

Technical Manual no. 10

Managing and Enhancing the Use of Germplasm – Strategies and Methodologies

Hari D Upadhyaya and CL Laxmipathi Gowda



**International Crops Research Institute
for the Semi-Arid Tropics**

Patancheru 502 324, Andhra Pradesh, India
2009

Acknowledgments

We gratefully acknowledge the scientific and technical inputs of Mr DVSSR Sastry, Mr KN Reddy, Mr V Gopal Reddy and Mr Sube Singh, Lead Scientific Officers, Genetic Resources, and Mr AG Girish, Scientific Officer, Plant Quarantine. Our sincere thanks are due to all the Genetic Resources staff past and present, particularly Mr M Thimma Reddy, for completing this manuscript. Finally, the secretarial help of Mr B Ashok Kumar, Senior Executive Associate is greatly appreciated.

Contents

Foreword	vi
Preface	vii
Introduction	1
History of ICRISAT germplasm collections.....	1
Facilities for germplasm conservation and management at ICRISAT	3
Genebank operations.....	6
Audit of Genebank operations at ICRISAT, Patancheru	8

Section 1

Germplasm Assembly.....	11
1A. Germplasm assembly by correspondence	11
1B. Germplasm assembly by exploration and collection	12
1C. Germplasm assembly from center's research	21
1D. Current policy on germplasm acquisition.....	22
1E. Identification of gaps in the germplasm collections	22

Section 2

Plant Quarantine	45
2A. Requirements for seed import.....	45
2B. Post-entry quarantine	46

Section 3

Registering New Germplasm	51
3A. Minimum standards for registration	51
3B. Procedure for registration of samples	52
3C. Documentation of information.....	55

Section 4

Seed Processing	61
4A. Seed cleaning	61
4B. Seed moisture testing.....	65
4C. Seed drying.....	70
4D. Seed viability testing.....	76
4E. Seed health testing.....	87
4F. Seed packing.....	89

Section 5

Seed Storage	99
5A. Medium- and long-term conservation.....	99
5B. Safety duplication.....	103
5C. Storage policy of ICRISAT genebank.....	104
5D. Documenting inventory data.....	105

Section 6

Germplasm Distribution	109
6A. Procedures for germplasm distribution within India.....	109
6B. Procedures for germplasm distribution to other countries.....	111
6C. Procedures for germplasm distribution within ICRISAT.....	112
6D. Feedback on germplasm distribution.....	113
6E. Documenting germplasm distribution data.....	113
6F. Germplasm repatriation.....	114

Section 7

Germplasm Utilization	125
7A. Enhancing germplasm utilization.....	125
7B. Core collections.....	126
7C. Mini core collections.....	130
7D. Identification of new sources.....	132
7E. Composite collections.....	132

Section 8

Monitoring	135
8A. Monitoring seed viability.....	135
8B. Monitoring seed quantity.....	136

Section 9

Germplasm Regeneration	139
9A. Reasons for regeneration.....	139
9B. Procedures for regeneration.....	140
9C. Sorghum regeneration.....	147
9D. Pearl millet regeneration.....	151
9E. Chickpea regeneration.....	156
9F. Pigeonpea regeneration.....	159
9G. Groundnut regeneration.....	163

9H. Small millets regeneration.....	167
9I. Documentation on regeneration.....	168

Section 10

Characterization and Preliminary Evaluation	171
10A. Phenotypic characterization and evaluation	171
10B. Molecular characterization	197
10C. Multilocation evaluation	199
10D. Diversity assessment	200

Section 11

Taxonomic Classification	203
11A. Key to the identification of <i>Sorghum</i> species	203
11B. Key to the identification of <i>Pennisetum</i> species.....	207
11C. Key to the identification of <i>Cicer</i> species	209
11D. Key to the identification of <i>Cajanus</i> species	211
11E. Key to the identification of <i>Arachis</i> species.....	213

Further Reading	219
------------------------------	------------

Appendix 1	223
-------------------------	------------

Appendix 2	226
-------------------------	------------

Foreword

Genebanks conserve a large number of *ex situ* collections that form the backbone of crop improvement programs. The technical manual of procedures and operations of the genebank is an essential document that provides the practices and procedures for various genebank operations. In the first such attempt at ICRISAT, a “Manual of Genebank Operations and Procedures” was published in 2000. The manual was edited by N Kameswara Rao and PJ Bramel. Since then, significant progress has been made globally, and at ICRISAT particularly in research on enhancing use of genetic resources in crop improvement. The concept of mini core collection, postulated by ICRISAT scientists, is now part of the International Public Goods and serves as a gateway for increasing germplasm use. The areas of assessment of diversity using both phenotypic and molecular characterization and identification of trait-specific germplasm for use by crop improvement programs are now a major focus of research at ICRISAT. The changes occurring at the global level, particularly since the implementation of the International Treaty on Plant Genetic Resources for Food and Agriculture, which came into force in June 2004, and meant for enhancing the sharing of germplasm and related information, has added a new dimension to the paradigm. All these, and the recommendations by the Internal Audit of ICRISAT, prompted us to revise the genebank manual and present it in its current form. We do hope that this publication will be helpful to the people dealing with the genetic resources of our five mandate crops and six small millets and to numerous other people who work on other crops. We sincerely acknowledge the contributions of the editors of the earlier version of the manual, and other genetic resources’ staff, past and present.

Hari D Upadhyaya
CL Laxmipathi Gowda

Preface

Germplasm or genetic resources are the foundation of crop improvement programs globally to ensure food security. Over 6 million accessions of different crops are conserved in about 1400 genebanks in different countries. The genebanks are sources of biodiversity for crop improvement, and hence contribute significantly towards achieving food security, poverty alleviation, environmental protection and sustainable development. Crop diversity that evolved over millennia in the farmers fields, is being eroded rapidly in most of the food crops mainly because of replacement of traditional landraces by high yielding cultivars, natural catastrophes (droughts, floods, fire hazards, etc), as well as large scale destruction and modification of natural habitats as a result of development. Large-scale efforts were made across the globe to collect and conserve landraces, cultivars that were becoming obsolete, genetic stocks and wild species important for crop improvement before these were lost forever. ICRISAT conserves nearly 120,000 accessions of five mandate crops (sorghum, pearl millet, chickpea, pigeonpea, groundnut) and six small (finger-, foxtail-, barnyard-, kodo-, little- and proso-) millets. The large *ex situ* collections were assembled and characterized for morphoagronomic traits, and resistance to various biotic and abiotic stresses. Regeneration and supply of seed are routine activities of the genebank. In spite of availability of large collections, their use in crop improvement efforts has been very limited. This is mainly because of non-availability of data of economic traits that require replicated multilocational evaluations to identify useful parents. Core collections, which contain about 10% of accessions but still representing about 70% diversity, have been proposed as a means to enhance use of germplasm. However, in most large collections, such as at ICRISAT, even a core collection would be unwieldy for meaningful evaluation. To overcome this, the concept of a mini core collection, which contains only 1% of the entire collection, was postulated by ICRISAT scientists. This has captured the imagination of the global community. Mini core collections can be evaluated extensively to identify trait-specific genetically diverse germplasm lines for use in crop improvement.

This publication, *Managing and Enhancing the use of Germplasm - Strategies and Methodologies*, by Hari D Upadhyaya and CL Laxmipathi Gowda provides information regarding germplasm assembly, plant quarantine, seed processing and storage, germplasm distribution and utilization, monitoring, germplasm regeneration, characterization and preliminary evaluation, and taxonomic classification. I hope that this publication will help all genebank curators and researchers in their work for safeguarding plant biodiversity in *ex situ* collections and enhancing its use in crop improvement for food security.

William D Dar
Director General
ICRISAT

Managing and Enhancing the Use of Germplasm – Strategies and Methodologies

Introduction

Crop diversity is part of the biological diversity and contributes towards achieving food security, poverty alleviation, environmental protection and sustainable development. Crop diversity is being eroded rapidly in important food crops mainly because of replacement of traditional landraces by modern, high yielding cultivars, natural catastrophes (droughts, floods, fire hazards, etc), as well as large scale destruction and modification of natural habitats harboring wild species. Genetic variation in traditional landraces and wild species is essential for crop improvement, eg, to combat pests and diseases and to produce cultivars better adapted to constantly changing environments. The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) responded to this need by establishing a Genetic Resources Unit in 1979. The specific objectives were assembly, conservation, maintenance, characterization, evaluation, documentation and distribution of germplasm of the mandate crops [sorghum (*Sorghum bicolor* (L.) Moench), pearl millet (*Pennisetum glaucum* (L.) R. Br.), chickpea (*Cicer arietinum* L.), pigeonpea (*Cajanus cajan* (L.) Millsp.) and groundnut (*Arachis hypogaea* L.) and their wild relatives; and six small millets [finger millet (*Eleusine coracana* (L.) Gaertn.), foxtail millet (*Setaria italica* (L.) Beauv), barnyard millet (*Echinochloa crusgalli* (L.) Beauv.), kodo millet (*Paspalum scrobiculatum* L.), little millet (*Panicum sumatrense* Roch. ex Roem. & Schult) and proso millet (*Panicum miliaceum* L.)] (Fig.1).

ICRISAT genebank is one of the world's largest repositories for the genetic resources of its mandate crops, with over 119,000 accessions from 144 countries. The germplasm at ICRISAT genebank was assembled through donations from various institutes and by launching germplasm collection missions in areas of origin and diversity of the mandate crops. These were conducted jointly with national agricultural research systems (NARS), universities and international institutes. Since 1991, collections were also stored as base collection in the long-term conservation facilities at -20°C and at present, about 90% of the total collection is in the long-term storage.

History of ICRISAT germplasm collections

ICRISAT acquired about 8,961 sorghum accessions from the Indian Agricultural Program of the Rockefeller Foundation collection in 1974 through the All India Coordinated Sorghum Improvement Project (AICSIP) and another 3,000 accessions of the missing collections from the duplicate sets maintained in the USA (Purdue and Fort Collins) and Puerto Rico (Mayaguez). Initially, ICRISAT also acquired over 2,000 pearl millet germplasm accessions assembled by the Rockefeller Foundation in collaboration with the Indian Council of Agricultural Research (ICAR) in New Delhi, and another 2,000 accessions collected by the Institut Francais de Recherche Scientifique pour le Développement en Coopération (ORSTOM) in Francophone West Africa.

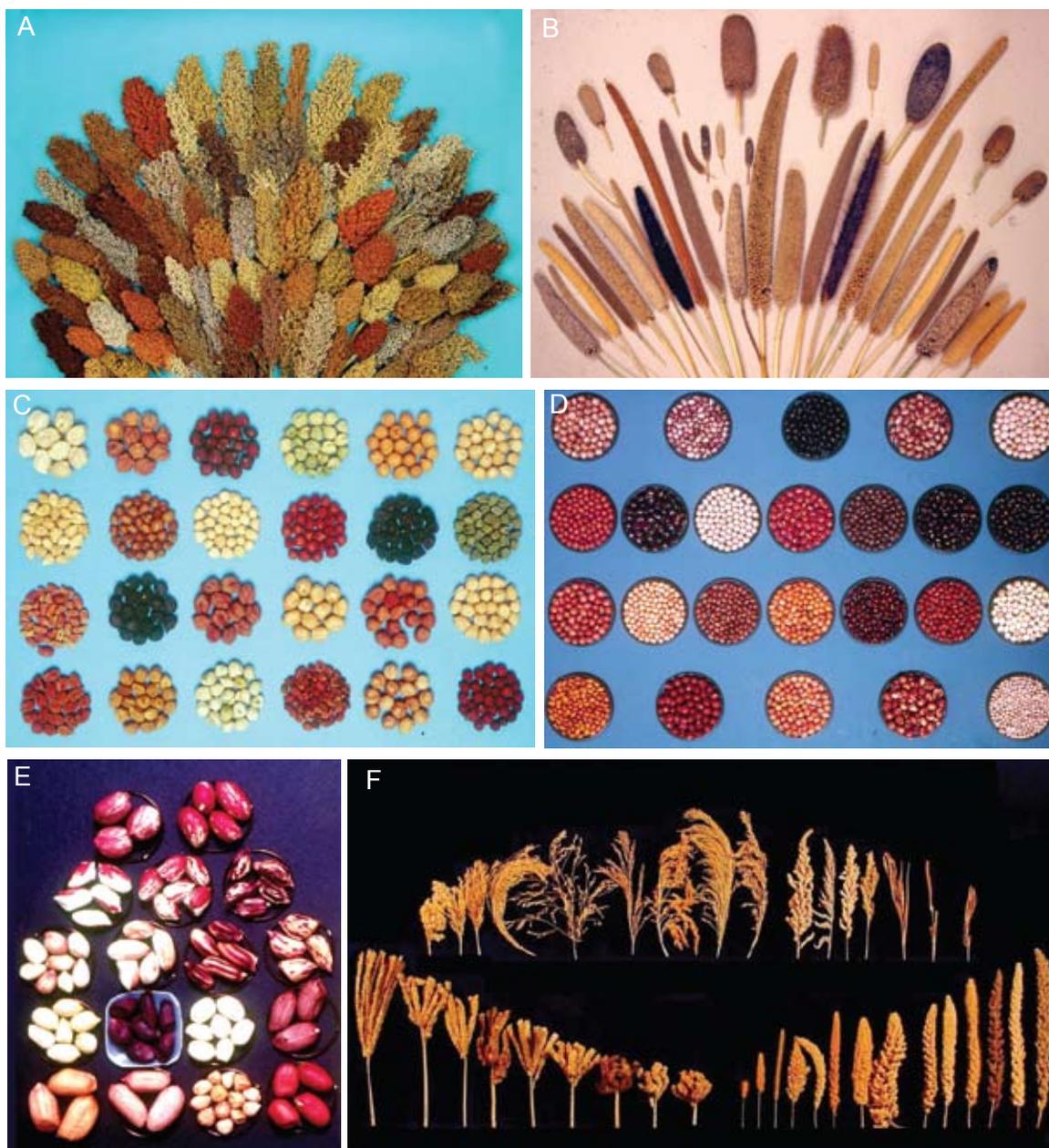


Figure 1. Mandate crops of ICRISAT: Sorghum (A), pearl millet (B), chickpea (C), pigeonpea (D) and groundnut (E), and small millets (F) – diversity for panicle and seed traits.

The chickpea and pigeonpea germplasm initially acquired by ICRISAT consisted of the material originally collected and assembled by the former Regional Pulse Improvement Project (RPIP), a joint project of the Indian Agricultural Research Institute (IARI); United States Department of Agriculture (USDA); and Karaj Agricultural University in Iran. Sets of this germplasm from different agricultural research institutes in India and Iran, and the USDA were donated to ICRISAT in 1972. ICRISAT also acquired over 1,200 chickpea accessions from the Arid Lands Agricultural Development Program (ALAD), which has

its headquarters in Beirut (Lebanon), and was supported by the Ford Foundation (USA), International Development Research Center (IDRC), Canada and of late from International Centre for Agricultural Research in Dry Areas (ICARDA), Syria and USDA, USA. Similarly, much of the groundnut germplasm initially assembled at ICRISAT was received from the collections maintained by the Indian national programs such as the National Research Center for Groundnut (NRCG), Junagadh, India, and the Southern Regional Plant Introduction Station and North Carolina State University, USA.

ICRISAT soon added germplasm to enlarge the world collections of the five mandate crops by collection or assembly of landraces and wild relatives from areas threatened by genetic erosion. Between 1974 and 2008, ICRISAT launched 216 collection missions in areas of diversity and collected 9,011 sorghum, 10,841 pearl millet, 4,228 chickpea, 3,873 pigeonpea and 2,776 groundnut accessions. Apart from ICRISAT's own collection efforts and the major donors cited above, several other international and national organizations, individuals and donations from Ethiopian Sorghum Improvement Project (Ethiopia), Gezira Agricultural Research Station (Sudan), NARS and agricultural universities in India were responsible for augmenting the collections.

All incoming germplasm samples are examined by the Indian Plant Quarantine Services with the assistance of the National Bureau of Plant Genetic Resources (NBPGR), India for exotic diseases and pests. The Indian Government has set up a quarantine unit within the ICRISAT, Patancheru campus to ensure prompt and expeditious inspection and clearance of seed shipments and receipts.

Facilities for germplasm conservation and management at ICRISAT

All the ICRISAT mandate crops and small millets produce orthodox seeds, which can withstand desiccation to low moisture content. Therefore, seed storage is the principle method of conservation of their genetic resources. Seeds are stored in controlled environments in the genebank to prolong seed viability. This process minimizes the frequency of regeneration, which is expensive and involves the danger of genetic shifts. Wild species of groundnut and chickpea, which do not produce adequate quantity of seeds, are maintained as live plants either in the special facilities created or in a greenhouse; and wild species of pigeonpea, sorghum and pearl millet are maintained in the field genebank at ICRISAT, Patancheru.

The genebank at Patancheru has the following facilities for germplasm conservation and maintenance:

- A short-term storage at 18–20°C and 30–40% RH, with a capacity of 680 m³ for temporary holding of seeds while they are dried and prepared for subsequent transfer to medium- and long-term storage.
- Two medium-term storage rooms with a capacity of 210 m³ each and three rooms with a volume of 125 m³ each, at 4°C and 20–30% RH to hold active collections (Fig. 2).



Figure 2. Inside view of the medium-term store at ICRISAT genebank, Patancheru, India.

- Four long-term storage rooms at -20°C each with a volume of 125 m^3 to store base collections of germplasm (Fig. 3).
- A seed drying room and two drying cabinets with a combined volume of about 100 m^3 at 15°C and 15% RH.
- An air-cooled screen house with an area of 402 m^2 and a special facility for maintaining and regenerating wild species of groundnut that do not produce and/or produce less seed. The special facility includes installation of 500 (90.0 cm diameter \times 83.3 cm high \times 5.5 cm thick) Reinforced Cement Concrete (RCC) rings for growing individual accessions, a 240 m^3 capacity underground water sump for collecting rainwater from the nearby buildings for irrigation, and a 1.2 meter high chain link fencing around, housing the area for RCC rings (Fig. 4).
- A field genebank with sufficient area allocated on long-term basis for wild species of sorghum, pearl millet and pigeonpea.
- A seed laboratory for conducting germination tests, seed technology research and cytological work.
- Access to sufficient field space in each cropping season on ICRISAT campus at Patancheru for regeneration, field characterization and evaluation of germplasm.

To meet the demand for germplasm of mandate crops from African countries and to facilitate easy access to the germplasm collections, ICRISAT also established medium-term cold stores run at 4°C and 20–30% RH at Nairobi, Kenya; Bulawayo, Zimbabwe and Niamey, Niger to hold working collections, core and mini core collections and reference sets of composite collections.



Figure 3. Inside view of the long-term store at ICRISAT genebank, Patancheru, India.



Figure 4. Facilities for regeneration and maintenance of groundnut wild relatives at ICRISAT, Patancheru, India.

At Patancheru, the storage chambers are constructed on a modular principle with prefabricated panels and have mobile shelving, each capable of accommodating about 20,000 seed accessions. The genebank has a standby generator to cope with power failure. Each medium- and long-term storage room has standby refrigeration and dehumidification systems. In addition, audible and visual electronic alarms and fire warning systems help maintain the desired conditions and safeguard the germplasm against fire hazards. All genebank cold rooms and seed drying facility are linked to a computer system and to the security services for safe and secured maintenance of genebank.

Genebank operations (Fig. 5)

Assembly of germplasm from other plant introduction centers and through collection in areas of known genetic diversity, is the first step in *ex situ* conservation of crop diversity. Collecting germplasm and its conservation are expensive. Therefore, collections are undertaken only after a critical assessment of the need and assembly is made only of unique landrace germplasm, which is not already represented in the collection. The Convention on Biological Diversity (CBD), which came into force on 29 December 1993 and the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) of 29 June 2004 provides the framework for acquisition and utilization of germplasm. Consistent with Article 15 of the CBD, which recognizes the sovereign rights of nations over their biodiversity, collection and acquisition of germplasm are undertaken with prior informed consent, using Material Transfer Agreement (MTA) on mutually agreed terms. Plant quarantine becomes an integral part of germplasm assembly since importation of exotic germplasm is subject to quarantine regulations of host country.

Following receipt at the genebank, the seed samples are registered and added to the collection if they meet the minimum standards for germination, seed quantity and accompanying passport information. The operational sequence to integrate an accession into the genebank involves cleaning, moisture determination, drying, viability testing and packing. The management of seed collections requires that germplasm accessions be maintained with a high proportion of viable seeds. This involves storage under optimal conditions, periodic monitoring of seeds for viability and quantity, and regenerating them when the situation warrants. Germplasm regeneration is done in post-rainy season to get quality seeds. Adequate number of plants are grown and sampled equally to minimize genetic drifts. During regeneration, the genetic integrity of cross-pollinating crops such as sorghum, pearl millet and pigeonpea is maintained by pollination control. In line with the policy of the Consultative Group on International Agricultural Research (CGIAR) on plant genetic resources, ICRISAT has been distributing germplasm free to all bona fide users. However, ICRISAT germplasm collections have been later placed under the auspices of the Food and Agricultural Organization of the United Nations (FAO) following an agreement signed on 26 October 1994 with the FAO. Germplasm samples are distributed under the Standard Material Transfer Agreement (SMTA) in accordance with the International Treaty (ITPGRFA) that prevents the recipients from claiming intellectual property rights (see Annexure 1.1).

