from West Asia and minimum from Oceania.

Table 4. Allele information on chickpea composite set based on seed types.

Seed type	No. of accessions	Allele information				
		Total	Range	Mean	Rare (p=0.01)	Unique
Desi	1668	1481	9~63	31	781	297
Kabuli	1167	1288	7~52	27	691	104
Pea-shaped	70	670	4~29	14	7	4
Wild species	10	341	1~10	7	0	69
Composite collection	2915	1683	14~67	35	935	-

Table 5. Allele information on chickpea composite set based on geographical region of chickpea.

Regions	No. of accessions	Allele information				
		Total	Range	Mean	Rare (p=0.01)	Unique
Africa	150	832	4~34	17	199	10
West Asia	720	1318	8~60	27	535	114
South & Southeast Asia	1138	1322	9~61	28	678	117
South America	49	524	3~23	11	0	3
North America	94	719	3~32	15	0	6
Europe	65	647	4~24	13	5	2
Oceania	2	88	1~3	2	0	0
Mediterranean	619	1241	9~48	26	573	114
Common wealth of Independent States (CIS)	44	557	3~21	12	0	1
Composite collection	2915	1683	14~67	35	935	-

Using molecular characterization of 852 of groundnut accessions using 21 SSR markers, we secured allele information on rare and unique alleles for hypogaea, and fastigiata cultivar groups and wild species accessions and accessions of geographic origin – Africa, America, Asia, Europe and Oceania. The gene diversity in groundnut composite collection was 0.819. Wild relatives were more diverse than sub-species hypogaea and fastigiata. Groundnut accessions from Americas were more diverse followed by Asia and the gene diversity was minimum in germplasm accessions from Oceania.

In pigeonpea, molecular characterization of 952 accessions using 20 SSR markers, we assembled information on rare and common alleles for accessions representing wild and cultivated types and accessions of different geographic origins – Africa, Americas, Asia (six groups), Caribbean and Europe. We identified 60 alleles specific to wild and 64 alleles specific to cultivated types and wild types shared 73 alleles with cultivated type. Thirty two alleles are specific to Asia 4 (India: Andhra Pradesh, Orissa, Karnataka, Kerala and Maharashtra) and two to Africa. Asia 3 (India: Daman and Diu, Gujarat, Madhya Pradesh and Maharashtra) shared 6 alleles with Asia 4 and Asia 4 shared 5 alleles with Asia 6 (Indonesia, Philippines, and Thailand).

Molecular characterization of finger millet composite collection using 959 accessions and 20 SSR markers revealed a total of 231 alleles (110 rare and 121 common) with an average 11.5 alleles per locus. The number of alleles observed was 42 in Africana, 80 in Spontanea, 148 in Compacta, 130 in Elongata, 160 in Plana, and 206 in Vulgaris types. Thirty seven alleles were specific to Vulgaris, 5 to Plana, and 4 to Africana and 2 to Compacta. Plana and Vulgaris shared 26 unique alleles. East Africa had 29 unique alleles, South Asia had 12, and Southern Africa had 11 unique alleles. In foxtail millet, molecular characterization of composite collection using 452 accessions and 19 SSR markers revealed rich allelic diversity (362 alleles, 19 alleles per locos, 196 common alleles and 166 rare alleles), group specific unique alleles and common alleles sharing between the races and geographical groups.

The composite collections of chickpea and groundnut were also field evaluated for various qualitative and quantitative traits at ICRISAT center, Patancheru, India. Reference sets based on SSRs, qualitative traits, quantitative traits and various combinations of SSR, qualitative and quantitative traits were compared in chickpea. A SSR based reference set of 300 accessions captured 78% alleles (1315) of the 1683 alleles in composite collection (Upadhyaya *et al* 2008a). The reference set based on 7 qualitative traits captured 73.5%

alleles (1237) while SSR and qualitative trait based reference set captured 80.5% alleles (1354). This demonstrated that 7 qualitative traits were as efficient as SSRs in chickpea (Upadhyaya *et al* 2008a). Similarly, in groundnut a reference set based on 21 SSRs captured 95.1% (466) 490 alleles of composite collection. The reference set based on 14 qualitative traits captured 93.3% (457) alleles while the SSR and qualitative trait based reference set captured 95.1 % alleles (466).

We have established reference sets of 300 accessions each in chickpea, groundnut, pigeonpea and finger millet, 384 accessions in sorghum and 200 accessions in foxtail millet. The chickpea and groundnut reference sets are being phenotyped for seed yield and related traits, drought and salinity and various biotic and abiotic stresses both in the field and controlled environmental conditions. The chickpea reference set is being genotyped for an additional 100 SSR markers to study trait-marker association.