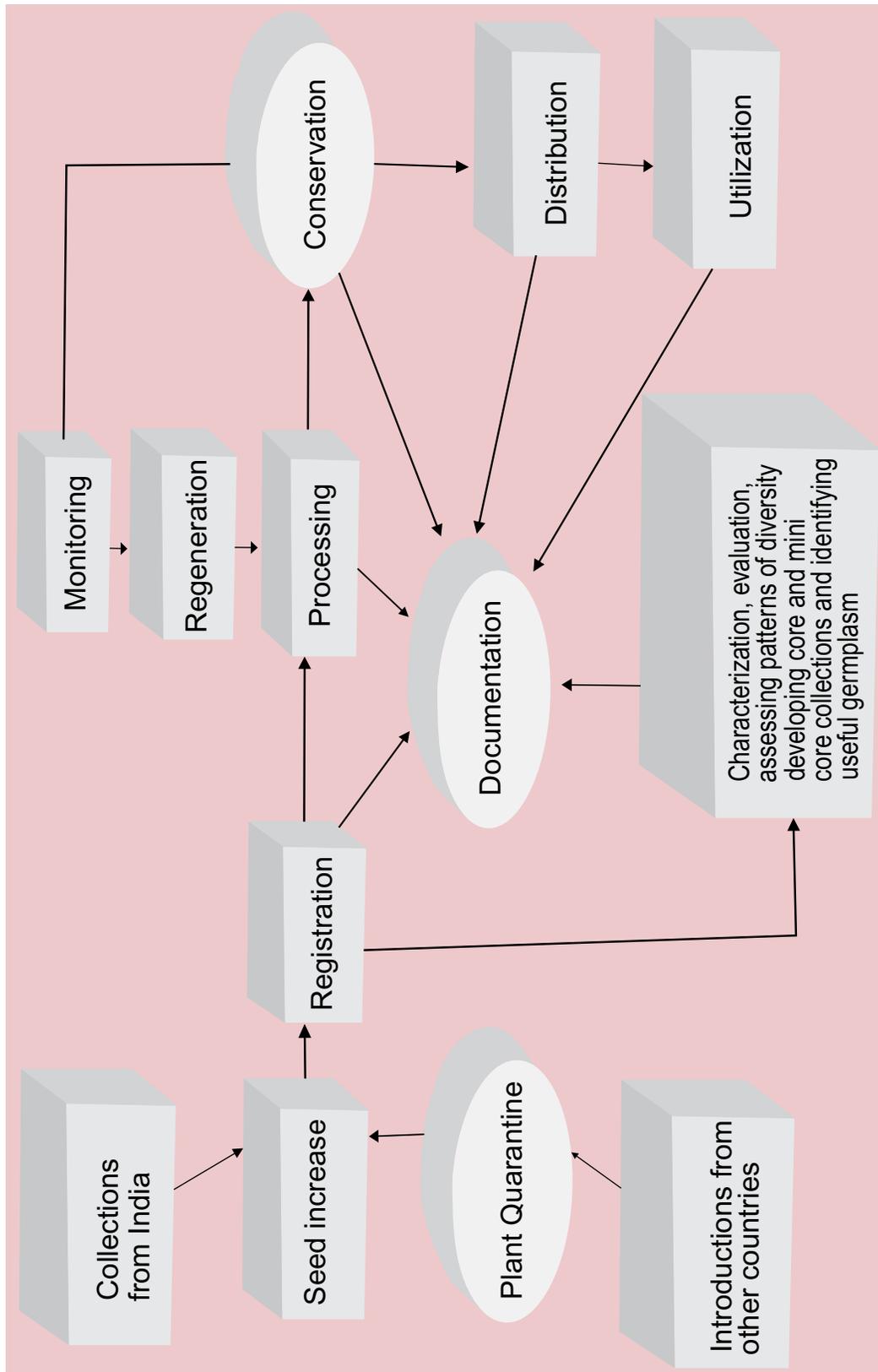


Figure 5. Operational flow chart of ICRISAT genebank.

Characterization and evaluation of the assembled germplasm is essential to facilitate its utilization. This is done using a set of internationally accepted descriptors for stable botanical characters and a few environmentally influenced agronomic and quality traits. The morphological and agronomic characters are scored in field plots during the rainy and post-rainy seasons. Characterization and evaluation data facilitate preliminary selection of germplasm by users, while information on country of origin, province and location of collection, and pedigree, among other passport data, permits the selection of germplasm on geographic basis and identification of gaps in the collection.

Work with genetic resources involves management of large volumes of information. Documentation is essential in good genebank management to allow efficient and effective use of germplasm. There are four categories of information associated with each accession related to passport, characterization and evaluation, inventory and distribution details. These data are maintained on computers using relational database management systems (Genebank Information Management System-GIMS), which facilitate sharing as well as easy retrieval of information on origin, morphological and agronomic traits based on predetermined criteria. The information on germplasm is also freely available to users. ICRISAT also ensures access to the passport and characterization data by participating in the CGIAR's System-Wide Information Network for Genetic Resources (SINGER). The data is accessible through <http://SINGER.grinfo.net>.

Audit of Genebank operations at ICRISAT, Patancheru

In terms of the Board-approved Internal Audit Plan for 2006-07, the Internal Audit Unit carried out an audit of the genebank operations at ICRISAT-Patancheru. It concluded that the internal control systems and risk management practices built into the genebank operations are of good level. Genebank activities are carried out with an appropriate degree of efficiency and effectiveness to meet its objectives. The audit observations and recommendations were discussed to strengthen and rationalize internal control systems, processes and risk management practices.

The various activities involved in germplasm conservation and management are described in detail in the following sections.

Section 1

Germplasm Assembly

The initial step in conservation of genetic resources of seed crops is germplasm assembly. This activity is done by:

- 1A. Germplasm assembly by correspondence.
- 1B. Germplasm assembly by exploration and collection.
- 1C. Germplasm assembly from center's research.

1A. Germplasm assembly by correspondence

Samples can be obtained by correspondence if it is known that diversity in an area of interest was already collected. However, in accordance with Article 15 of the CBD, which clearly states that access to genetic resources shall be on mutually agreed terms, material should not be acquired until its status with regard to conservation, distribution and use are clearly defined through formal agreements with the donors.

1A.1. Identification of unique samples for acquisition

Maintaining a sample in the genebank is expensive; therefore, the Genebank Curator should carefully check if the sample already exists in the collection before deciding on acquiring it. Since each genebank adopts its own numbering systems, it is possible that the same accession is available under different identities. Duplication in the collections is best identified by comparing relevant fields in databases, using a computer program such as Genebank Information Management System (GIMS), a stand-alone facility developed at ICRISAT.



Working with GIMS

- ***Select the tables from the resident and imported databases that contain passport information of the collections.***
- ***For comparison, select the fields that contain information on accession identity, alternate name from both the tables and run the program.***

The program checks for similar names/identities in imported data and lists them with relevant accession numbers in the resident database. List of unique accessions in the imported data can also be obtained through the program, if the user wants.

1A.2. Acquiring unique germplasm from other genebanks

- Obtain complete passport information of the collection, especially alternate names or identification numbers, pedigree, original source, etc, from the donating institute.

Most often, errors are made during data entry, especially with spaces, hyphenation, case and spelling, which require careful checking when comparing databases to identify duplicate accessions. The GIMS program was developed to handle such events.

- Prepare the final list of unique accessions to be acquired.
- If the material is to be received from other countries, obtain an Import Permit, for example, from the National Plant Quarantine Service, Government of India, by applying to the Director, NBPGR, New Delhi, India on a prescribed form (Annexure 1.2).
- Send the final list of unique accessions along with the Import Permit and green labels for affixing on seed packages, with the following guidelines to the consignor on seed export.

1A.3. Guidelines to be followed by consignor (when sending seeds to India)

- The original Import Permit and a Phytosanitary Certificate (PSC) issued by the National Plant Quarantine Services of the exporting country must accompany the material.
- Ask the consignor to complete and send the “Form for Advance Intimation of Import of Seed Samples to India” as advance intimation of export of the proposed seed material (Annexure 1.3).
- The green labels are to be affixed or pasted on the outside of the seed package (Annexure 1.4).
- The consignor should not address the seed consignment to ICRISAT, but he/she should send it directly to Director, NBPGR, New Delhi, India.
- The seed material should be free from soil.
- The seeds should be free of infections (of pathogens) or infestations (by pests) and free of weed seeds, crop residues and inert material.
- Seed samples should not be treated with chemicals.

1B. Germplasm assembly by exploration and collection

The main reasons for collecting germplasm are:

- Genetic erosion — loss of genetic diversity.
- Gap filling — when diversity is missing or insufficiently represented in an existing collection.
- Need based — for breeding, research, or developmental work.

Germplasm collecting missions are broadly of two kinds:

- Crop specific or targeted missions to collect material with attributes such as adaptation to high altitude, salinity and cold tolerance; or wild relatives, weedy types and related taxa of a crop, and
- Ecosystem focused missions to collect maximum diversity in different crops occurring and maturing almost at the same time in the region.



- ***Collecting germplasm is expensive. Therefore, make a critical review of the past collection activities of the crop before embarking on a collection trip.***
- ***If germplasm was already collected from the area, correspond with the collector(s) and obtain duplicate samples.***

1B.1. Planning collection missions

The focus of collection in the past has been mainly on conserving the broad range of diversity in the landraces or wild species. The collection team consists of an expert on the crop from the center and a local expert. The collection sites are selected based on the knowledge of the crop specialist or priorities set for the collection. Subsequent to the coming into force of the CBD, and the recognition of sovereignty of countries over their natural resources, access to genetic resources is subject to prior informed consent by the contracting party, ie, national governments. Further, access is granted on mutually agreed terms. It also requires taking measures to share the benefits of commercialization and utilization of genetic resources with provider countries. In line with the changed environment, ICRISAT has revised procedures for germplasm acquisition. The new procedures for acquisition of germplasm are as follows:

- Collections should be done at the specific requests from national agricultural research systems (NARS). The purpose of collection could be for:
 - national and international genebanks,
 - breeding programs,
 - other crop improvement programs, and
 - scientific study.
- Collection sites should be identified through participatory approach with broader group of users including:
 - scientists in NARS or at universities,
 - scientists in the private seed sector,
 - crop based national, regional or international networks,
 - crop and topic specific working groups,
 - development or food aid programs,
 - local, national or international non-governmental organizations (NGOs), and
 - community seed banks or other seed distribution agencies.

Technical and logistic planning begins once the decision for collecting is made. Collection missions should be planned at least one year in advance. The explorer must synthesize all available information such as:

- Environmental heterogeneity
- History and distribution of crop
- Cultural diversity
- History of movement of people
- Threats to genetic diversity
- Soundness of the political climate of the country to be explored.



- ***Consult state and regional reports, flora and latest published works to get familiarized with climate, ecology, vegetation and agriculture.***
- ***Study herbarium material, particularly of wild relatives to get visual impression of taxa targeted for collection.***
- ***Study critically, the provenance data on the herbarium sheets and take notes on the flowering, fruiting, habitat, altitude and other useful characteristics.***

An itinerary and provisional route should be established using information gathered from above. In wild species, harvesting needs to be done before seed shattering, therefore, timing of the collection mission is important. Crop cycle and seasonal workload should be considered to ensure cooperation by the farmers in the collection. Also efforts should be made to accommodate the farmer's traditional procedures for field entry and harvest, which may include significant ceremonies.

1B.2. Technical preparation

Collecting germplasm requires meticulous planning. The explorer needs to be in the right area at the right time. Prospective collectors should identify local or national collaborators, and have prior discussion or correspondence with them on practical arrangements including:

- Priorities of collecting
- Methodologies and strategies
- Information to be gathered during collection
- Processing and conservation arrangements
- Financial aspects of the mission.



In line with the principle of national sovereignty over plant genetic resources, and Article 7 of International Code of Conduct for Plant Germplasm Collecting and Transfer, germplasm collectors should secure from relevant authorized government body:

- ***Prior approval for collecting,***
- ***Material transfer agreements for transfer of the collected material incorporating provisions for further handling, storage, regeneration, utilization and distribution (sample Standard Material Transfer Agreement (SMTA) given as Annexure 1.1).***

1B.3. Collecting team

Collecting team should always be small — not more than three or four; and preferably include a woman. The team leader should be preferably a botanist or breeder/agronomist from the collecting organization. The team should consist of:

- Team leader from collecting organization
- Representative of NARS
- Local expert like extension officer
- Driver with some knowledge of the target region, who should be able to speak local language and carry out basic repairs to the vehicle.

A single team can conduct the collection or it can be coordinated through local or regional staff, depending on the range of maturity encountered in the region. The single team model requires careful planning to coincide the time of collection with seed harvest. If the local extension agents or NGOs are requested to make the final collection, special instructions need to be given for sampling and seed handling.

1B.4. Equipment

The basic equipment needed for collection is as follows:

1. Harvesting

- | | |
|---|--|
| <input type="checkbox"/> cloth bags or nylon net bags | <input type="checkbox"/> drying stove and stand |
| <input type="checkbox"/> seed envelopes | <input type="checkbox"/> packing sacks |
| <input type="checkbox"/> strong knife or secateurs | <input type="checkbox"/> digger and sieves for sifting soil (important for collection of groundnut and its wild species) |
| <input type="checkbox"/> scissors | <input type="checkbox"/> pencil, pens and permanent markers of different colors |
| <input type="checkbox"/> field or collector's notebooks | <input type="checkbox"/> stapler and staples |
| <input type="checkbox"/> rubber bands for closing bags | <input type="checkbox"/> pocket knife |

- labels (preferably tear-off tags) for labeling specimens
- plant press with corrugated aluminum sheets
- absorbent paper for pressing specimen
- gloves
- local currency in small denomination
- receipt pad

2. Scientific equipment

- portable altimeter
- Geographical Positioning System (GPS)
- field compass for emergency use
- cameras with close-up lenses and filters
- digital camera with charger
- light meter
- pocket lenses
- soil sample kit
- binoculars
- calendar

3. Printed material

- regional flora
- road maps
- vegetation or climate maps
- list of rest houses or hotels
- information on fuel points
- printed slips with institute's address
- collection data sheets
- herbarium and quarantine labels
- visiting cards
- Import Permit or other required permits

4. Personal care

- light-weight tents with sealed ground sheet if collecting in areas with no accommodation
- mosquito net
- sleeping bags, pillow and blanket
- cooking equipment and stove
- eating utensils
- large and small water containers
- high quality water filter or purifier system
- electric torch and lamp with spare batteries
- matches
- candles
- tarpaulin

5. Other equipment

- cardboard boxes
- hunter shoes
- plastic water shoes
- wide brimmed hat
- sun glasses
- plastic bottles of various sizes
- formaldehyde
- alcohol

6. Medicines

- anti-malarial pills appropriate for the region
- insecticide sprays or repellent creams
- first-aid kit
- pain-killer pills
- snake bite kit for commonly found poisonous snakes
- antipyretics (paracetamol or aspirin)
- anti-itch creams or antihistamines
- antacid tablets
- antiseptic cream or liquid
- anti-diarrhea pills

Follow medical advice about vaccinations. Preventive inoculations for typhoid, yellow fever, cholera and other endemic diseases in targeted location(s) for germplasm collections should be taken according to the health laws and regulations of the visiting country or area.

7. Transport

- a four-wheel drive motor vehicle with roof rack
- puncture repair kit
- one set of spare parts and tools
- two jerricans for diesel/petrol
- two spare tyres
- engine driven winch and chain or nylon rope
- pump and pressure gauge

When explorations are planned in foreign countries, transportation arrangements and acquisition of articles listed above should be finalized well ahead of time.

8. Clothing

- drip-dry clothes that can be layered for warmth and protection
- strong high boots for snake infested areas
- lightweight jackets and long sleeved shirts with plenty of pockets
- sweater and water proof clothing if collecting during rainy season.

In general, clothing should suit the region being visited. Remember that high altitudes can be very cold during nights and in the mornings, even if it is warm during the day.



When collecting abroad, do not forget to carry with you:

- ***Permission letter of government, addresses of contact persons and copies of previous correspondence.***
- ***Passport with visas, health certificate and other travel documents.***
- ***Import Permit from home country if the material is to be transported back.***

1B.5. Sampling strategy

The sampling strategy should be based on purpose of collection. For example, if it is for a genebank, then concentrate on maximum diversity both among and within landraces, with a minimum number of samples. If it is for a breeding program, focus on identifying sources of improved farmers' varieties, and if it is for development programs, farmers' varieties along with related information would be more important.

1B.6. Collection sites

Overall sampling strategy depends on the breeding system and ecological diversity of the area.

- Collections should not be made from sites that are less than 10 km apart, unless,
 - landraces grown are morphologically different,
 - there is marked change in altitude or cropping systems,
 - a formidable barrier such as a mountain or a river exists, or
 - local people are ethnically different from previous collection site.
- Disjunct populations occupying remote and distinct ecological habitats should be collected.
- Priority for taking sample should be farmers' field. Samples can be taken from farmers' store, local markets if there is not enough time to cover fields over a whole region. Local tribal markets offer enormous diversity, including little known cultigens of local distribution.
- Samplings must be made over as many different environments and regions as possible.
- Collect away from main roads since introduction of advanced cultivars begins in regions close to main roads.
- Ascertain from local farmers and ***avoid collecting introduced and improved cultivars.***
- Avoid collecting duplicates. However, landraces or 'old' cultivars with the same name and essential features, if grown in ecologically distinct sites, could be different eco-strains and, therefore, can be sampled.

1B.7. Sample size

As a rule of thumb, obtain random sample by taking heads or pods every three paces, along a number of transects through the crop.

- Collect not less than 50 and not more than 100 panicles from each field, in case of cereals. If the species produces heads with large number of seeds (eg, sorghum and millets), collect only small portions of the heads.
- Take five ripe pods from each of three adjacent plants every three paces, in case of legumes.

It is best to collect a larger sample keeping in mind that seeds may be needed for base and active collections as well as for duplicate conservation. Larger quantity is also needed in case of genetically heterogeneous samples than for more uniform samples. Additional

non-random samples may be collected if the collector sees interesting forms, which are not included by random sampling.

1B.8. Useful tips when collecting

- It is advisable to start work in the morning after an early breakfast. Carry packed lunch. Get back to the camping site before sunset.
- Note down meter reading before the vehicle starts for work each day. Keep record of distances covered daily, petrol filling and other expenses incurred (distilled water, coolant and oil) and enter these in the logbook.
- Hold discussions with local officers, block or village extension workers and old farmers, and assemble relevant information on crops and locations for collecting diversity. Do this each evening a day ahead of the collecting itinerary and prepare a tentative program to be followed — villages/route/distances to be covered by vehicle and on foot.
- If the team is coming back to the same camping site, it is advisable to follow a circuitous route so that more villages can be covered.
- Do not plan to cover more than 100 or 200 km on “bad” roads and 300 km on “good” roads.
- Spare time for market survey, backyard surveys, visit to farmers’ homes to see stored produce and other observations. Also, allot time for discussions with farmers, extension workers and others.
- Allow enough time to collect herbarium specimens (especially wild species) and take photographs.
- On reaching the camp each evening, take out the collection, check and label them properly, press herbarium specimens, and complete notes in the logbook as well as in the field data book.
- Make it a principle to complete your daily work the same evening, re-equip your bag with items needed for the next day’s collecting before retiring.



- ***Be inquisitive to acquire information on anything interesting.***
- ***Do not be over zealous to take more material than that agreed with the farmer.***
- ***Money may have to be paid occasionally to collect the desired germplasm from farmers.***
- ***Never forget to convey your gratitude to the farmer before leaving.***

1B.9. Documentation

Data gathering is an important part of collection. Absolute minimum information to be recorded is:

- Collectors' and collection number
- Date and site of collection
- Geographical coordinates of collection sites
- Status of sample (wild, weedy, cultivated)
- Source of collection (field, market sample or farm store)
- Label the collection bags both within and outside.

For convenience in the field, carry standardized collecting record books (specimen page presented as Annexure 1.5).

Use tear-off tags for this purpose. The label inside accompanies the sample when it is cleaned, threshed or placed in storage containers. The label outside helps in initial sorting of the samples.

- Identify the collection site precisely using a Geographical Positioning System (GPS). If the site is not obvious from the map, then record the names of adjacent villages and kilometer reading of the vehicle at known places before and after the site.
- Document information on traditional knowledge from the farmers growing the varieties (Fig.1B.9.1), including:
 - farmer's name and description of environment,
 - landrace or cultivar characteristics as described by the farmer,
 - end use of the landrace or cultivar and its specific properties,
 - normal cultural practices used with the landrace or cultivars, and
 - history of the landrace or cultivar with the farmer.

A farmer's survey form incorporating the above details developed and used by Bramel-Cox and Christinck (1998) is in Annexure 1.6.



Figure 1B.9.1. Collecting information along with germplasm sample.

1B.10. Handling and processing of collected samples

- Collect the seeds in small paper bags (15 cm × 7 cm) with metal or ordinary hand fold.
- Use cloth bags that allow circulation of air (eg, muslin bags) when panicles or mature pods are to be collected.
- Thresh, winnow and clean the seeds if time permits and facilities are available at the base camp.
- Dry the seeds under shade or with a drying agent or by allowing ample aeration within the sample to reduce the moisture content.

If it becomes necessary to travel for collecting new material while holding the already collected germplasm, the exploration team should ensure safety of the collected material until collection is completed and it is transported to the genebank.

Primary focus of collection is to meet the needs of the National Programs or the local communities. The collected material should be first evaluated by growing in the local environment. The grow-out could be used for seed multiplication and characterization of the collection in the country of origin in cooperation with the partners. Field days are arranged to identify locally-adapted cultivars, so that the benefits of the collection are shared in the country of origin and among the local communities. Ensure that materials are acquired using SMTA (see Annexure 1.1).



Exposing seeds to unfavorable environmental conditions during transportation can be very damaging. Therefore, care must be taken:

- ***to maintain the material at optimum temperature and moisture content even when the distance for transportation is short, and***
- ***to see that the container or box is cushioned, and no damage is done to the seeds during transport.***

Recruit couriers to accompany the team when collecting on long expeditions in remote places, and send perishable material or seeds with limited viability to the base camp for onward transmission to headquarters.