## Conclusions

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 and the responsibilities for 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. ICRISAT needs to ensure that the assembled germplasm is maintained in a safe, secure and cost-effective manner and provided to all bonafide users (under SMTA) 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 genetic enhancement are the primary need of the plant breeder. Agronomically superior 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 the usefulness of germplasm accessions in allele mining. Another dimension of breeders' requirements is agronomic desirability of the germplasm lines. This

helps them maintaining or even improving the agronomic performance of breeding lines while enhancing the traits expression. Thus our aim is to identify the trait-specific genetically diverse and agronomically superior germplasm lines for use in crop improvement programs to develop high yielding cultivars with a broad genetic base. The easy and convenient evaluation of mini core even for agronomic traits would help identifying such lines.

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## Appendix 1

### ICRISAT germplasm collection data sheet



#### ICRISAT – GENETIC RESOURCES UNIT – COLLECTION DATA

1. Collection number  2. ICRISAT Accession No.

3. Crop species \_\_\_\_\_

4. Collector(s) \_\_\_\_\_ 5. Date \_\_\_\_\_ 19 \_\_\_\_\_

6. Country \_\_\_\_\_ 7. State \_\_\_\_\_ 8. District \_\_\_\_\_

9. Village \_\_\_\_\_ 10. Precise locality \_\_\_\_\_

11. Altitude \_\_\_\_\_ m 12. Latitude \_\_\_\_\_ 13. Longitude \_\_\_\_\_

14. Soil & topography \_\_\_\_\_

15. Precipitation: < 450 mm  451-650 mm  651-900 mm  > 900 mm

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

17. Local name \_\_\_\_\_ 18. Type/ Race etc: \_\_\_\_\_

19. Ethnic group \_\_\_\_\_ 20. Donor's name \_\_\_\_\_  
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: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_



Figure 1. Regeneration in cross-pollinated crops by bagging:  
Sorghum (top) and Pearl millet (bottom)



Figure 2. Regeneration in Pigeonpea: bagging (top), and under cages (bottom)