1C. Germplasm assembly from center's research

1C.1. Acquisition of genetic stocks

Germplasm accessions screened and 'purified' through selection for desirable characteristics, and mutants identified in germplasm grow-outs serve as important raw material for crop improvement. These include sources of resistance to biotic and abiotic constraints, male sterile lines, dwarfs and other genetic stocks. Genebanks should acquire such material along with complete pedigree information.

1C.2. Acquisition of elite breeding material

Elite germplasm generated in the breeding programs for specific traits or with proven high yield such as released cultivars and genetic stocks may also be acquired by the genebank. While acquiring, ensure that the material has complete pedigree information and key morphological data.

1D. Current policy on germplasm acquisition

The genebank should have a clear policy on acquisition so that the volume of material acquired is within limits of the management capacity of the genebank. When storage space or the resources to maintain the collections are limiting, acquire germplasm based on priority.

Prioritization

Germplasm usually consists of the whole range of genetic variation found in the crop, contained in:

- Primitive cultivars,
- Landraces,
- Wild and weedy forms,
- Genetic stocks,
- Elite breeding material, and
- Improved varieties (both obsolete and modern).

Acquisition of germplasm should be based on value or perceived threat of extinction. Value can be assessed by the usefulness of traits, and adaptation to unique environments. Landraces, wild and weedy species should receive high priority for acquisition due to the imminent threat of replacement, followed by genetic stocks. Consider the ability to be able to manage the species before acquiring wild species.

1E. Identification of gaps in the germplasm collections

Identification of gaps in the germplasm collections is necessary to assess completeness of the collection and exploration for further collection.

Procedure to identify gaps:

- Using Microsoft Encarta®, an electronic atlas (MS Encarta® Interactive World Atlas 2000), retrieve the geographic coordinates of locations to fill the gaps for accessions not having the information.
- Using Arc View, a GIS tool, check the accuracy of the coordinates by plotting all accessions on latest political boundary map of each country.

- Using the FloraMap, a GIS tool, create the probability distribution map for each species in different countries.
- Overlay the collection sites or sampled sites on the probability map and identify the districts without and/or with few collection sites and high probability (>70%).
- Using Diva-GIS, assess the diversity in the assembled germplasm for each trait.
- Overlay the probability map, collection sites and the diversity index of assembled germplasm and identify the gaps in trait-wise diversity.
- Use land cover maps of FAO to know the type of vegetation and land cover in the areas identified.
- Consult local government officials and extension officers working in targeted area for crop cultivation and cropping pattern and then finalize the area for exploration.

 STANDARD MATERIAL TRANSFER AGREEMENT*

PREAMBLE

WHEREAS

The International Treaty on Plant Genetic Resources for Food and Agriculture (hereinafter referred to as “the **Treaty**”)¹ was adopted by the Thirty-first session of the FAO Conference on 3 November 2001 and entered into force on 29 June 2004;

The objectives of the **Treaty** are the conservation and sustainable use of **Plant Genetic Resources for Food and Agriculture** and the fair and equitable sharing of the benefits arising out of their use, in harmony with the Convention on Biological Diversity, for sustainable agriculture and food security;

The Contracting Parties to the **Treaty**, in the exercise of their sovereign rights over their **Plant Genetic Resources for Food and Agriculture**, have established a **Multilateral System** both to facilitate access to **Plant Genetic Resources for Food and Agriculture** and to share, in a fair and equitable way, the benefits arising from the utilization of these resources, on a complementary and mutually reinforcing basis;

Articles 4, 11, 12.4 and 12.5 of the **Treaty** are borne in mind;

The diversity of the legal systems of the Contracting Parties with respect to their national procedural rules governing access to courts and to arbitration, and the obligations arising from international and regional conventions applicable to these procedural rules, are recognized;

Article 12.4 of the **Treaty** provides that facilitated access under the **Multilateral System** shall be provided pursuant to a Standard Material Transfer Agreement, and the **Governing Body** of the **Treaty**, in its Resolution 1/2006 of 16 June 2006, adopted the Standard Material Transfer Agreement.

¹ *Note by the Secretariat:* as suggested by the Legal Working Group during the Contact Group for the Drafting of the Standard Material Transfer Agreement, defined terms have, for clarity, been put in bold throughout.

* In the event that the SMTA is used for the transfer of Plant Genetic Resources for Food and Agriculture other than those listed in Annex I of the Treaty:

The references in the SMTA to the “Multilateral System” shall not be interpreted as limiting the application of the SMTA to Annex I Plant Genetic Resources for Food and Agriculture, and in the case of Article 6.2 of the SMTA shall mean “under this Agreement”;

The reference in Article 6.11 and Annex 3 of the SMTA to “Plant Genetic Resources for Food and Agriculture belonging to the same crop, as set out in Annex I to the Treaty” shall be taken to mean “Plant Genetic Resources for Food and Agriculture belonging to the same crop”.

ARTICLE 1 — PARTIES TO THE AGREEMENT

1.1 The present Material Transfer Agreement (hereinafter referred to as “**this Agreement**”) is the Standard Material Transfer Agreement referred to in Article 12.4 of the **Treaty**.

1.2 **This Agreement** is:

BETWEEN: *(name and address of the provider or providing institution, name of authorized official, contact information for authorized official*)* (hereinafter referred to as “the **Provider**”),

AND: *(name and address of the recipient or recipient institution, name of authorized official, contact information for authorized official*)* (hereinafter referred to as “the **Recipient**”).

1.3 The parties to **this Agreement** hereby agree as follows:

ARTICLE 2 — DEFINITIONS

In **this Agreement** the expressions set out below shall have the following meaning:

“**Available without restriction**”: a **Product** is considered to be available without restriction to others for further research and breeding when it is available for research and breeding without any legal or contractual obligations, or technological restrictions, that would preclude using it in the manner specified in the **Treaty**.

“**Genetic material**” means any material of plant origin, including reproductive and vegetative propagating material, containing functional units of heredity.

“**Governing Body**” means the **Governing Body** of the **Treaty**.

“**Multilateral System**” means the **Multilateral System** established under Article 10.2 of the **Treaty**.

“**Plant Genetic Resources for Food and Agriculture**” means any **genetic material** of plant origin of actual or potential value for food and agriculture.

“**Plant Genetic Resources for Food and Agriculture under Development**” means material derived from the **Material**, and hence distinct from it, that is not yet ready for **commercialization** and which the developer intends to further develop or to transfer to another person or entity for further development. The period of development for the **Plant Genetic Resources for Food and Agriculture under Development** shall be deemed to have ceased when those resources are **commercialized** as a **Product**.

* *Insert as necessary. Not applicable for shrink-wrap and click-wrap Standard Material Transfer Agreements.*

A “shrink-wrap” Standard Material Transfer Agreement is where a copy of the Standard Material Transfer Agreement is included in the packaging of the **Material**, and the **Recipient's** acceptance of the **Material** constitutes acceptance of the terms and conditions of the Standard Material Transfer Agreement.

A “click-wrap” Standard Material Transfer Agreement is where the agreement is concluded on the internet and the Recipient accepts the terms and conditions of the Standard Material Transfer Agreement by clicking on the appropriate icon on the website or in the electronic version of the Standard Material Transfer Agreement, as appropriate.

“Product” means **Plant Genetic Resources for Food and Agriculture** that incorporate² the **Material** or any of its genetic parts or components that are ready for **commercialization**, excluding commodities and other products used for food, feed and processing.

“Sales” means the gross income resulting from the **commercialization** of a **Product** or **Products**, by the **Recipient**, its affiliates, contractors, licensees and lessees.

“To commercialize” means to sell a **Product** or **Products** for monetary consideration on the open market, and **“commercialization”** has a corresponding meaning. **Commercialization** shall not include any form of transfer of **Plant Genetic Resources for Food and Agriculture under Development**.

ARTICLE 3 — SUBJECT MATTER OF THE MATERIAL TRANSFER AGREEMENT

The **Plant Genetic Resources for Food and Agriculture** specified in *Annex 1* to **this Agreement** (hereinafter referred to as the **“Material”**) and the available related information referred to in Article 5b and in *Annex 1* are hereby transferred from the **Provider** to the **Recipient** subject to the terms and conditions set out in **this Agreement**.

ARTICLE 4 — GENERAL PROVISIONS

- 4.1 **This Agreement** is entered into within the framework of the **Multilateral System** and shall be implemented and interpreted in accordance with *the objectives* and provisions of the **Treaty**.
- 4.2 The parties recognize that they are subject to the applicable legal measures and procedures, that have been adopted by the Contracting Parties to the **Treaty**, in conformity with the **Treaty**, in particular those taken in conformity with Articles 4, 12.2 and 12.5 of the **Treaty**.³
- 4.3 The parties to **this Agreement** agree that (*the entity designated by the Governing Body*),⁴ acting on behalf of the **Governing Body** of the **Treaty** and its **Multilateral System**, is the third party beneficiary under **this Agreement**.
- 4.4 The third party beneficiary has the right to request the appropriate information as required in Articles 5e, 6.5c, 8.3 and *Annex 2, paragraph 3*, to **this Agreement**.
- 4.5 The rights granted to the (*the entity designated by the Governing Body*) above do not prevent the **Provider** and the **Recipient** from exercising their rights under **this Agreement**.

² As evidenced, for example, by pedigree or notation of gene insertion.

³ In the case of the International Agricultural Research Centers of the Consultative Group on International Agricultural Research (CGIAR) and other international institutions, the Agreement between the Governing Body and the CGIAR Centers and other relevant institutions will be applicable.

⁴ *Note by the Secretariat*: by Resolution 2/2006, the Governing Body “invite[d] the Food and Agriculture Organization of the United Nations, as the Third Party Beneficiary, to carry out the roles and responsibilities as identified and prescribed in the Standard Material Transfer Agreement, under the direction of the Governing Body, in accordance with the procedures to be established by the Governing Body at its next session”. Upon acceptance by the FAO of this invitation, the term, “the entity designated by the Governing Body”, will be replaced throughout the document by the term, “the Food and Agriculture Organization of the United Nations”.

ARTICLE 5 — RIGHTS AND OBLIGATIONS OF THE PROVIDER

The **Provider** undertakes that the **Material** is transferred in accordance with the following provisions of the **Treaty**:

- a) Access shall be accorded expeditiously, without the need to track individual accessions and free of charge, or, when a fee is charged, it shall not exceed the minimal cost involved;
- b) All available passport data and, subject to applicable law, any other associated available non-confidential descriptive information, shall be made available with the **Plant Genetic Resources for Food and Agriculture** provided;
- c) Access to **Plant Genetic Resources for Food and Agriculture under Development**, including material being developed by farmers, shall be at the discretion of its developer, during the period of its development;
- d) Access to **Plant Genetic Resources for Food and Agriculture** protected by intellectual and other property rights shall be consistent with relevant international agreements, and with relevant national laws;
- e) The **Provider** shall periodically inform the **Governing Body** about the Material Transfer Agreements entered into, according to a schedule to be established by the **Governing Body**. This information shall be made available by the **Governing Body** to the third party beneficiary.⁵

ARTICLE 6 — RIGHTS AND OBLIGATIONS OF THE RECIPIENT

- 6.1 The **Recipient** undertakes that the **Material** shall be used or conserved only for the purposes of research, breeding and training for food and agriculture. Such purposes shall not include chemical, pharmaceutical and/or other non-food/feed industrial uses.
- 6.2 The **Recipient** shall not claim any intellectual property or other rights that limit the facilitated access to the **Material** provided under **this Agreement**, or its genetic parts or components, in the form received from the **Multilateral System**.
- 6.3 In the case that the **Recipient** conserves the **Material** supplied, the **Recipient** shall make the **Material**, and the related information referred to in Article 5b, available to the **Multilateral System** using the Standard Material Transfer Agreement.
- 6.4 In the case that the **Recipient** transfers the **Material** supplied under **this Agreement** to another person or entity (hereinafter referred to as “the **subsequent recipient**”), the **Recipient** shall

⁵ *Note by the Secretariat:* The Standard Material Transfer Agreement makes provision for information to be provided to the **Governing Body**, in the following Articles: 5e, 6.4b, 6.5c and 6.11h, as well as in *Annex 2*, paragraph 3, *Annex 3*, paragraph 4, and in *Annex 4*. Such information should be submitted to:

The Secretary

International Treaty on Plant Genetic Resources for Food and Agriculture
Food and Agriculture Organization of the United Nations
I-00100 Rome, Italy

- a) do so under the terms and conditions of the Standard Material Transfer Agreement, through a new material transfer agreement; and
- b) notify the **Governing Body**, in accordance with Article 5e.

On compliance with the above, the **Recipient** shall have no further obligations regarding the actions of the **subsequent recipient**.

- 6.5 In the case that the **Recipient** transfers a **Plant Genetic Resource for Food and Agriculture under Development** to another person or entity, the **Recipient** shall:
 - a) do so under the terms and conditions of the Standard Material Transfer Agreement, through a new material transfer agreement, provided that Article 5a of the Standard Material Transfer Agreement shall not apply;
 - b) identify, in *Annex 1* to the new material transfer agreement, the **Material** received from the **Multilateral System**, and specify that the **Plant Genetic Resources for Food and Agriculture under Development** being transferred are derived from the **Material**;
 - c) notify the **Governing Body**, in accordance with Article 5e; and
 - d) have no further obligations regarding the actions of any **subsequent recipient**.
- 6.6 Entering into a material transfer agreement under paragraph 6.5 shall be without prejudice to the right of the parties to attach additional conditions, relating to further product development, including, as appropriate, the payment of monetary consideration.
- 6.7 In the case that the **Recipient commercializes** a **Product** that is a **Plant Genetic Resource for Food and Agriculture** and that incorporates **Material** as referred to in Article 3 of **this Agreement**, and where such **Product** is not **available without restriction** to others for further research and breeding, the **Recipient** shall pay a fixed percentage of the **Sales** of the **commercialized Product** into the mechanism established by the **Governing Body** for this purpose, in accordance with *Annex 2* to **this Agreement**.
- 6.8 In the case that the **Recipient commercializes** a **Product** that is a **Plant Genetic Resource for Food and Agriculture** and that incorporates **Material** as referred to in Article 3 of **this Agreement** and where that **Product** is **available without restriction** to others for further research and breeding, the **Recipient** is encouraged to make voluntary payments into the mechanism established by the **Governing Body** for this purpose in accordance with *Annex 2* to **this Agreement**.
- 6.9 The **Recipient** shall make available to the **Multilateral System**, through the information system provided for in Article 17 of the **Treaty**, all non-confidential information that results from research and development carried out on the **Material**, and is encouraged to share through the **Multilateral System** non-monetary benefits expressly identified in Article 13.2 of the **Treaty** that result from such research and development. After the expiry or abandonment of the protection period of an intellectual property right on a **Product** that incorporates the **Material**, the **Recipient** is encouraged to place a sample of this **Product** into a collection that is part of the **Multilateral System**, for research and breeding.

- 6.10 A Recipient who obtains intellectual property rights on any Product developed from the Material or its components, obtained from the Multilateral System, and assigns such intellectual property rights to a third party, shall transfer the benefit-sharing obligations of this Agreement to that third party.
- 6.11 The **Recipient** may opt as per *Annex 4*, as an alternative to payments under Article 6.7, for the following system of payments:
- a) The **Recipient** shall make payments at a discounted rate during the period of validity of the option;
 - b) The period of validity of the option shall be ten years renewable in accordance with *Annex 3* to **this Agreement**;
 - c) The payments shall be based on the **Sales** of any **Products** and of the sales of any other products that are **Plant Genetic Resources for Food and Agriculture** belonging to the same crop, as set out in *Annex 1* to the **Treaty**, to which the **Material** referred to in *Annex 1* to **this Agreement** belongs;
 - d) The payments to be made are independent of whether or not the **Product** is **available without restriction**;
 - e) The rates of payment and other terms and conditions applicable to this option, including the discounted rates are set out in *Annex 3* to **this Agreement**;
 - f) The **Recipient** shall be relieved of any obligation to make payments under Article 6.7 of **this Agreement** or any previous or subsequent Standard Material Transfer Agreements entered into in respect of the same crop;
 - g) After the end of the period of validity of this option the **Recipient** shall make payments on any **Products** that incorporate **Material** received during the period in which this Article was in force, and where such **Products** are not **available without restriction**. These payments will be calculated at the same rate as in paragraph (a) above;
 - h) The **Recipient** shall notify the **Governing Body** that he has opted for this modality of payment. If no notification is provided the alternative modality of payment specified in Article 6.7 will apply.

ARTICLE 7 — APPLICABLE LAW

The applicable law shall be General Principles of Law, including the UNIDROIT Principles of International Commercial Contracts 2004, the objectives and the relevant provisions of the **Treaty**, and, when necessary for interpretation, the decisions of the **Governing Body**.

ARTICLE 8 — DISPUTE SETTLEMENT

- 8.1 Dispute settlement may be initiated by the **Provider** or the **Recipient** or the (*the entity designated by the **Governing Body***), acting on behalf of the **Governing Body** of the **Treaty** and its **Multilateral System**.
- 8.2 The parties to **this Agreement** agree that the (*the entity designated by the **Governing***

Body), representing the **Governing Body** and the **Multilateral System**, has the right, as a third party beneficiary, to initiate dispute settlement procedures regarding rights and obligations of the **Provider** and the **Recipient** under **this Agreement**.

- 8.3 The third party beneficiary has the right to request that the appropriate information, including samples as necessary, be made available by the **Provider** and the **Recipient**, regarding their obligations in the context of **this Agreement**. Any information or samples so requested shall be provided by the **Provider** and the **Recipient**, as the case may be.
- 8.4 Any dispute arising from **this Agreement** shall be resolved in the following manner:
- a) Amicable dispute settlement: The parties shall attempt in good faith to resolve the dispute by negotiation.
 - b) Mediation: If the dispute is not resolved by negotiation, the parties may choose mediation through a neutral third party mediator, to be mutually agreed.
 - c) Arbitration: If the dispute has not been settled by negotiation or mediation, any party may submit the dispute for arbitration under the Arbitration Rules of an international body as agreed by the parties to the dispute. Failing such agreement, the dispute shall be finally settled under the Rules of Arbitration of the International Chamber of Commerce, by one or more arbitrators appointed in accordance with the said Rules. Either party to the dispute may, if it so chooses, appoint its arbitrator from such list of experts as the Governing Body may establish for this purpose; both parties, or the arbitrators appointed by them, may agree to appoint a sole arbitrator, or presiding arbitrator as the case may be, from such list of experts. The result of such arbitration shall be binding.

ARTICLE 9 — ADDITIONAL ITEMS

Warranty

- 9.1 The **Provider** makes no warranties as to the safety of or title to the **Material**, nor as to the accuracy or correctness of any passport or other data provided with the **Material**. Neither does it make any warranties as to the quality, viability, or purity (genetic or mechanical) of the **Material** being furnished. The phytosanitary condition of the **Material** is warranted only as described in any attached phytosanitary certificate. The **Recipient** assumes full responsibility for complying with the recipient nation's quarantine and biosafety regulations and rules as to import or release of **genetic material**.

Duration of Agreement

- 9.2 **This Agreement** shall remain in force so long as the **Treaty** remains in force.

ARTICLE 10 — SIGNATURE/ACCEPTANCE

The **Provider** and the **Recipient** may choose the method of acceptance unless either party requires **this Agreement** to be signed.

Option 1 –Signature*

I, (*Full Name of Authorized Official*), represent and warrant that I have the authority to execute **this Agreement** on behalf of the **Provider** and acknowledge my institution’s responsibility and obligation to abide by the provisions of **this Agreement**, both by letter and in principle, in order to promote the conservation and sustainable use of **Plant Genetic Resources for Food and Agriculture**.

Signature..... Date.....

Name of the **Provider**

I, (*Full Name of Authorized Official*), represent and warrant that I have the authority to execute **this Agreement** on behalf of the **Recipient** and acknowledge my institution’s responsibility and obligation to abide by the provisions of **this Agreement**, both by letter and in principle, in order to promote the conservation and sustainable use of **Plant Genetic Resources for Food and Agriculture**.

Signature..... Date.....

Name of the **Recipient**

Option 2 – Shrink-wrap Standard Material Transfer Agreements*

The **Material** is provided conditional on acceptance of the terms of **this Agreement**. The provision of the **Material** by the **Provider** and the **Recipient’s** acceptance and use of the **Material** constitutes acceptance of the terms of **this Agreement**.

Option 3 – Click-wrap Standard Material Transfer Agreement*

I hereby agree to the above conditions.

* Where the Provider chooses signature, only the wording in Option 1 will appear in the Standard Material Transfer Agreement. Similarly where the Provider chooses either shrink-wrap or click-wrap, only the wording in Option 2 or Option 3, as appropriate, will appear in the Standard Material Transfer Agreement. Where the “click-wrap” form is chosen, the Material should also be accompanied by a written copy of the Standard Material Transfer Agreement.

LIST OF MATERIALS PROVIDED

This *Annex* contains a list of the **Material** provided under **this Agreement**, including the associated information referred to in Article 5b.

This information is either provided below or can be obtained at the following website: (*URL*).

The following information is included for each **Material** listed: all available passport data and, subject to applicable law, any other associated, available, non-confidential descriptive information.