Figure 3. Medium-term storage facility at ICRISAT

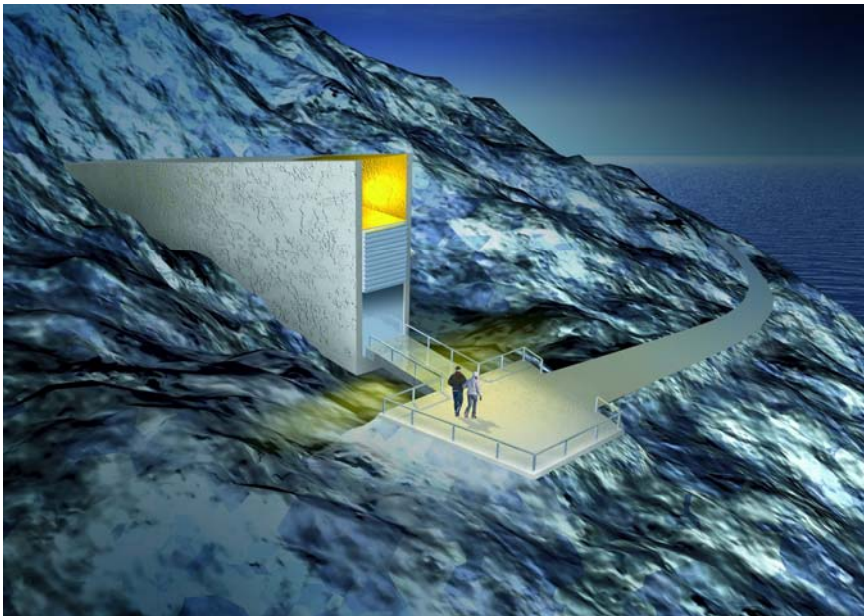


Figure 4. Svalbard Global Seed Vault, Norway for germplasm safety backup



Figure 5. Controlled environment facility for germplasm seed drying at ICRISAT



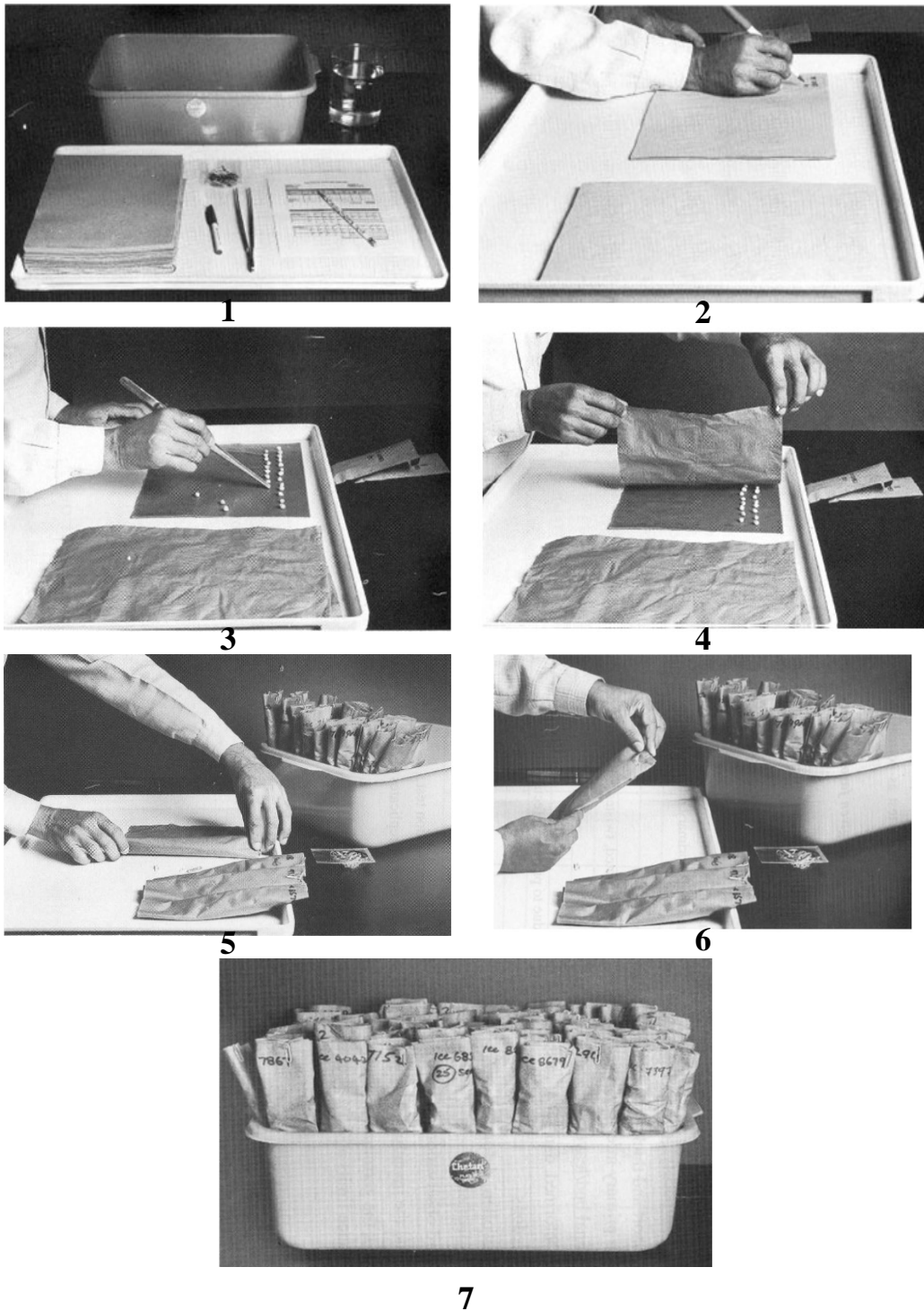


Figure 6. Testing germination of seeds using moist paper towels (1~7)