(*List*)

**RATE AND MODALITIES OF PAYMENT UNDER ARTICLE 6.7
OF THIS AGREEMENT**

1. If a **Recipient**, its affiliates, contractors, licensees and lessees, **commercializes a Product or Products**, then the **Recipient** shall pay one point-one percent (1.1 %) of the **Sales** of the **Product or Products** less thirty percent (30%); except that no payment shall be due on any **Product or Products** that:
 - (a) are **available without restriction** to others for further research and breeding in accordance with Article 2 of **this Agreement**;
 - (b) have been purchased or otherwise obtained from another person or entity who either has already made payment on the **Product or Products** or is exempt from the obligation to make payment pursuant to subparagraph (a) above;
 - (c) are sold or traded as a commodity.
2. Where a **Product** contains a **Plant Genetic Resource for Food and Agriculture** accessed from the **Multilateral System** under two or more material transfer agreements based on the Standard Material Transfer Agreement only one payment shall be required under paragraph 1 above.
3. The **Recipient** shall submit to the **Governing Body**, within sixty (60) days after each calendar year ending December 31st, an annual report setting forth:
 - (a) the **Sales** of the **Product or Products** by the **Recipient**, its affiliates, contractors, licensees and lessees, for the twelve (12) month period ending on December 31st;
 - (b) the amount of the payment due; and
 - (c) information that allows for the identification of any restrictions that have given rise to the benefit-sharing payment.
4. Payment shall be due and payable upon submission of each annual report. All payments due to the **Governing Body** shall be payable in (*specified currency*)⁶ for the account of (*the Trust Account or other mechanism established by the Governing Body in accordance with Article 19.3f of the Treaty*).⁷

6 *Note by the Secretariat:* The Governing Body has not yet considered the question of currency of payment. Until it does so, Standard Material Transfer Agreements should specify United States dollars (US\$).

7 *Note by the Secretariat:* This is the Trust Account provided for in Article 6.3 of the Financial Rules, as approved by the Governing Body (*Appendix E* to this Report). The details of the Trust Account when established, will be introduced here, and communicated to Contract Parties.

**TERMS AND CONDITIONS OF THE ALTERNATIVE PAYMENTS SCHEME
UNDER ARTICLE 6.11 OF THIS AGREEMENT**

1. The discounted rate for payments made under Article 6.11 shall be zero point five percent (0.5 %) of the **Sales** of any **Products** and of the sales of any other products that are **Plant Genetic Resources for Food and Agriculture** belonging to the same crop, as set out in Annex 1 to the **Treaty**, to which the **Material** referred to in *Annex 1* to **this Agreement** belong.
2. Payment shall be made in accordance with the banking instructions set out in paragraph 4 of *Annex 2* to **this Agreement**.
3. When the **Recipient** transfers **Plant Genetic Resources for Food and Agriculture under Development**, the transfer shall be made on the condition that the **subsequent recipient** shall pay into the mechanism established by the **Governing Body** under Article 19.3f of the **Treaty** zero point five percent (0.5 %) of the **Sales** of any **Product** derived from such **Plant Genetic Resources for Food and Agriculture under Development**, whether the **Product** is **available or not without restriction**.
4. At least six months before the expiry of a period of ten years counted from the date of signature of **this Agreement** and, thereafter, six months before the expiry of subsequent periods of five years, the **Recipient** may notify the **Governing Body** of his decision to opt out from the application of this Article as of the end of any of those periods. In the case the **Recipient** has entered into other Standard Material Transfer Agreements, the ten years period will commence on the date of signature of the first Standard Material Transfer Agreement where an option for this Article has been made.
5. Where the **Recipient** has entered or enters in the future into other Standard Material Transfer Agreements in relation to material belonging to the same crop[s], the **Recipient** shall only pay into the referred mechanism the percentage of sales as determined in accordance with this Article or the same Article of any other Standard Material Transfer Agreement. No cumulative payments will be required.

**OPTION FOR CROP-BASED PAYMENTS UNDER THE ALTERNATIVE PAYMENTS
SCHEME UNDER ARTICLE 6.11 OF THIS AGREEMENT**

I (*full name of **Recipient** or **Recipient's** authorized official*) declare to opt for payment in accordance with Article 6.11 of **this Agreement**.

Signature

Date.....

**NATIONAL BUREAU OF PLANT GENETIC RESOURCES
INDIAN COUNCIL OF AGRICULTURAL RESEARCH
NEW DELHI, INDIA**

**APPLICATION FOR PERMIT TO IMPORT GERMPLASM/
TRANSGENICS/GENETICALLY MODIFIED ORGANISMS (GMOs)
(FOR RESEARCH PURPOSES)**

**The Director,
National Bureau of Plant Genetic Resources
Pusa Campus, New Delhi 110012**

I hereby apply for a permit in accordance with provisions of Clause-6(2) of the Plant Quarantine (Regulation of Import in to India) Order, 2003 issued under the Sub-section(1) of Section (3) of the Destructive Insects & Pests Act, 1914 (2 of 1914), authorizing the import of plants/planting materials for research purposes as per details given below:

1. Name and Address of the applicant:
2. Research and Development (R&D) status/affiliations of the organization:
3. Exact description of seeds/planting material to be imported:
 - (a) Common and Botanical name:
 - (b) Germplasm/variety/hybrid/composite/ synthetic provenance/clone/others
 - (c) Form of material required (seed/rooted plants/ scions /tubers/cuttings/bulbs in vitro cultures)
 - (d) Parentage, if known
 - (e) Place of collection/origin of the material to be imported (country/state)
4. Whether transgenic/GMO or not?
[If yes, attach the approval letter issued by RCGM (DBT) in original]
5. Name and address of the organization/institution producing the material
6. Number of samples to be imported
7. Quantity to be imported (separately for each accession/ variety/hybrid/transgenic/GMO)
8. Suggested source of availability of material including published reference, if known

9. (a) Whether the aforesaid germplasm/variety/ hybrid was imported by you earlier? If so, details there of (year, quantity, source, etc)
(b) Was the material shared with other scientists/ National Gene Bank at NBPGR?
10. Expected date and mode of arrival in India
11. Mode of shipment (airmail/air freight/ accompanied baggage)
12. Place where imported seeds/planting material will be grown and scientists under whose supervision the seeds/planting material will be grown

DECLARATION

1. I hereby declare that the germplasm under import has no commercial value/exclusive ownership and may be shared freely for research purpose.
2. This germplasm does not contain any terminator genes.
3. I undertake that the material is exclusively for research purposes.

Place:

Date:

Signature of the applicant & address

For further information contact Tel. No.91-11-25843697 or Fax. 91-11-25844295 or Email-director@nbpgr.ernet.in and web address-http://nbpgr.ernet.in

Form for Advance Intimation of Export of Seed Samples to India

To:
The Director
National Bureau of Plant Genetic Resources
IARI Campus
NEW DELHI 110 012

The following consignment has been dispatched separately to you for plant quarantine clearance and forwarding to ICRISAT.

- 1. Name and address of consignor _____
 - 2. i) Crop (with botanical name) _____
 - ii) No. of boxes/bags/cartons _____
 - iii) Distinguishing marks _____
 - 3. Weight _____
 - 4. Mode of dispatch _____
 - 5. Particulars of Phytosanitary certificate _____
 - 6. General health, pest incidence/intensity on crop at the time of seed collection _____
 - 7. Date(s) of collection _____
 - 8. Remarks, if any _____
- Date _____

Signature: _____
Name: _____

Cc: Chief Plant Quarantine Officer, ICRISAT, Patancheru, Andhra Pradesh 502 324, India

Note: Duplicate copy of Phytosanitary Certificate should be attached with this letter to facilitate release of the seed material.

Seed for Experimental Purposes
No Commercial Value
for the Use of
ICRISAT
International Crops Research Institute for the Semi-Arid Tropics
Patancheru, Andhra Pradesh 502 324, India



Seed Unfit for Consumption
Phytosanitary Certificate
and Import Permit Enclosed



DIRECTOR
NATIONAL BUREAU OF PLANT GENETIC RESOURCES
(NBPGR)
PUSA CAMPUS
NEW DELHI-110 012, INDIA

From:
.....
.....
(Please use capital letters)


ICRISAT – Genetic Resources Unit – Collection Data Sheet

1. Collection Number 2. ICRISAT Accession No.

3. Crop Species _____

4. Collector(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

Diseases _____

Insects _____

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

29. Remarks: _____

Farmer's survey form*

Farmer's name _____

Village/district _____

Site characteristics _____

Topography (local name) _____

Site (local name) _____

Soil (local name) _____

Farmer's description of landraces

Landrace name _____

Type within landrace characteristics _____

Name of farmers: _____

Name of collectors: _____

Morphological

Trait	Observation			
Maturity	Early	Medium	Late	
Plant height	Short	Medium	Tall	very tall
Tillering	None	1 or 2	Many	very many
Head compactness and shape	Curved	Bent	Erect	
Head size	Very small	Small	Medium	large
Plant color	Pigment	Tan		
Grain color	White	Yellow	Red	Brown
Glume color	Tan	Red	Purple	
Glume coverage	0-25	25-50	50-75	complete
Race				
Stem thickness	Thin	Average	Thick	
Leaf type	Thin, short	Thin, long	Thick, short	thick, long

Agronomic

Grain Yield	Low	Medium	High	
Fodder Yield	Low	Medium	High	

Disease problems

Problem	Susceptible	Resistance

Insect problems

Problem	Susceptible	Resistance

Weed, storage pest, or bird problems

Problem	Susceptible	Resistance

*(adapted from Bramel-Cox and Christinck 1998)

Stress reactions

	Sowing	Seedling	Heading	Maturing
Month				
Drought				
Water logging				

End use

Local bread	Poor	Good
Injera	Poor	Good
Porridge	Poor	Good
Local beer	Poor	Good
Popping	Poor	Good

Quality

Taste	Bitter	Sweet
Cooking time	Poor	Good
Threshing ease	Poor	Good
Dehulling	Poor	Good
Storability	Poor	Good

Cultural practices

Planting time	_____
Number of weedings	_____
Harvest time	_____
Irrigated or rainfed	_____
Number of times irrigated	_____
Fertility requirements	_____
Intercropping or sole cropping	_____
Normal crop rotation	_____

Source of seed

Own crop	_____
Number of years	_____
Before that	_____
Received from relative	_____
When	_____
Purchased from others	_____
When	_____
Given as gift	_____
When	_____
Relief Agency	_____
When	_____

Seed selection and conservation practices

Timing of selection for seed	_____
Field prior to harvest	_____
Field at harvest	_____
Prior to threshing	_____
Save from bulk after threshing	_____
Harvest or production of seed stocks	_____
-in same field as crop	_____
-in separate area of field or	_____
separate field	_____
Any special cultural practices	_____
Thresh separately	_____
Drying procedures	_____
Selection criteria used for seed stock	_____
Selection criteria used for seed plant	_____

Section 2

Plant Quarantine

Plant quarantine services facilitate safe introduction of new germplasm samples from other countries. Plant quarantine regulations aim to prevent the introduction of destructive pests and diseases of plants from other countries. All plants or plant parts (including seed) are subject to plant quarantine. In India, the plant quarantine activities are done with assistance from NBPGR, the plant quarantine authority of the Government of India. The Plant Quarantine Laboratory (PQL) at ICRISAT, Patancheru coordinates with NBPGR in safe introduction and exchange of the center's germplasm samples.

2A. Requirements for seed import

- Seeds cannot be imported into India without an Import Permit (IP) issued by the NBPGR (see Annexure 1.2).
- The incoming seeds must be accompanied by a Phytosanitary Certificate (PC) obtained from the National Plant Quarantine Service of the exporting country (see Annexure 6B.2).
- A customs declaration at the port of entry and release by the National Plant Quarantine System (NPQS) are essential.
- The Genebank Curator must obtain the required permits and send to the donating institution before ordering the seeds.
- IP initially issued is valid for six months, and the competent authority can extend the period of validity for another six months.
- Import permits are not transferable and no permits are issued for landed consignments.
- A PC is also required for seeds brought in as accompanied baggage.
- Seed consignment should be dispatched within 14 days of inspection and issue of the PC.

The seed package should not be addressed to ICRISAT, carried on one's person or brought as undeclared accompanied baggage at the port of entry. Exceptionally, when bringing the seed package to ICRISAT, a certificate of fumigation/examination from plant quarantine officials must be obtained at the port of entry.



The Genebank should acquire no imported seed, unless it is cleared by National Plant Quarantine Services.

Additional declarations are required for entry of seeds into India as listed below:

Sorghum: Certification that seeds are free of bacterial leaf stripe and bacterial leaf streak.

Pearl millet: Certification that seeds are free of downy mildew, smut and ergot.

Chickpea: Certification that seed samples were collected from mother plants free of *Ascochyta rabiei* and fusarium wilt.

Pigeonpea: Certification that seeds are free from anthracnose, fusarium wilt and bacterial diseases.

Groundnut: Certification that seeds are produced in areas free from rust and scab diseases, certification that parent crop was regularly inspected during active growth and found free of symptoms of peanut stunt, peanut stripe, marginal and ring spot viruses.

Groundnut cuttings, if imported, must pass through the growth stage in intermediate or third country quarantine.

2B. Post-entry quarantine

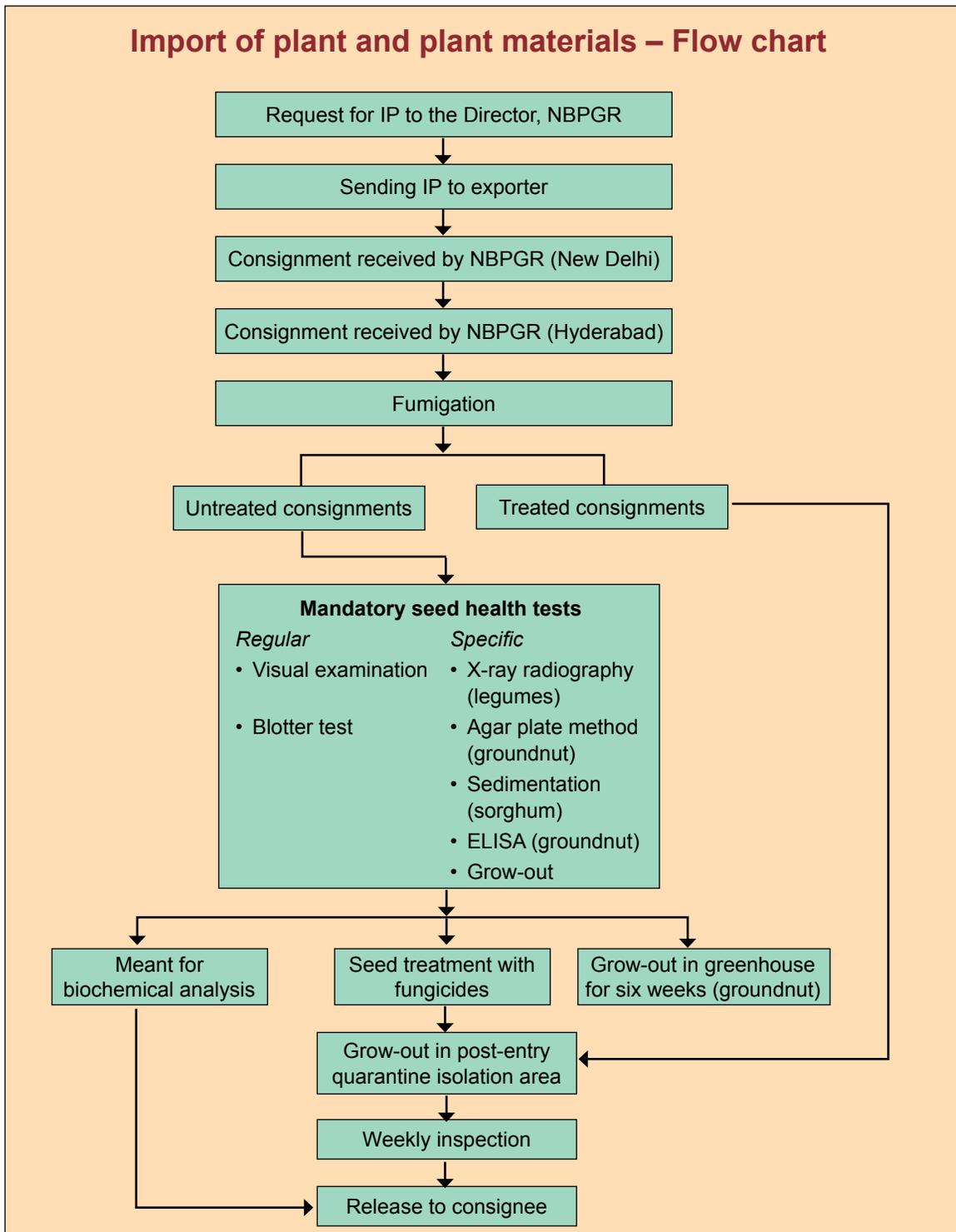
Seed materials received at NBPGR are subjected to visual and microscopic examination. Once a pest, pathogen or weed is detected, appropriate eradication treatments such as fumigation, heat treatment or chemical dressing are given before release of the material for sowing.

All exotic crop germplasm samples are required to be grown in Post-Entry Quarantine Isolation Area (PEQIA), at ICRISAT, Patancheru to avoid possible introduction of seed borne diseases and pests. Sowings are done under the close supervision of the Plant Quarantine Officer, Government of India. Grow optimum number of plants (minimum sample size) to harvest sufficient quantity of seeds for storage and to maintain genetic integrity of the sample. Save a few seeds for replanting in case of crop failure. If the number of plants available is less than the number required for maintenance of genetic integrity, use all seeds and record the bottleneck in genebank documentation. Use optimal planting and crop management practices to ensure production of high quality seeds (see Section 9). These include:

- Uniform plots with good drainage, free from weeds, pests and pathogens.
- Fertility and water management to provide suitable conditions for growth that ensure maximum possible survival of plants.
- Weekly inspections are undertaken to detect exotic pests and diseases associated with growing plants.
- Elimination of alien pollen by bagging inflorescence where needed.
- Ensuring appropriate female-male combinations.
- Harvesting at optimal maturity.
- Equal contribution of seed from each parent.

Groundnut seeds are first sown in a glasshouse to detect viruses and 4 weeks-old seedlings are released for transplantation in PEQIA. Weekly inspections are carried out until the plants are mature. Plants infected with objectionable diseases are uprooted and burnt in an incinerator. Seed samples free from diseases and pests are released to the genebank for registration.

In the event of crop failure, Plant Quarantine should be contacted to replant the remnant seed. If seed is not available, duplicate sample of the same seed lot is obtained from the donor.



Section 3

Registering New Germplasm

Registration is carried out to allow the genebank curator to keep a record of samples held in the genebank and to produce inventory lists for conservation, distribution, and other aspects of germplasm management. Registration is done by assigning each sample a **unique accession number**, which distinguishes it from all other accessions in the genebank.

Register the sample when it first enters the genebank. Register only those samples acquired with appropriate SMTA and/or whose status with regard to conservation and further use is clearly defined.

3A. Minimum standards for registration

3A.1. Passport data

The minimum passport data required for collected/donated samples include:

- Information on source country.
- Location of collecting site (if relevant).
- Local name or cultivar name.
- Pedigree information for breeding lines and improved varieties.
- Source of collection (farmers' field, farm store, market, etc, if the sample is collected on a germplasm expedition).
 - record the accompanying data on country of origin, location of collection site, local names and other basic information
 - request for any missing passport data when the material is logged-in, otherwise, it may be forgotten and unavailable at a later date
 - if the accompanying data is incomplete or seeds are insufficient or poor in quality, assign a temporary number until such time the sample is ready for a permanent number after regeneration.

3A.2. Seed health and quality

- Seeds should be absolutely free from pathogens, insects, insect damage, fungal growth, damaged, broken, or shriveled seeds.
- If the seeds are in poor condition, then discard the sample and make note in the files of the action taken and reasons for it. Write to the donor for fresh samples.
- Percentage germination should not be < 85% for cultivated and < 75% for the wild species accessions (see Section 4D.1 for germination testing).

3A.3. Seed quantity

- Seed quantity should be sufficient to conduct at least four regenerations.

3B. Procedure for registration of samples

Arrange the material received by the genebank according to alphabetical order of name of samples or numeric order of number depending on the identification provided on the packets/bags.

- Check all the packets against the list provided along with the samples.
- If a list was not provided or seeds do not correspond with the list, prepare a new list. Check again to confirm that all packets have been included.
- Check the collectors' number, alternate accession identifier and other information to ensure that the sample does not exist already in the genebank.
- If the material is already represented in the genebank, grow the putative duplicates alongside the existing accession to compare the morphoagronomic characters and if possible use molecular markers. If convinced that they are duplicates, they can be bulked and maintained under the existing accession number.
- If two samples have identical or very similar names, identical grain characteristics, maturity and other morphological and agronomical features, bulk them to make one sample and assign an accession number.



- ***For suspected duplicates and samples with insufficient seeds, assign a temporary number to identify the sample until a final decision is made on registering it.***
- ***If the germination percentage of the received sample is < 85% or seed quantity is insufficient, regenerate immediately. Assign accession number when it meets the standards.***

If the seed samples meet all the standards for registration and do not exist in the genebank, assign accession number following the procedure described below:

3B.1. Procedure for assigning accession number

- Check the passport data file to determine which was the last accession number used for the crop.
- Assign the next ascending accession number to the first sample on the list and consecutive numbers to each sample (see Table 3B.1 for crop identity codes).
- Write the accession number clearly on the packet using a permanent marker and on the list of the new samples.
- Enter the details in the passport database of the genebank.

- for each accession, record the entire passport and original identification data and date of registration in the designated fields of the passport database
- when the data are missing, leave the field blank and try to fill the data at a later date, by writing to the donor to supply the missing data.
- Once the sample is registered, clean, dry and pack the seeds in appropriate containers for storage as outlined in Section 4F.
- Pack a small sample (5–10 seeds or pods in legumes, 5-10 g in cereals) of the original seed separately in a transparent resealable plastic envelop to serve as a future reference to verify genetic integrity after regeneration, and during seed transfer.

3B.2. Useful tips on numbering

The numbering system should be simple and practical to use.

- Use a strictly numeric system that is sequential in operation, eg, 1, 2, 3 and so on. Additional information such as year of acquisition or crop code **should not** be incorporated in the accession number.
- If large collections of germplasm are maintained, give separate but sequential accession numbering for each crop (eg, IS 1, IS 2, IS 3.... for sorghum, IP 1, IP 2, IP 3.... for pearl millet, as practiced in ICRISAT genebank) (see Table 3B.1).

This approach however, is not recommended if the genebank is small or has many crops.

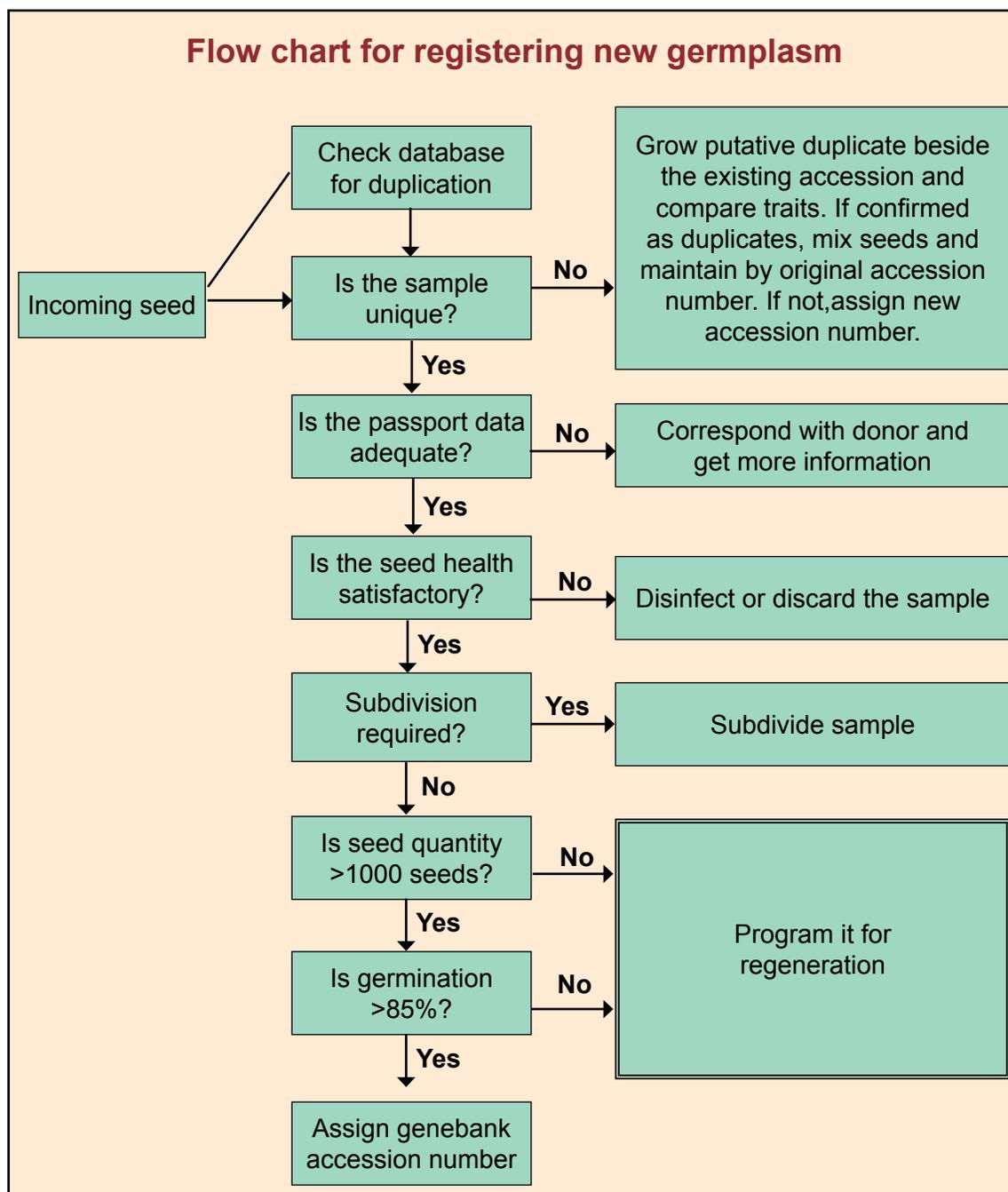
- Avoid assigning 'reserved' numbers for particular crops (for instance, 1–100 for sorghum, 101–200 for pearl millet) or for wild species, etc, when using a single numbering system.

Table 3B.1. Botanical names and crop identity codes used for germplasm accessions at ICRISAT genebank.

Crop	Botanical name	Prefix code
Sorghum	<i>Sorghum bicolor</i> (L.) Moench	IS
Pearl millet	<i>Pennisetum glaucum</i> (L.) R. Br.	IP
Chickpea	<i>Cicer arietinum</i> L.	ICC
Pigeonpea	<i>Cajanus cajan</i> (L.) Millsp.	ICP
Groundnut	<i>Arachis hypogaea</i> L.	ICG
Finger millet	<i>Eleusine coracana</i> (L.) Gaertn.	IE
Foxtail millet	<i>Setaria italica</i> (L.) Beauv.	ISe
Kodo millet	<i>Paspalum scrobiculatum</i> L.	IPs
Barnyard millet	<i>Echinochloa crusgalli</i> (L.) Beauv.	IEc
Proso millet	<i>Panicum miliaceum</i> L.	IPm
Little millet	<i>Panicum sumatrense</i> Roch. ex Roem.& Schult	IPmr

3B.3. Subdivision of the sample

- If the sample consists of genetically distinct subtypes, subdivide and maintain them as distinct accessions.
- Subdivision **should not** be undertaken if variation in the original sample is continuous as with highly cross-pollinating crops such as pearl millet and pigeonpea.



3C. Documentation of information

Documenting the information received with the sample is an important part of the registration procedure. Much of the information will be passport data, which is recorded when the sample was originally collected or the data accompanying the sample when it is received from other sources (Fig. 3C.1).

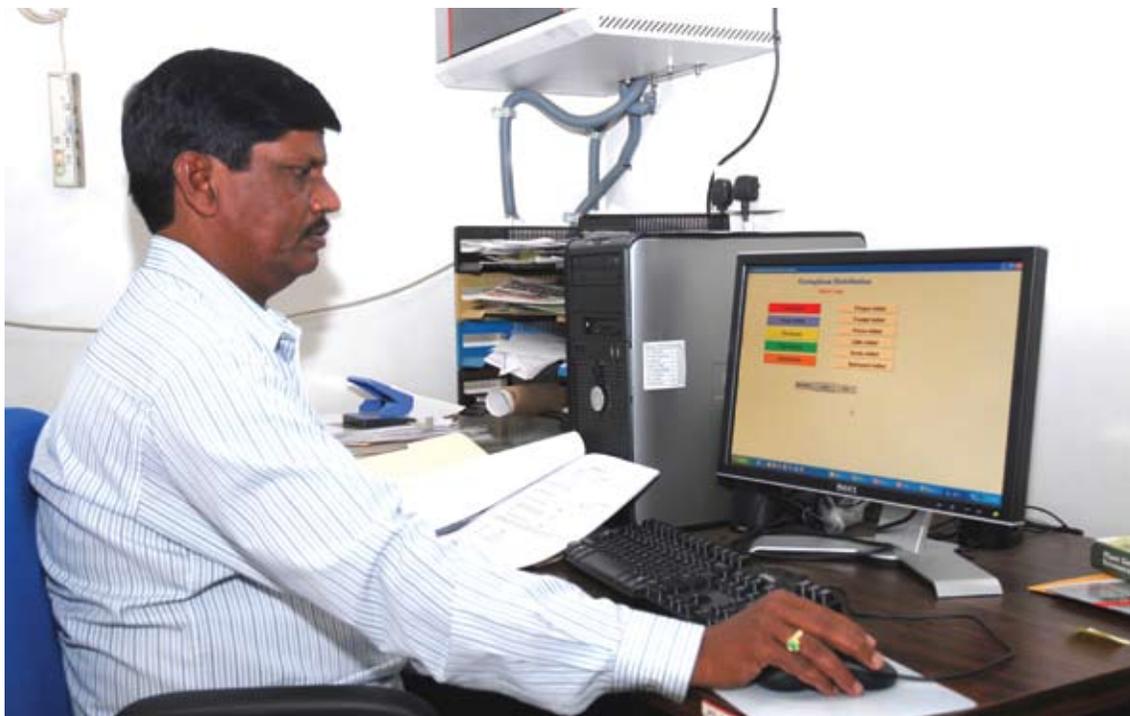


Figure 3C.1. Documenting information related to germplasm collections.

Passport data (based on IPGRI/FAO Multi-crop passport descriptors)

Include information relating to the identification of each accession in the genebank.

Accession number: This number serves as a unique identifier for accessions and is assigned when an accession is entered into the collection. Once assigned, this number should never be reassigned to another accession in the collection. Even if an accession is lost, its assigned number should never be reused.

Mission code: A code for the mission that consists of the Center's acronym plus a unique code for the mission as given by the Center.

Collector's number: Original number assigned by collector(s) of the sample, normally composed of initials of the collector(s) followed by a number. It should be unique and always accompany subsamples wherever they are sent.

Alternate accession identifier: Any name(s) or alternate identifier(s) given by the donor or any other identification number known to exist in other collections for this accession other than the cultivar name and collector's number. Letter should be used before the number to identify the donating genebank or national system.

Genus: Genus name for the taxon. Initial uppercase letter required.

Species: Species name in lowercase letters plus authority.

Subtaxa: To store additional taxonomic information. The following abbreviations are allowed. “ssp. (for subspecies); “var.” (for variety); “race” (for race).

Local name: Mostly the name given by the farmers. Not the registered name or the cultivar name.

Cultivar name: Either a registered name or other formal designation given to the accession. First letter uppercase. Multiple names separated with semicolon.

Country of origin: Name of the country in which the sample was originally collected or derived. Use the ISO 3166 extended codes.

Province of the collecting site: Name of province information below the country level that describes where the accession was collected.

Precise location of the collecting site: Location information below the province level that describes name of village or town or the exact location where the accession was collected. Such information may include distance in kilometers and direction from the nearest town, or village (eg, 10 km south of Patancheru).

Latitude of collecting site: Degrees and minutes after + (for North) or – (for South) (eg, – 10.30). Missing data (minutes) should be indicated with hyphen (eg, 10.-).

Longitude of collecting site: Degrees and minutes after + (for East) or - (for West) (eg, -076.25). Missing data (minutes) should be indicated with hyphen (eg, 076.-).

Elevation of collecting site: Elevation of collecting site expressed in meters above sea level (masl).

Date of collection (MMDDYYYY): Collecting date of the original sample, where, MM is the month, DD is the day and YYYY is the year.

Date received (MMDDYYYY): Date on which the accession was registered in the genebank, where, MM is the month, DD is the day and YYYY is the year.

Biological status of sample: Also referred as improvement status of the accession.

- 1 Wild
- 2 Weedy
- 3 Landrace/traditional cultivar
- 4 Breeder's material
- 5 Advanced/released cultivar
- 0 Unknown
- 99 Others

Source of sample: Source from where the sample was collected/drawn. Recorded as numeric codes (eg, 1,2,3). Most common sources of germplasm are:

- 1 Wild habitat
- 2 Farmer's field
- 3 Farmer's seed store
- 4 Threshing floor
- 5 Markets
- 6 Institute/research organization
- 0 Unknown
- 99 Others

Donor institute code: Code for the donor institute. The code consists of a 3-letter ISO code of the country where the institute is located.

Donor geographic code: The unique, internal number for the country from which the accessions were derived or originally collected, ie, its biological origin.

Remarks: The remarks field is used to add notes or to elaborate on descriptors with value "99" (= Others). Prefix remarks with the field name they refer to and a colon (eg, Collecting source: roadside). Remarks referring to different fields are separated by a semicolon.

Section 4

Seed Processing

Seed processing involves cleaning the seed samples of extraneous materials, drying them to optimum moisture levels, testing their germination and packaging them in appropriate containers for conservation and distribution.

- Seeds received at the genebank are first checked for insect infestation and purity, and then sent for cleaning if required (see Section 4A).
- The moisture content of the seeds is estimated using the oven drying method and if it is not within the limits recommended for safe storage, the seed lots are sent for drying as described in Section 4C. Drying continues for several days and when expected seed moisture content reaches optimum levels (8–10% for medium-term storage and 3–7% for long-term storage), subsamples are tested to accurately determine the moisture content using the methods outlined in Section 4B.
- Subsamples are taken and the seed viability is tested using appropriate methods listed in Section 4D after drying. Seeds with poor viability are sent for regeneration, while good quality seeds are sent for packaging.
- Seed health tests are conducted on representative samples as described in Section 4E. Only seed lots that meet the minimum prescribed standards for seed health are accepted for long-term conservation. Seed lots with a high percentage of infection are rejected.
- Dry seeds are packed in appropriate containers. The type of container depends on the purpose of storage. For example, plastic bottles or aluminum cans with screw caps are used to store the collections that are regularly used or distributed (see Section 4F).
- Packed seeds are placed in the genebank and data on inventory is recorded, along with seed viability and related information.

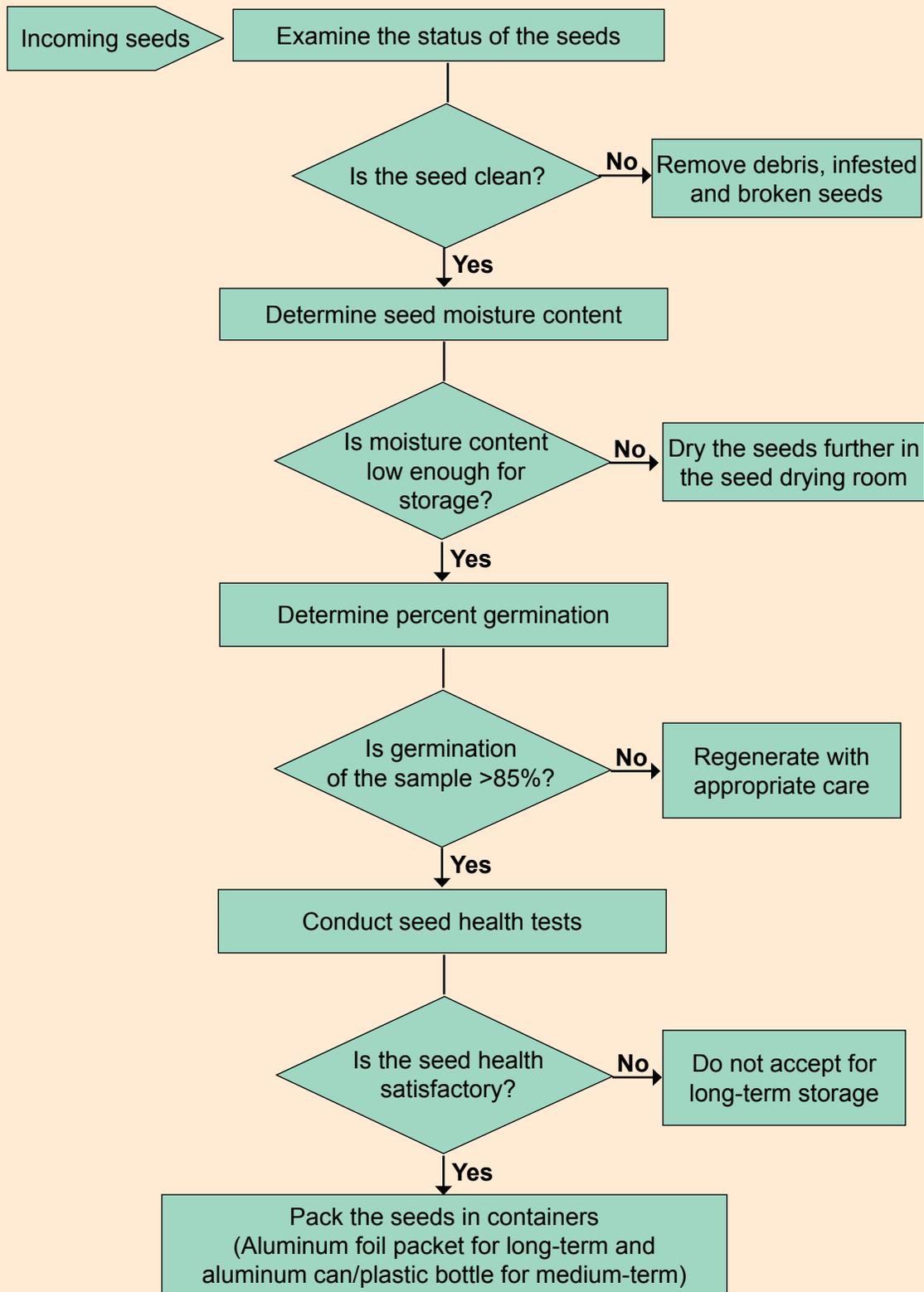
4A. Seed cleaning

The cost of maintaining an accession in a genebank is high and space is limited. Debris and damaged seeds can spread infection. Therefore, place only good quality viable seeds in storage.

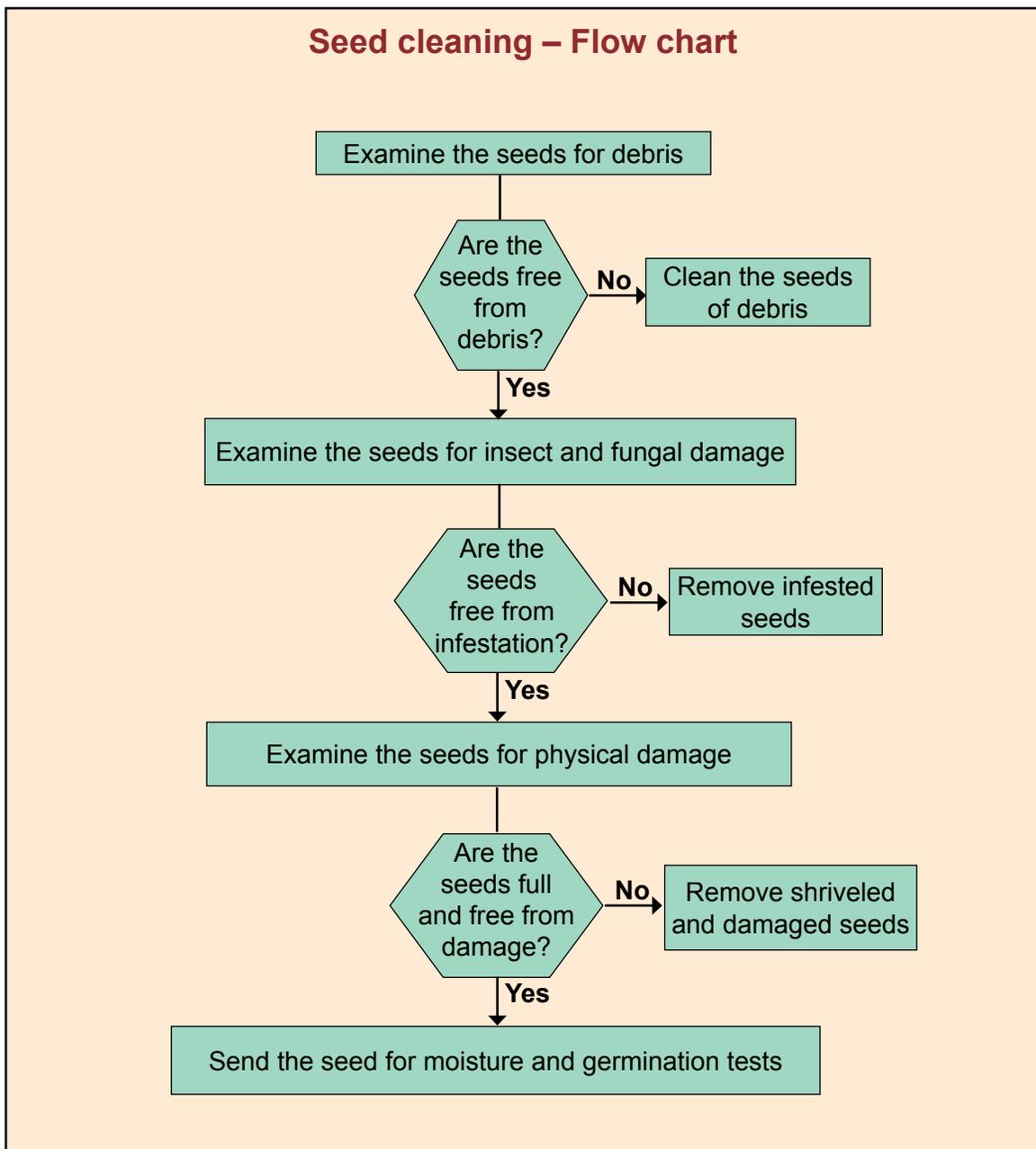
Seed cleaning involves removal of debris, low quality, infested or infected seeds and seeds of different species (weeds).

- Clean the seeds immediately after registration (if required) or harvest.
- Cleaning should be done in a way that causes least damage to the sample and does not waste good seeds.
- Clean chickpea, pigeonpea and groundnut seeds preferably by hand.
- Use seed blower to clean sorghum and millet seeds (Fig. 4A.1).
 - clean the blower between accessions to prevent mixing of seeds from different accessions.

Seed processing for conservation – Flow chart



Seed cleaning – Flow chart



- Groundnut requires shelling before storage.
 - shell the seeds manually.

The following procedure can be used as a guide for seed cleaning:

- Empty the contents of each packet into a labeled tray and assess the need for cleaning.
- If cleaning is required, check that the seeds are dry enough to be cleaned without damage.
- If the samples are moist, place them in the drying room to reduce the moisture content to <15%.