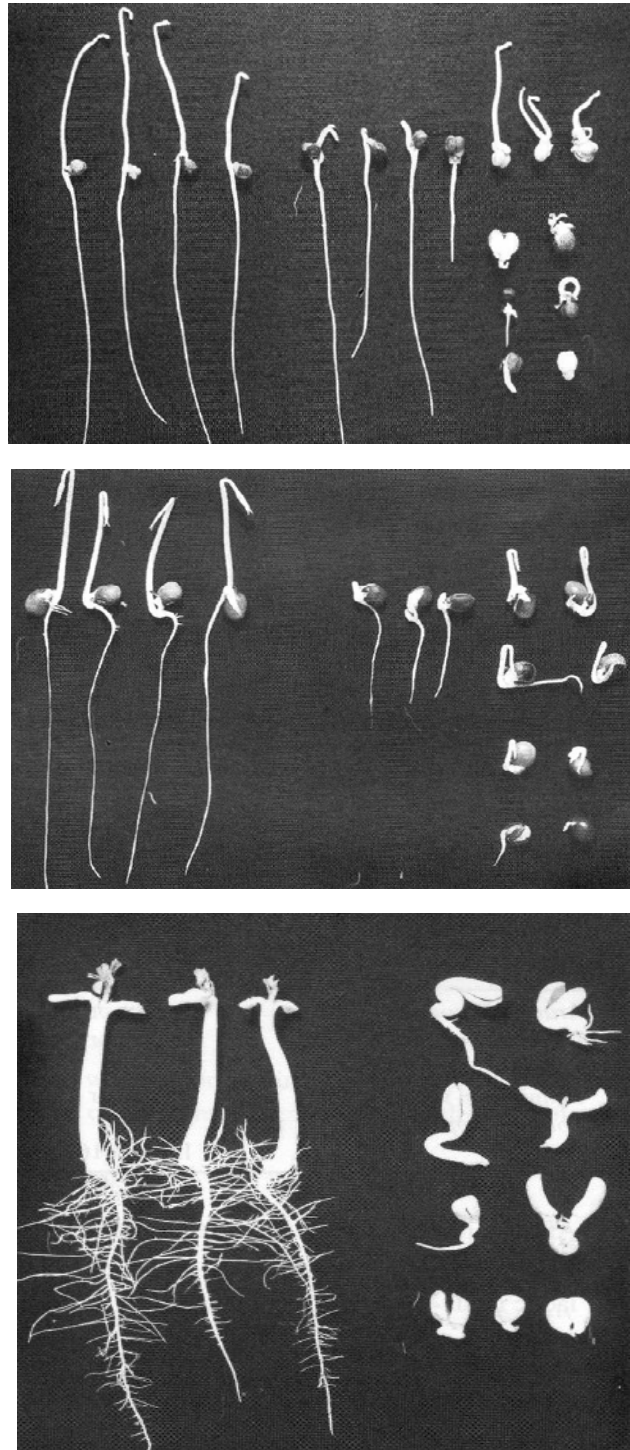


Figure 7. Seed germination test – evaluation of seedlings – normal (left) and abnormal (right) in different crops: Chickpea (top), Pigeonpea (middle), and Groundnut (bottom)

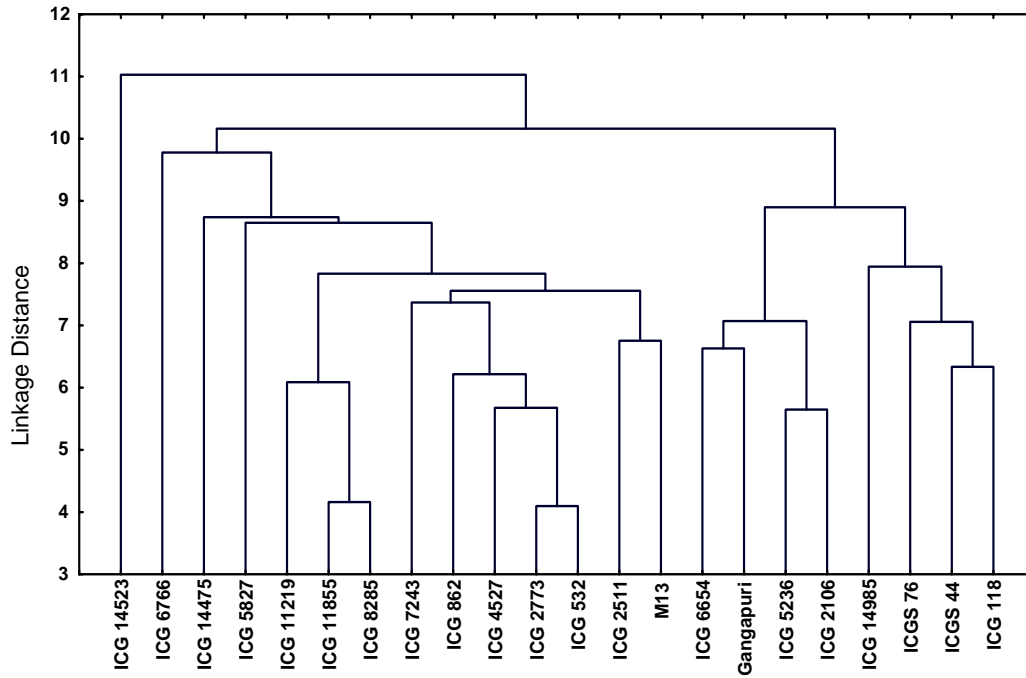


Figure 8. Dendrogram based on first fifteen principal components of 18 selected groundnut accessions and four control cultivars (Upadhyaya 2005)