1. Seed blower.



2. Graded sieves.



3. Purity workboard.

Figure 4A 1-3. Seed cleaning equipment used in genebanks.

- Remove debris from seeds by graded sieves (Fig. 4A.2), ensuring that small sized seeds in the sample are not discarded.
- Separate lightweight material and empty glumes by gentle winnowing or using the seed blower.
- Spread the seeds on a flat well-lit surface of contrasting color such as an illuminated table and examine the seeds for any physical damage or infestation with insects and fungi (Fig. 4A.3).
- Discard any visually damaged, shriveled, infected or infested seeds.
- Destroy any waste material to prevent the spread of the disease or insects to other material.

4B. Seed moisture testing

The moisture content is the amount of water in the seed, 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. For a given species, there is a definable equilibrium relationship between relative humidity and seed moisture content. The equilibrium relationship between seed moisture content and relative humidity at 25°C for ICRISAT mandate crops is presented in Fig. 4B.1:

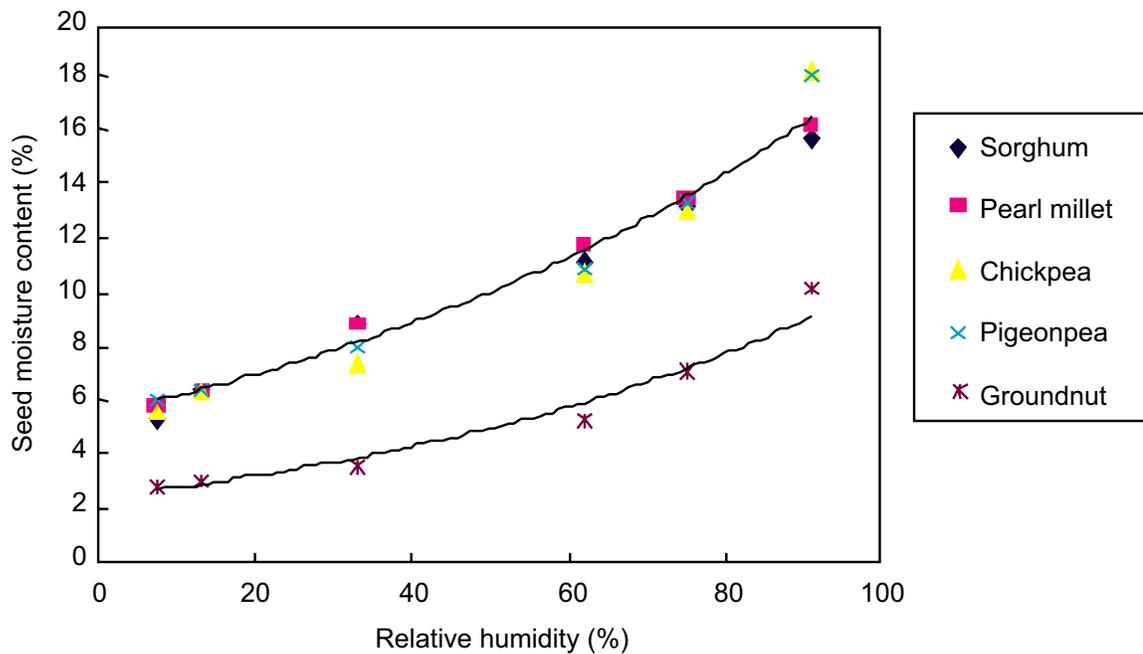


Figure 4B.1. Equilibrium relationship between seed moisture content and relative humidity at 25°C for ICRISAT mandate crops.

The data were obtained by allowing seeds to equilibrate in environments with known RH maintained by saturated salt solutions of NaOH (7.5%), LiCl (13%), MgCl₂ (45%), NH₄NO₃ (65%), NaCl (75%) and KNO₃ (91%).

Even small changes in moisture content have a large effect on storage life of seeds.

Therefore,

- Determine the moisture content after final drying, but before packing them in containers and placing them in seed storage.

Moisture content can be expressed on either a wet weight basis (as percentage of the wet weight of the seeds) or on a dry weight basis (expressed as percentage of the dry weight of the seeds).

For genebank purpose, moisture content is usually expressed on a wet weight basis.

Seed moisture content can be determined by various methods.

Methods prescribed by the International Seed Testing Association (ISTA) are used for determining the seed moisture content in genebanks.

ISTA has prescribed two kinds of oven-drying methods for determining moisture content:

- Low-constant temperature oven method for groundnut (oily seeds), and
- High-constant temperature oven method for sorghum, millets, chickpea and pigeonpea (non-oily seeds).

Grinding is required for determination of moisture content in all ICRISAT mandate crops, except millets.

Moisture content determination

Equipment

The following equipment is necessary for determining the moisture content (see Fig. 4B.2):

1. A mechanical-convection (forced-drought) oven
 - With recovery time 15 min or less
 - Capable of maintaining the required temperature within $\pm 1^\circ\text{C}$
 - Fitted with a thermometer accurate to 0.5°C .



Figure 4B.2. Equipment used to determine seed moisture content.

2. Noncorrosive drying containers (metal or glass) with tight fitting lid
 - Size of the container should be such that the height of the evenly distributed sample does not exceed 0.3 g cm^{-2} .
3. Grinder
 - Should be adjustable to obtain specified particle sizes (0.5 and 4.0 mm)
 - Should not expose the sample to air
 - Should not cause undue heating.

4. Analytical balance
 - Capable of weighing 0.0001 g.
5. Desiccators
 - Fitted internally with a thick metal or ceramic plate to promote rapid cooling of the containers, and
 - Containing a desiccant such as silica gel or calcium chloride at the bottom.
6. Tongs or hand gloves
 - To handle hot containers.

Sample size

Oven-drying method is destructive and considering that seed quantity is limited in most germplasm accessions, small sample weights are used whilst increasing the accuracy of the weighing.

- Use two replicates of 0.5–1.0 g of seeds for moisture determination.

Grinding

Seeds of sorghum, chickpea, pigeonpea and groundnut must be ground. At least 50% of the ground material should pass through a sieve with mesh of

- 0.5 mm for sorghum, or
- 4.0 mm for chickpea, pigeonpea and groundnut.

Predrying

Predrying is obligatory if moisture content of seeds is expected to be above 17%.

If predrying is required:

- Weigh two subsamples of 1.0–1.5 g of seeds.
- Predry the samples overnight in a warm dry place such as on a lab bench.
- Weigh again to determine the loss of weight.

Determine the moisture content as described below:

- Dry the containers at 130°C for 1 h and allow them to cool in the desiccator for 1 h.
- Label and weigh each dish with the lid.
- Place about 1–0.5 g of the sample (predried and ground if necessary) in the container, replace the lid and weigh again.
- Place dishes with lids removed in the oven maintained at 130°–133°C for samples of non-oily seeds and at 103° ± 2°C for groundnut.

- Dry:
 - sorghum and pearl millet samples for 2 h,
 - chickpea and pigeonpea for 1 h, and
 - groundnut for 16 h.

The drying period commences when the oven has regained the required temperature after the samples are kept in the oven and the oven door is closed.

- Place back the lid on each dish from which it is removed at the end of the drying period.
- Move the containers to a desiccator and allow them to cool for about 45 min.
- Record the weight of the containers.
- Calculate the moisture content on wet weight basis and express it as percentage to one decimal place.
- Repeat the test if the moisture content between the two replicates differs by more than 0.2%.

Calculation of moisture content

- Use the following equation for samples tested without predrying:

$$\text{Percent (\%)} \text{ moisture content} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

where, W_1 is the weight of the container with lid, W_2 is the weight of container and sample before drying and W_3 is the weight of container and sample after drying.

- Use the following equation for samples which have been predried:

$$\text{Final moisture content (\%)} = S_1 + S_2 - \frac{(S_1 \times S_2)}{100}$$

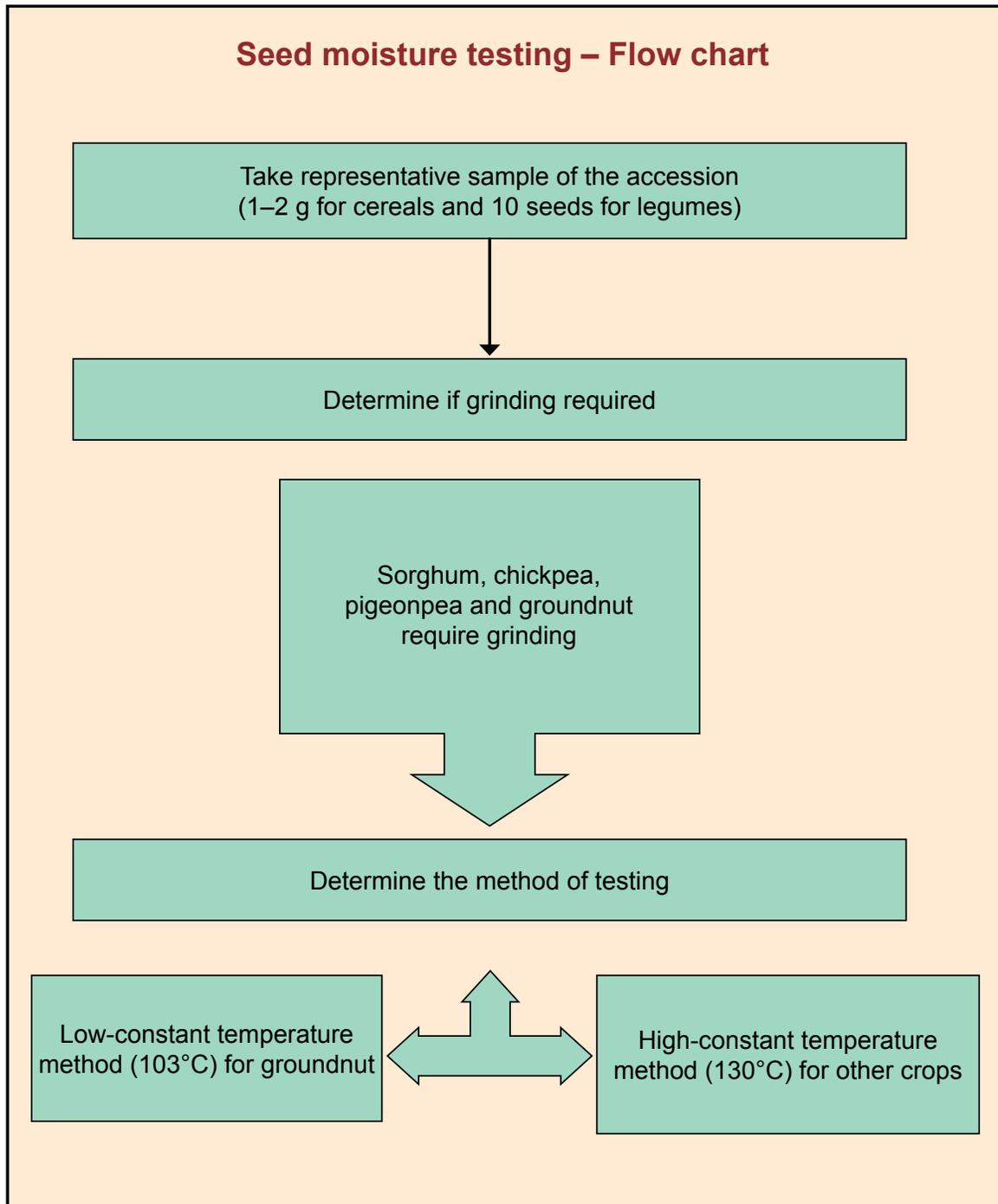
where, S_1 is the percentage moisture content from first-stage drying and S_2 is the percentage moisture content from second-stage drying.

The data sheet used to record moisture content is given as Annexure 4B.1.



During moisture determination, exposure of the sample to atmosphere of the laboratory should be reduced to the absolute minimum.

Seed moisture testing – Flow chart



4C. Seed drying

Seed drying involves reduction of moisture content to the recommended levels for storage.

- Commence drying process as soon as possible after the receipt of the seeds to avoid unnecessary deterioration.

Seed-drying procedures

- Estimate the moisture content of seeds using methods described in Section 4B and assess the need for drying depending on where the seed is going to be stored.
 - moisture content of the seeds stored as base collections (conserved under long-term storage conditions, seed not used for routine distribution) should be between 3 and 7%, and
 - moisture content of the seeds stored as active collections (conserved under medium-term storage conditions, used for regeneration, distribution, characterization and evaluation) should be around 6–8% for groundnut and 8–10% for other crops.

If the moisture is above these limits, further drying is required.

- If drying is required, place the seeds in labeled cloth bags.

The bags used for drying should allow moisture to escape easily. Muslin cloth bags are best suited for this purpose.

- Do not keep too many seeds in the same bag.
- Close the bags properly to ensure there is no spillover and mixing of seeds.
- Use methods that minimize loss of viability during drying.

Several methods are available for drying seeds. One should be chosen depending on the equipment available. The most common and safe methods used for drying are dehumidified drying and silica gel drying.

4C.1. Dehumidified drying

The FAO/IPGRI Genebank Standards recommend the use of $15 \pm 5\%$ relative humidity and $15 \pm 5^\circ\text{C}$ temperature for drying seeds. For smaller genebanks, seed-drying cabinets designed to provide these environmental conditions are available in the market (Fig. 4C.1.1). Larger genebanks, however, need modular walk-in seed-drying rooms as shown in Fig. 4C.1.2.

- Place the seeds packed in muslin cloth bags on the open racks of the drying room or seed-drying cabinet.
- Leave the seeds in the drying room or cabinet until the moisture content is likely to be in the range required for storage.

The length of the drying period can be predicted by one of the two methods:



Figure 4C.1.1. Seed-drying cabinet at ICRISAT genebank.



Figure 4C.1.2. Walk-in seed drying room at ICRISAT genebank.



The drying cabinet should have safety devices to regulate the temperature and prevent overheating in the event of failure of thermostat.

Prediction of drying period by weight loss

- Determine the moisture content of the seed sample using the methods described in Section 4B.
- Weigh the seed sample that requires drying.
- Calculate the weight of the seeds at required moisture content by the equation:

$$\text{Final seed weight} = \text{Initial weight of seeds} \times \frac{(100 - \text{Initial \% moisture content})}{(100 - \text{Final \% moisture content})}$$

- Keep the sample in a muslin cloth bag and allow it to dry until the required weight is attained.

If there is no previous experience of drying seeds of particular species, it may be necessary to do some experimental work to predict the appropriate drying period.

Prediction of drying period from mean drying curves

- Determine the moisture content of the seed lots using methods described in Section 4B.
- Keep the seed lots in labeled muslin cloth bags and place them in drying environment.
- Remove a small sample and repeat moisture determination of the seed lot every day.
- Plot the moisture content of the seeds on a graph with percentage moisture content on Y-axis and drying time on X-axis.

Seeds dry at an exponential rate until equilibrium moisture content is reached. The rate of drying of different seed lots of the same species will be more or less similar.

The drying curves under a constant drying environment of 15°C and 15% RH for seeds of sorghum, pearl millet, chickpea, pigeonpea and groundnut are shown below (Fig. 4C.1.3).

The following graphs can be used for predicting the drying period of all seed lots of a particular species dried under similar conditions.

- Draw a horizontal line each from the initial and desired moisture contents on the Y-axis across to the drying curve.
- Mark or read the day on X-axis for the two points of intersection.

The difference between the two points gives the drying time required to achieve the desired moisture content.

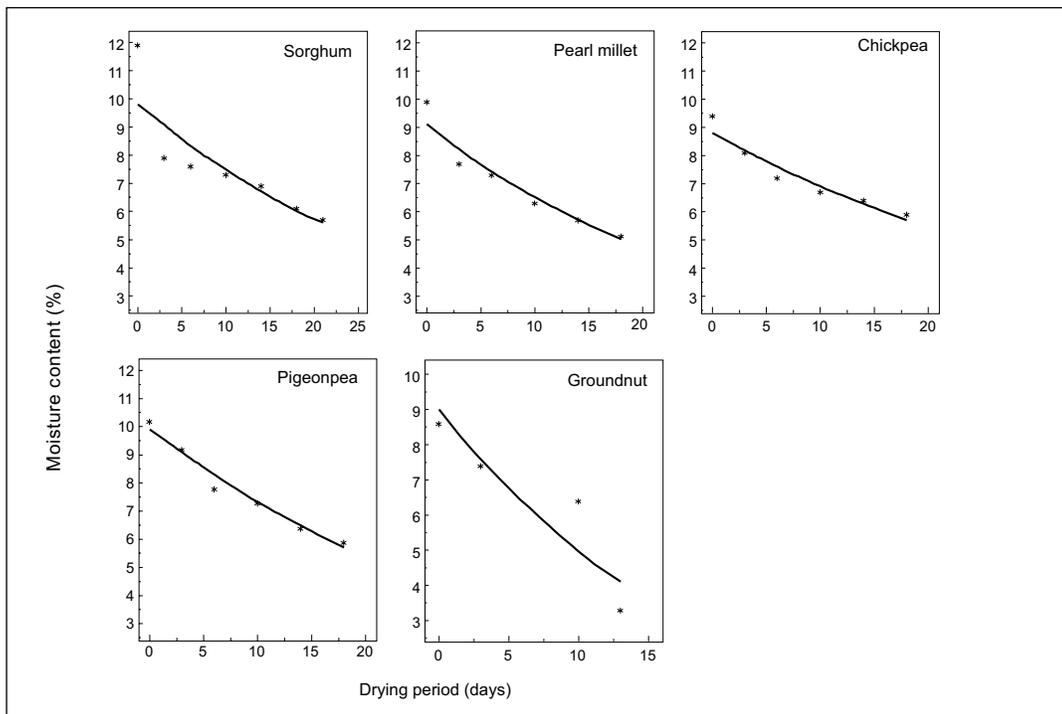


Figure 4C.1.3. Seed drying curves under a constant drying environment of 15°C and 15% RH for seeds of ICRISAT mandate crops.

4C.2. Silica gel drying

Small samples can be dried using silica gel (see Fig. 4C.2.1).

- Place dried silica gel (deep blue in color) in desiccators or glass jars with an airtight seal. The weight of the silica gel used should be equal to the seeds for efficient drying.
- Place the seeds in muslin bags and keep them in close proximity to the silica gel.
- Keep the desiccator at 15°–20°C.
- Change the silica gel daily or when the color changes from deep blue to pink or pale blue.
- Regenerate the silica gel by heating at 100°C until it turns deep blue again and allow it to cool in an airtight container for reuse.
- Leave the seeds with fresh changes of silica gel in the container until the moisture content of the seeds is in the range required for storage.
 - if the initial moisture content and weight of seed lot are known, the weight of seeds at required moisture content can be calculated by the weight loss using the following equation:

$$\text{Final weight} = \frac{\text{Initial weight} \times 100 - \text{Initial moisture content (\%)}}{100 - \text{Final moisture content (\%)}}$$

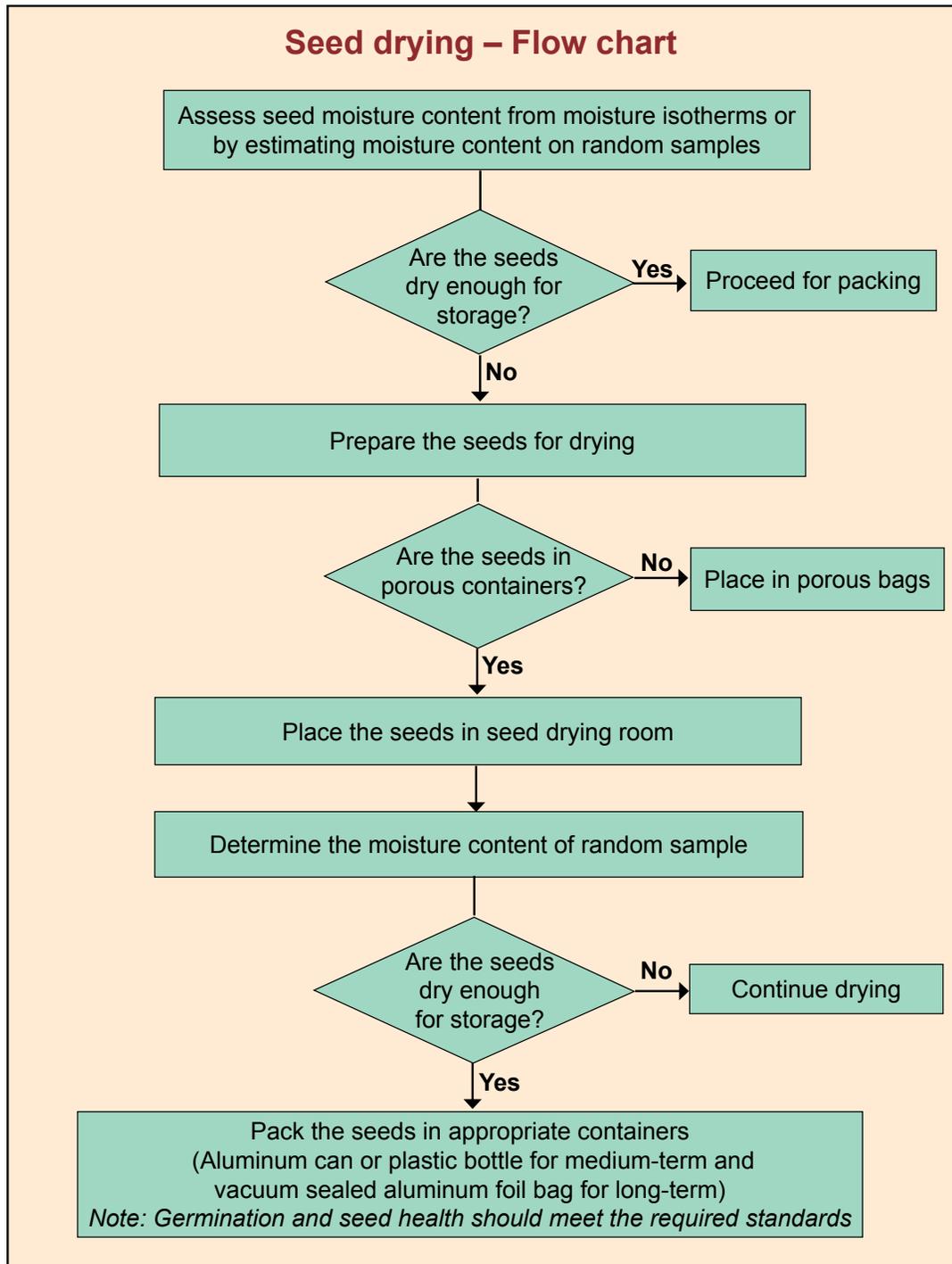
- alternatively, remove a subsample and determine whether or not the required moisture content is attained, using methods described in Section 4B.

- Pack the seeds in appropriate containers once the recommended moisture content or the equilibrium seed weight is attained and if the germination and seed health are acceptable.
- If the moisture content is not low enough for storage, continue further drying.



Figure 4C.2.1. Seed drying using silica gel at ICRISAT genebank.

Seed drying – Flow chart



4D. Seed viability testing

Viability tests measure how many seeds germinate and develop into plants, which reproduce themselves.

- Viability of accessions should be tested:
 - before seeds are packaged and placed in the genebank, and
 - at regular intervals during storage.

Many methods are available to test seed-viability. The most accurate method to test seed viability is the germination test using appropriate procedure.

4D.1. Germination test

Complete germination can be achieved only under optimum conditions of light, temperature and water. The requirements for germination vary with species as shown in Table.4D.1.1.

Table 4D.1.1. Recommended conditions for germinating seeds of ICRISAT mandate crops.

Crop	Substrate*	Temperature	Special requirements**
Sorghum	BP	20/30°C (16/8h); 20°C	0.2% KNO ₃ for wild species
Pearl millet	TP	20/30°C (16/8h); 20°C	
Chickpea	BP	20°C	Mechanical scarification for wild species
Pigeonpea	BP	25°C	Mechanical scarification for wild species
Groundnut	BP	25°C	Remove shell, 0.2% ethrel***
Finger millet	TP	20/30°C (16/8h)	
Foxtail millet	TP	20/30°C (16/8h)	
Little millet	TP	Not available	
Proso millet	TP	20/30°C (16/8h)	Light $180 \times 10^{-6} \text{ m}^{-2} \text{ s}^{-1}$, 12 h/d
Barnyard millet	TP	20/30°C (16/8h)	Prechill, light
Kodo millet	TP	20/30°C (16/8h)	

* TP = Top of Paper, BP = Between Paper (Paper towel method)

** Freshly harvested seeds and wild species of most crops show dormancy, ie, the seeds remain hard and firm during the germination test. Special treatments are required to overcome dormancy.

*** Prepared by diluting 2 mL ethrel (2-chloroethylphosphonic acid) with 998 ml distilled water.

Sample size

- Use a minimum of two replicates each of 50 or 100 seeds for testing initial germination and two replicates each of 25 or 50 seeds for subsequent tests, depending on available seed quantity.
- Take a random sample of seeds from the container.
- If the seeds are very dry (moisture content <8%) expose them to ambient atmosphere for 24 h to raise the moisture content before testing for germination.

Two methods are used for testing germination:

- A. Top of paper method for millets.
- B. Between paper (Rolled towel) method for sorghum, chickpea, pigeonpea and groundnut.

Paper towel is used as substrate for germination in both these methods.

Quality of paper towel

- The paper used as substrate should not be toxic to developing seedlings.
- It should be able to absorb and supply sufficient moisture to the seeds to germinate.
- It should be strong enough not to fall apart when handled and not to be penetrated by the roots of developing seedlings.



All new batches of paper towel substrate should be tested for their quality on receipt.

Simple test for paper quality

Presence of toxic substances

- Cut the paper to size and place in a 9-cm petri dish.
- Moisten the paper with sufficient water.
- Test the seeds of sensitive species like Bermuda grass (*Cynodon dactylon*), if available, or finger millet (*Eleusine coracana*) for germination on the moistened paper:
- Evaluate the root development after 5 days.
 - classic symptoms of paper toxicity are shortened and discolored root tips.

Paper strength

- Moisten the paper and hold it in the air from one corner.
 - paper should not fall apart.

Moisture absorption

- Cut the paper into strips about 10 mm wide.
- Hold vertically with about 20 mm of the paper immersed in water.
- Measure the height above the water level that the moisture has risen to.
 - minimum standard is a 30 mm rise in 2 min.

4D.1.1. Top of paper method

Seeds are germinated on top of moist paper (Whatman Grade 181) in a petri dish (Fig. 4D.1.1.1-4).

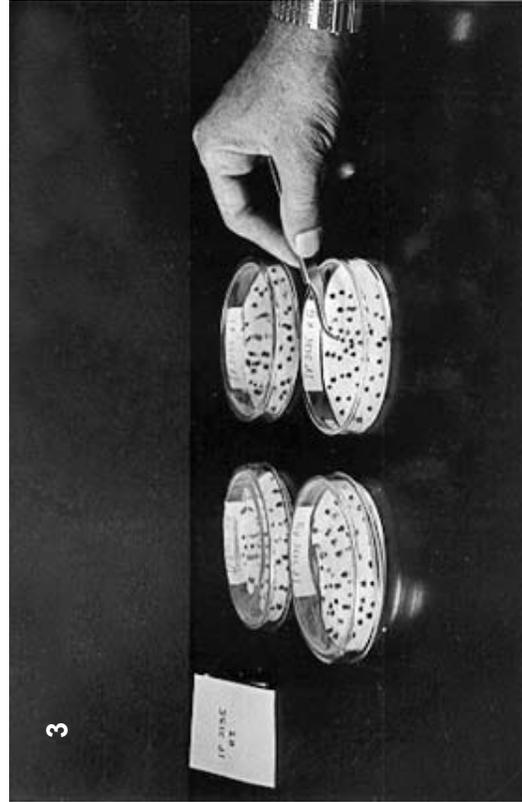


Figure 4D.1.1.1-4. Testing germination of seeds on the top of filter paper.

- Place the paper in 9-cm petri dishes.
- Moisten it with about 4 ml of distilled water.
- Put a label in the petri dish with accession number, number of replicate and date of the test.
- Spread the seeds at regular distance on the surface of the paper.
- Cover the petri dishes and keep them in a plastic bag to prevent drying.
- Place the petri dishes in an incubator maintained at the recommended optimum temperature.

4D.1.2. Between Paper (Paper Towel) method

Seeds are germinated between two layers of moist paper towels (Fig. 4D.1.2.1-7).

- Cut the paper to a convenient size to hold one replicate of the seeds (Fig. 4D.1.2.1.1).
- Label the paper on the outside at one end with the accession number, replicate number and the date of testing (Fig. 4D.1.2.1.2).
- Moisten the paper towels with water.
- Arrange the seeds in rows at regular intervals 4 cm from the top edge, leaving 3–4 cm gap on the sides (Fig. 4D.1.2.1.3).

Scarify (puncture the seed coat with a razor blade or scalpel without damaging the embryo) the seeds of Cicer and Cajanus species before sowing.

- Cover the seeds with another sheet of dry paper towel (Fig. 4D.1.2.1.4).
- Roll the paper loosely from the label end (Fig. 4D.1.2.1.5).
- Put a paper clip to hold the rolled paper towels from falling apart (Fig. 4D.1.2.1.6).
- Keep the rolls in a plastic tray (Fig. 4D.1.2.1.7).
- Add sufficient quantity of distilled water (covering the bottom 3-cm of rolls) to the tray.
- Place the tray in an incubator maintained at recommended temperature (Fig. 4D.1.2.2) (see Table 4D.1.1).

Fungal contamination is common when testing germination of legume seeds. Adopt the following laboratory practices to minimize infection:

- Use proper spacing of seeds — increase the distance between seeds and use greater number of replicates.
- Provide optimum environment for germination — temperature regime should be suitable and the test environment must be well aerated.
- Ensure cleanliness of germination test media and containers — making sure that these are not sources of inoculum.
- Avoid imbibition injury (by prior humidification of the seeds) that could lead to leakage of cell contents and provide source of nutrients to fungi.
- Promptly remove decaying seeds to prevent the spread of fungi to neighboring seeds.

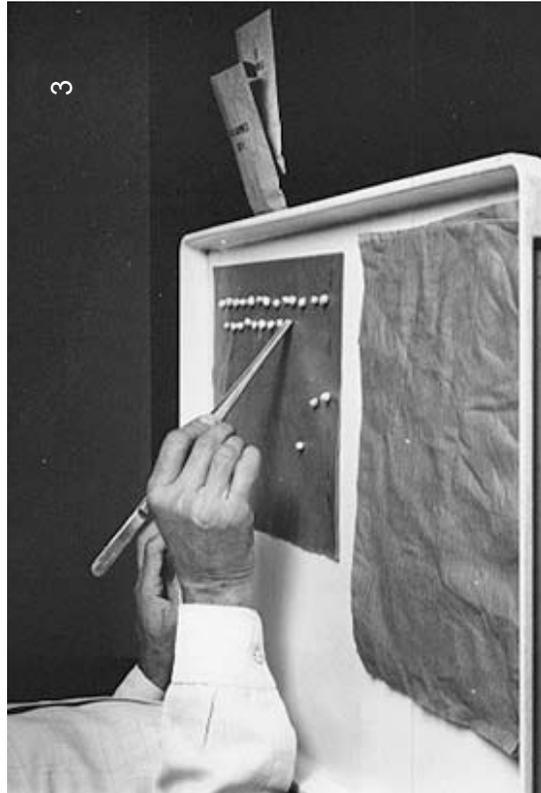
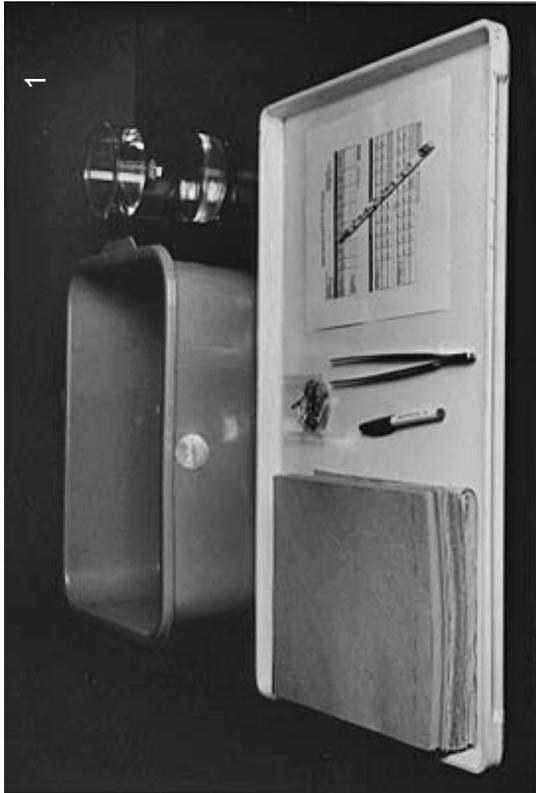
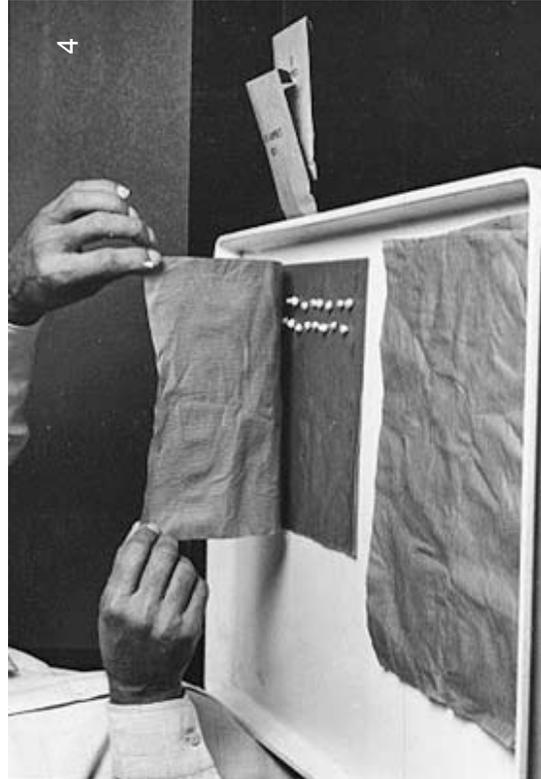




Figure 4D.1.2.1-7. Testing germination of seeds between moist paper towels.

- Remove seed covering structures before tests when these are found to be sources of infection.
- Remove sprouted seeds (seeds that germinated before harvest and subsequently dried), which can be a source of severe infection.
- Treat seed with Thiram (tetramethyl thioperoxy dicarbonic diamide).

4D.1.3. Evaluation of germination tests

- Evaluate the seedlings 7 days after sowing.
- Scarify the hard and ungerminated seeds of chickpea and pigeonpea and evaluate at 14 days after sowing.
- Classify the seedlings removed during course of germination test as normal seedlings and abnormal seedlings.
 - **normal seedlings are capable of developing into plants given favorable conditions and possess adequate root and shoot structures,**
 - **abnormal seedlings are those incapable of further development and suffer deficiency, decay or weakness in their root or shoot system.**

Seedlings with the following defects are classified as abnormal (see Figs. 4D.1.3.3 and 4):

- **Roots**
 - primary root stunted, stubby, missing, broken, split from the tip, spindly, trapped in the seed coat, with negative geotropism, glassy, decayed due to primary infection, and with less than two secondary roots.
- **Shoot** (hypocotyl, epicotyl and mesocotyl)
 - short and thick, split right through, missing, constricted, twisted, glassy, and decayed due to primary infection.
- **Terminal bud/leaves**
 - deformed, damaged, missing, and decayed due to primary infection
- **Cotyledons**
 - swollen, deformed, necrotic, glassy, separated or missing, and decayed due to primary infection
- Record observations in the data sheet shown as Annexure 4D.1.
- Update the inventory database with information from germination test.
- Repeat the germination test if the difference between the two replicates exceeds the maximum tolerance limits at 2.5% probability (Appendix 1).



Figure 4D.1.2.2. Trays containing rolled paper towels placed in an incubator.

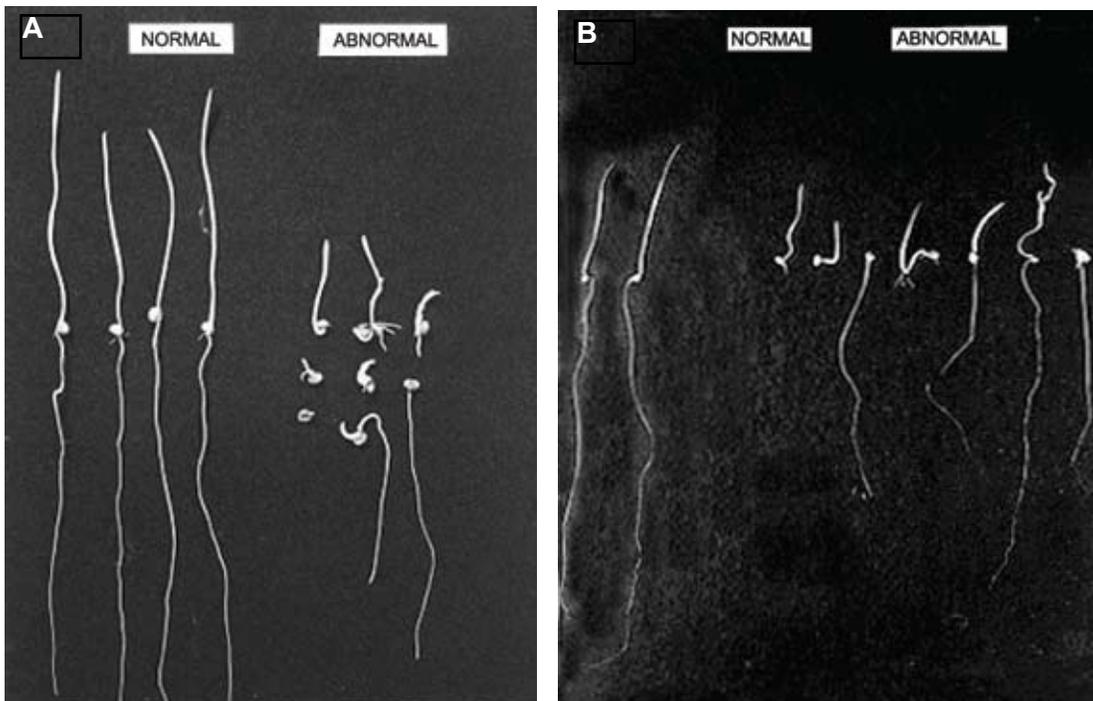


Figure 4D.1.3.3. Normal and abnormal seedlings of sorghum (A) and pearl millet (B).

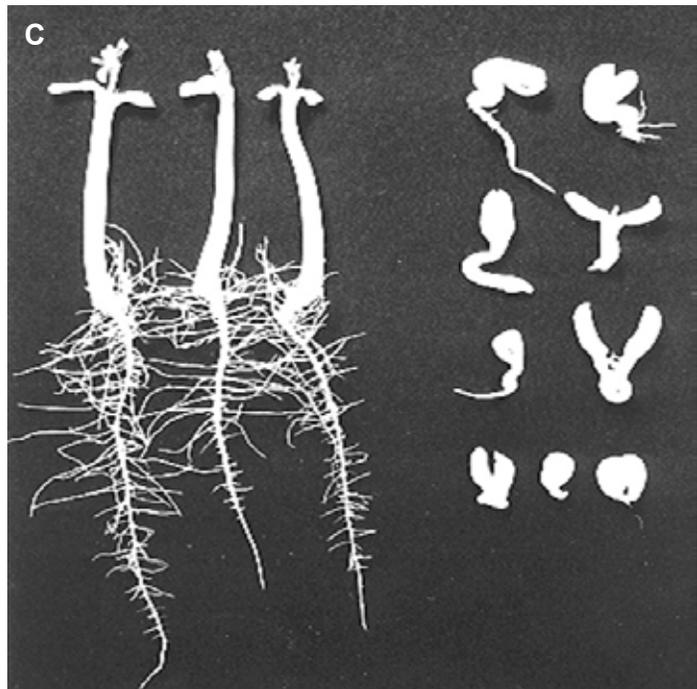
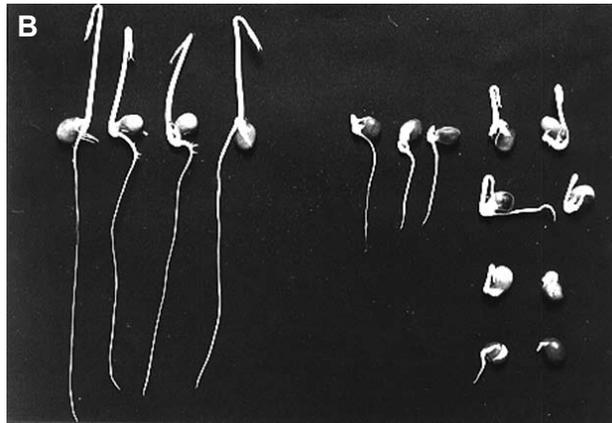
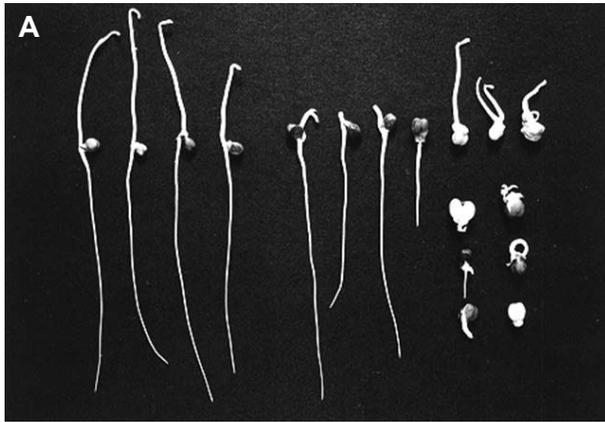


Figure 4D.1.3.4. Normal (left) and abnormal (right) seedlings in chickpea (A), pigeonpea (B) and groundnut (C).

4.D.2. Topographical tetrazolium test for viability

The tetrazolium test can be used as a backup procedure to germination tests in genebanks.

It can be applied to firm seeds, which have failed to germinate at the end of germination test.

The tetrazolium test procedure includes the following steps:

Preconditioning

- Remove the seed covering structures (glumes, etc).
- Precondition the seeds by first soaking in water or by placing them on a moist medium at 30°C.



No preconditioning is necessary when nongerminated seeds are evaluated at the end of a germination test.

Staining

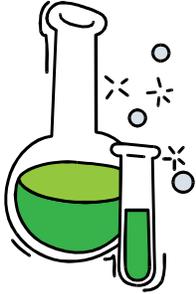
- Bisect the seeds longitudinally through the embryo with a razor blade.
- Discard one-half of the seed and place the other half in the staining solution at recommended concentration (Table 4D.2.1) in a glass vial.
- Place the vials in an incubator maintained in the dark at recommended temperatures and duration (Table 4D.2.1).

Table 4D.2.1. Concentration, temperatures and period of staining with tetrazolium solution.