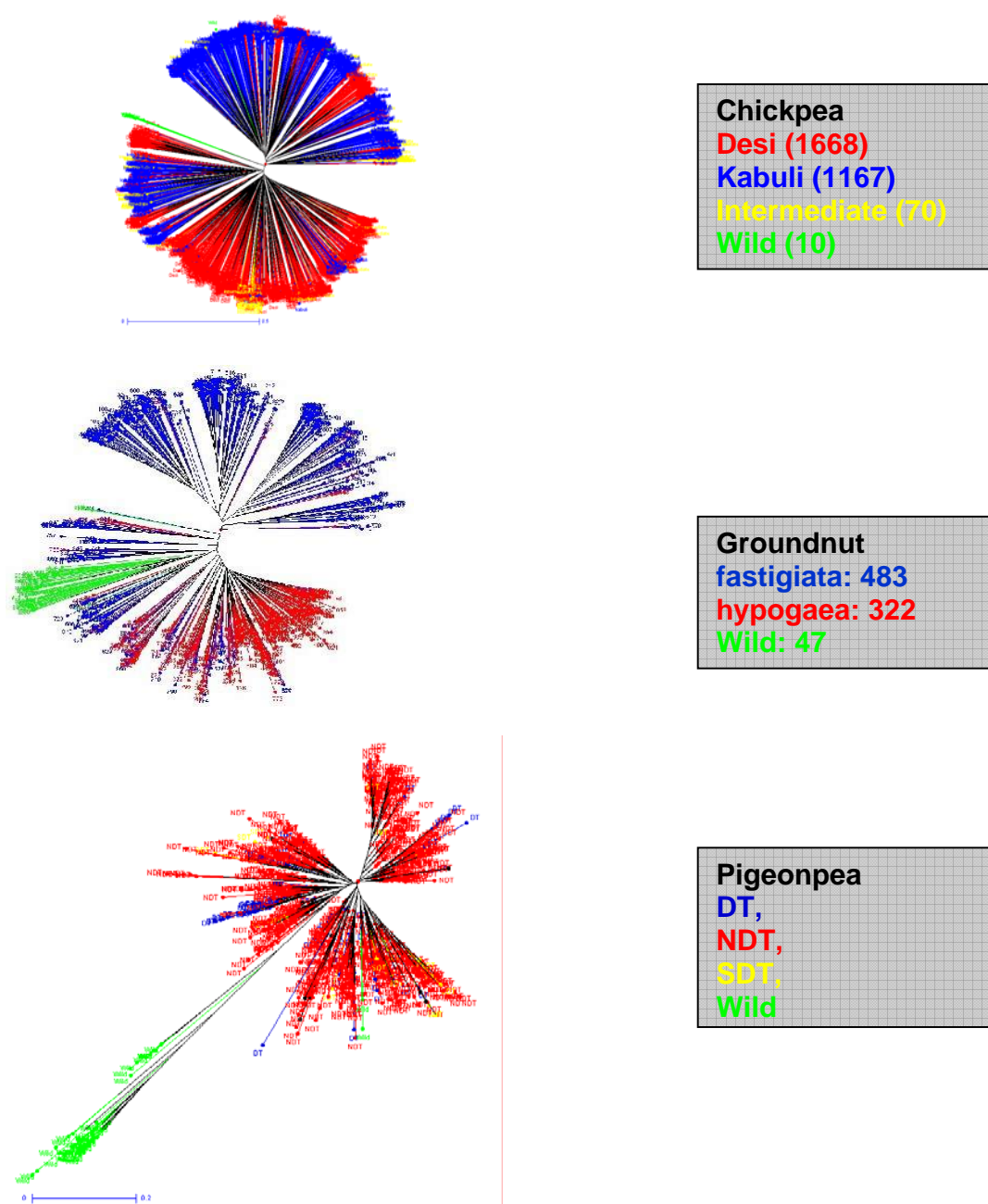


Figure 9. Tree diagrams of genotyping composite sets – top: Chickpea (2915 accessions and 48 SSR markers); middle: Groundnut (852 accessions and 21 SSR markers); and bottom: Pigeonpea (952 accessions and 20 SSR markers).