Crop	Preconditioning	Staining
<i>Arachis hypogaea</i>	Imbibe or soak, 18 h	1%, 40°C, 24 h
<i>Cicer arietinum</i>	Imbibe or soak, 18 h	1%, 30°C, 24 h
<i>Cajanus cajan</i>	Scarify seed, imbibe or soak, 48 h	1%, 30-35°C, 24 h
<i>Pennisetum spp.</i>	Imbibe or soak, 6–8 h	0.5–1%, 30°C, 6–24 h
<i>Sorghum spp.</i>	Imbibe, 16 h, 30°C	0.5–1%, 30°C, 3–24 h

- After staining, wash the seeds several times in distilled water to remove excess stain.
- Immerse the seeds in lactophenol (1 L of lactophenol prepared from 200 ml phenol, 200 ml lactic acid, 400 ml glycerine, and 200 ml water) solution for 1–2 h before evaluation of the seeds.
- Evaluate the seeds for staining pattern under a low power binocular microscope.
- **Viable tissues stain bright red. Pink and very dark red stains are indicative of dead tissue.**

- Classify the seeds into three categories depending on staining pattern:
 - completely stained and viable seeds,
 - completely unstained seeds that are nonviable, and
 - partially stained seeds.



Preparation of 1% tetrazolium chloride solution

The tetrazolium solution should be between pH 6 and 8 to achieve best results.

Prepare 1 L of buffered 1% tetrazolium chloride* solution as follows:

- Dissolve 3.631 g KH_2PO_4 in 400 ml of distilled water
- Dissolve 7.126 g $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ in 600 ml of distilled water
- Mix the two solutions to prepare the buffer
- Dissolve 10 g of 2,3,5,-triphenyl tetrazolium chloride in the 1 L of buffer solution.

* To dilute the 1% tetrazolium buffered stock solution to produce 0.5% tetrazolium solution, mix one part of the stock solution with one part of distilled water.



***The tetrazolium test is not an absolute test of seed viability.
To gain confidence the test must be first calibrated with the results
of germination test for each species.***

4D.3. Seed vigor tests

Vigor is the sum total of all those properties in seed which upon sowing result in rapid and uniform production of healthy seedlings under a wide range of environments, including both favorable and stress conditions. Vigor tests supplement information about seed quality.

Selected tests for vigor

Speed of germination

Speed of germination is an important measure of vigor. It depends on the time taken to reach 50% germination at constant temperature. Seeds with low vigor take longer time to germinate.

- Place 25–50 seeds over filter paper (Whatman No. 1) moistened with 4 ml distilled water inside a petri dish.
- Count and remove the germinated seeds every 12 h. Germination is considered to have occurred when the radicle protrudes by 2–4 mm.

- Calculate germination index using the equation $\sum(t \times n)/\sum n$, where n is the number of germinated seeds and t is the number of hours from the beginning of the germination test (Annexure 4D.3.1).

Seedling growth test

Measurements of seedling growth (root and shoot) at specific number of days after sowing give an indication of their vigor (Annexure 4D.3.1). Slow seedling growth (shorter roots and shoots) indicates low vigor.

- Conduct the germination test as described earlier and measure the length of the root and shoot.

The seedlings may be cut and dried at 110°C for 17 h to record their dry weights, which is more for better quality seeds.

Membrane integrity

The test is based on measuring the concentration of leachates by electrical conductivity (Annexure 4D.3.1). Low-vigor seeds generally possess poor membrane structure. When such seeds are soaked in water, greater electrolyte loss occurs, leading to higher conductivity of water. The test is mainly used for grain legumes.

- Soak 10 seeds in 50 ml of distilled water in a beaker at room temperature.
- Measure leachate conductivity after 24 h using a digital conductivity bridge.
- Record the reading in $\mu\text{S ml}^{-1}$ water g^{-1} dry weight of the seed sample.
- A lower reading indicates seeds with higher vigor and vice versa. It is important to note that many species (eg, legumes) have seeds that are impermeable or only slowly permeable to water. This can affect the leaching of electrolytes from seeds in a conductivity test.

4E. Seed health testing

Seed borne fungi such as *Alternaria*, *Fusarium*, *Penicillium*, *Aspergillus* and *Rhizopus* spp. affect longevity during storage. Curators should ensure that seeds prepared for long-term conservation are free from the seed borne pathogens. The methods employed to detect the pathogens are referred to as seed health testing methods.

The commonly used seed health testing methods are:

4E.1. Visual examination

Seeds are examined under an illuminated magnifying lens (2×) or under low-power stereo-binocular microscope (Fig. 4E.1.1). By this method, it is possible to detect sclerotia, smut balls, fungal spores and other fructifications such as pycnidia, perithecia, etc.

4E.2. Blotter test

Blotter tests are similar to germination tests in that seeds are placed on moistened layers of blotter paper and incubated under conditions that promote fungal growth.



Figure 4E.1.1. Seed health testing in laboratory using binocular microscope.

- Line the lower lid of the petri dishes with three layers of blotter paper moistened with sterile water.
- Drain off excess water and place 20–25 seeds manually with a forceps.
- Evenly space the seeds to avoid contact with each other.
- Incubate the seeds under near ultraviolet light in alternating cycles of 12-h light/darkness for 7 days at $20 \pm 2^{\circ}\text{C}$.
- Examine the petri dishes under a stereo-binocular microscope for fungi developing on the seeds.

Profuse seedling growth may make interpretations difficult. This may be overcome by adding 2,4-D sodium salt to provide a 0.2% moistening solution.

4E.3. Agar plate method

This is the most common method used for identification of seed borne fungi.

- Prepare the medium by mixing Potato Dextrose Agar (PDA) powder with appropriate quantity of water.

- Sterilize the mixture in an autoclave at 121°C for 15–20 minutes with 15 lb pressure and cool to about 50°C.
- Carefully pour the mixture into petri dishes by lifting the lid enough only to pour in the agar to avoid contamination.
- Allow it to cool and solidify for 20 min.
- Surface-disinfect the seed by pre-treating for 1 min in a 1% sodium hypochlorite (NaOCl) solution prepared by diluting 20 parts of laundry bleach (5.25% NaOCl) with 85 parts of water.
- Place about 10 seeds (depending on size) on the agar surface with a forceps.
- Incubate the petri dishes at 20–25°C for about 5–8 days.
- Identify the seed borne pathogens on the basis of colony and spore characteristics.

Sometimes, bacterial colonies develop on the agar and inhibit fungal growth making identification difficult. This can be overcome by adding an antibiotic such as streptomycin to the autoclaved agar medium after it cools to 50–55°C.

4E.4. Seed health standard

- Examine each seed for the presence of pathogens.

If the percentage of seeds infected by one or more of the following fungi is >5%, the seeds are unsuitable for conservation as base collection:

Alternaria, Aspergillus, Cladosporium, Curvularia, Fusarium, Macrophomina, Penicillium Phoma and Rhizopus spp.

4F. Seed packing

Seed packaging involves placing the dry seeds of an accession into a container for storage. Seeds are packaged to:

- Prevent them from absorbing moisture from air
- Avoid mix up of individual accessions
- Prevent contamination by insects and diseases.

Pack the seeds immediately after drying.

4F.1. Types of containers

Different types of containers are available for packaging. The choice depends on storage conditions and species. The packing material should be impermeable to moisture and suitable for long-term use. If the relative humidity of the storage room is not controlled, it is imperative to use moisture proof containers.

Some frequently used containers in genebanks are: glass bottles, aluminum cans, aluminum foil packets and plastic bottles.

- Glass bottles are good but fragile.
- Aluminum cans are difficult to reseal once opened.
- Aluminum foil packets can be resealed and occupy less space. However, seeds with sharp projections can pierce the packets and render them moisture permeable. The packets used for conservation therefore, should have sufficient strength to withstand piercing.
- Plastic bottles are moisture resistant but not moisture proof. So they should be used with caution if relative humidity is not controlled.

The Bioversity International (BI), formerly the International Plant Genetic Resources Institute (IPGRI) has recommended the following specifications for aluminum foil packets to be used for genebanks:

- An outer layer of 17 g m⁻² Melinex, 4 g m⁻² lacquer,
- A middle layer 33 g m⁻² (12 μm) aluminum foil, 4 g m⁻² lacquer, and
- An inner layer of 63 g m⁻² polyethylene.

Always obtain containers from a reliable manufacturer. The quality of containers may vary with batches, therefore test each batch for quality before using. When using laminated aluminum foil packets, ensure that the packets are adequately sealed after filling.

Simple test for determining the quality of containers

The quality of the containers and sealing can be tested as follows:

- Fill the container with regenerated silica gel and seal it.
- Accurately determine the weight of the container with an analytical balance.
- Keep the container over water in a desiccator for about a week.
- Remove the container from desiccator and allow the surface to dry.
- Weigh the container and record the change in weight, if any.

The weight of the container remains constant if the container is moisture proof and the sealing is good. Increase in weight of the container indicates poor quality of the container or inadequate sealing.

- Adjust sealing time and repeat the test to confirm the quality of the container.

Alternatively, the container quality can be tested by filling it with water and holding over silica gel in a desiccator or a ventilated oven at 40°C. Change in weight of the container indicates its poor quality sealing.

At ICRISAT genebank,

- Active collections which are frequently sampled for use are stored in:
 - rust-proof aluminum cans with screw caps and rubber gaskets for sorghum, pearl millet, chickpea, pigeonpea and small millets (Fig. 4F.1.1A), or
 - large size plastic bottles with inner lid and screw cap for groundnut (Fig. 4F.1.1A).

- Base collections conserved for long-term are stored in re-sealable laminated aluminum foil packets* (Fig. 4F.1.1B)

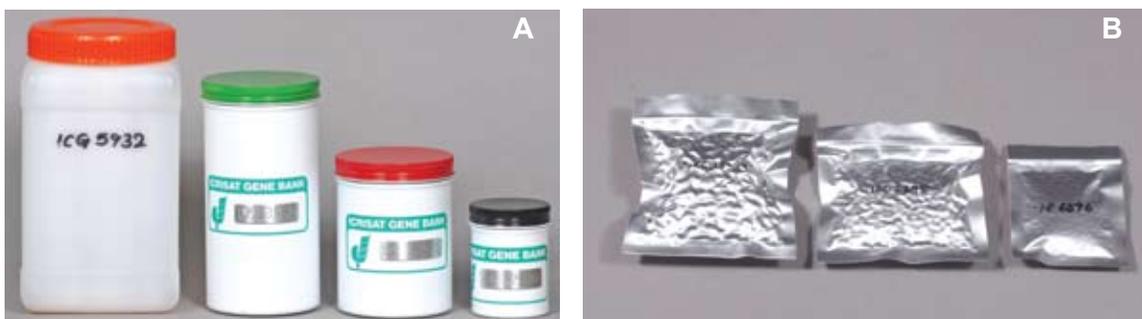


Figure 4F.1.1. Containers used in medium-term (A) and long-term (B) storage at ICRISAT.

4F.2. Packing procedures

Base collections:

Prepare and label the aluminum foil packets with computer generated self-adhesive labels (eg, Z-Label Computer Labels). The label should contain the following information:

- Accession number.
- Identity.
- Season of harvest.

Use aluminum packets of the following size to accommodate the recommended minimum sample size for each crop.

Aluminum packet sizes and approximate maximum quantity of seeds stored in base collections at ICRISAT genebank are as follows:

Crop	Packet size (mm)	Approx. seed quantity (g)
Sorghum and pearl millet	100 × 140	75
Chickpea, pigeonpea and groundnut	190 × 160	200
Small millets	110 × 80	25

- Take out a few samples at a time from the drying room to minimize reabsorption of moisture by the seeds.
- Weigh the amount of seed being prepared for storage.
- Fill the container with seeds leaving at least 2.5 cm headspace for sealing.
- Inside the container, place a non adhesive label with:
 - accession number,
 - identity, and
 - season of harvest - to help in identification of the accession if the label outside is lost.

* Barrier Foils Products Co., Hollands Mill, 61 Shaw Heath, Stockport, SK3, 8BH, UK.

- Seal the container at 0.6 mbar vacuum using Audionvac sealer (Fig. 4F.2.1 A and B).
- Check for any deficiencies in packets and in sealing.
- Print the date of sealing on the packet (if available).
- Move the packets into long-term store.

Active collections:

The following type, size and cap color of containers are used at ICRISAT genebank:

Crop	Type of container	Size/Capacity	Cap color*
Sorghum	Aluminum can	10 × 7.5 cm	Red
Pearl millet	Aluminum can	10 × 7.5 cm	Blue
Chickpea	Aluminum can	10 × 7.5 cm	Yellow
Pigeonpea	Aluminum can	15 × 7.5 cm	Green
Groundnut	Plastic bottle	1.5 kg	Orange
Small millets	Aluminum can	7 × 5 cm	Black

* Color code helps in easy identification when seeds of several crops are to be stored in the same room.

- Paste adhesive metallic labels pre-engraved with accession number or use permanent marking pens for labeling the containers.
- Weigh the amount of seed being prepared for storage.
- Place a label with accession number, identity and season inside the container.
- Fill the container with seed and close the cap tightly.
- Move the containers into storage room.



- **Do not mix seeds from different seasons.**
- **Keep them separate in cloth or resealable plastic bags within the same container.**
- **Do not forget to retain a small sample of the original seed to serve as a reference for future verification of accession identity.**



Figure 4F.2.1. Vacuum sealer (A) and packing seeds (B) for long-term conservation at ICRISAT genebank.

ICRISAT Genebank

Crop/species:

Accession number:

Date of storage:

Date of testing:

Substrate: **BP/TP**

Temperature:

Incubation time: **7d****Germination data**

Rep		I	II	III	IV	Total	Remarks
No. of seeds		25/50	25/50	25/50	25/50	100/200	
Date	Days						
Total germinated							
Abnormal							
Hard/Dormant							
Dead							

% Germination

Seed vigor studies

Crop:

Accession number:

Date of storage:

Date of testing:

Temperature: 20/25°C

Radicle emergence data

Rep	I	II	III	IV	Total (n)	t x n	Remarks
No. of seeds tested	50	50	50	50			
Date	h/d (t)						
	12						
	24/1d						
	36						
	48/2d						
	60						
	72/3d						
	84						
	96/4d						
	108						
	120/5d						
	132						
	144/6d						
	156						
	168/7d						
Total							

Mean time (t X n)/ n

Seedling vigor data

Rep I

Seedling no.	1	2	3	4	5	6	7	8	9	10
Root length, mm										
	11	12	13	14	15	16	17	18	19	20
	21	22	23	24	25	Mean				

Rep II

Seedling no.	1	2	3	4	5	6	7	8	9	10
Root length, mm										
	11	12	13	14	15	16	17	18	19	20
	21	22	23	24	25	Mean				

Mean radicle length (after 5/7d)

Electrical conductivity

Soaking duration: 24 hrs

Temperature: 25°C

Rep	I	II	III	IV	Remarks
Seed #/Water (mL)					
EC (µS)					

Mean (µS)

Section 5

Seed Storage

Seed collected in the field should be quickly processed, packaged in appropriate containers and stored as soon as possible.

5A. Medium- and long-term conservation

Maintaining genetic integrity, which is the main priority of a genebank curator, can be achieved by storing the original seeds (or from initial multiplication) as **base collections** under long-term conditions in sufficient quantity.

If the genebank has distribution of germplasm as a function, adopt a two-step storage system and maintain **active collections** of the sample under medium-term conditions.

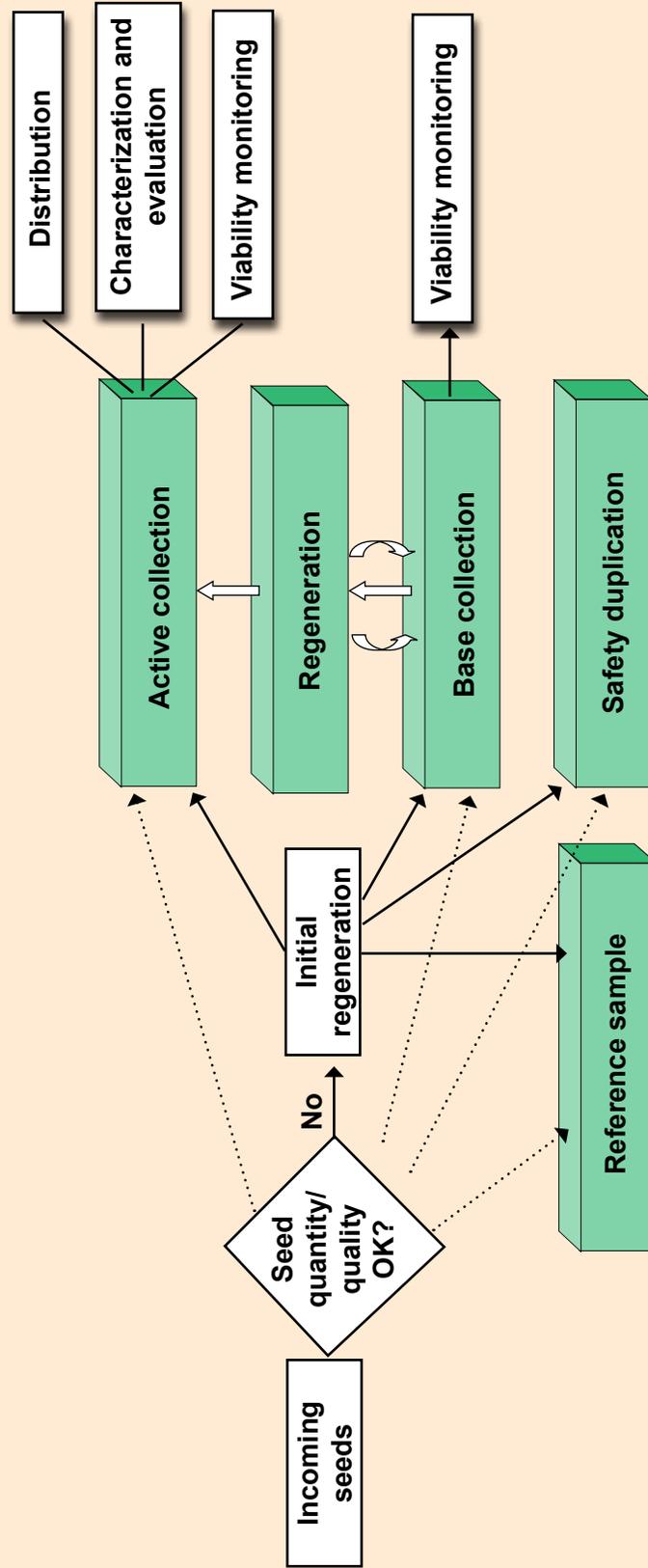


If the genebank has distribution as a function, adopt a two-step seed storage system to avoid repeated regeneration. In this system, two separate seed samples are maintained - one for multiplication (Base collection) and the other for distribution (Active or Working collection).

- *Base collections are stored under better storage conditions (long-term, -20°C) than those for Active collections (medium-term, 4°C and 20-30% RH).*
- *Demand for seeds are met by distributing the samples from the medium-term store until the seed is about to be exhausted or the seed viability begins to decline. Then some seeds are taken out from long-term store and given for multiplication. The multiplied seeds are stored in medium-term for further distribution.*
- *These cycles are repeated until the seeds in long-term are about to be exhausted or the viability of seeds has declined.*
- *When the seeds in long-term storage are due for regeneration, fresh seeds produced by multiplication are used both to replenish seed stock in medium-term and storage in long-term.*

The number of regenerations through which seeds are passed before distribution is thus reduced. Consequently, high quality seeds with minimal genetic change are made available for distribution, while original or near original seeds are available for regeneration.

Diagram of a two-step storage system



5A.1. Base collection

Base collection is a set of accessions preserved for long-term future. Each accession is distinct, and in terms of genetic integrity is as close as possible to the sample originally collected or acquired.

Seeds **are not** distributed from the base collection.

- Preferred storage conditions are:
 - -20°C with 3–7% seed moisture content, depending on species.
- Acceptable storage conditions are:
 - sub-zero temperature with 3–7% moisture content.

Accession size

- At least 1,000 viable seeds, but preferably 1,500–2,000 seeds should be stored for materials showing little morphological variation (genetically homogeneous accessions) as with chickpea and groundnut.
- For materials showing large morphological variation (genetically heterogeneous accessions) the accession should consist of at least 4,000 seeds, but preferably 12,000 seeds as with sorghum, pearl millet and pigeonpea.

Seed viability

- Seed placed in base collection should have >85% germination in groundnut and >90% in other crops. The minimum germination standard for wild species is 75%.



Seeds in base collection are not used for distribution. They are used only for regeneration.

5A.2. Active collection

Active collection comprises accessions that are available for immediate multiplication, distribution and use. Since these accessions are accessed frequently, they are maintained under medium-term conditions, which ensure that accession viability remains above at least 65% for 10–20 years. A combination of storage temperature and moisture content is given below:

Temperature (°C)	Moisture content (%)	
	Groundnut	Sorghum, millets, chickpea, pigeonpea
25	2.0	6.5
20	3.5	7.5
15	5.0	8.0
10	6.0	9.0
5	7.0	10.0
0	8.0	11.0

Active collections at ICRISAT genebank are maintained at 4°C and 20–30% RH.

Accession size

Accession size depends on the demand for the accessions. Frequently requested materials can be stored in larger quantities than others. The maximum sample size held in active collections at ICRISAT genebank is given below:

- Sorghum, pearl millet, chickpea, pigeonpea: 400 g.
- Groundnut: 1.5 kg.



The weight of seeds can be converted into seed number using the 100-seed weight. For example, if 100-seed weight is 2.5 g, 400 g contain: $100 \times 400/2.5 = 16,000$ seeds

Seed viability

Seeds placed in the active collection should have >80% germination in groundnut and >85% in other crops.

Location in storage

The physical location of the accession in the genebank should be coded to locate it easily for retrieval of seeds, etc. The location of an accession in the ICRISAT genebank is coded as follows:

- Room: 1–7.
- Rack: A–Z.
- Bay: I–IV.
- Tray: 1–999.

For example, the code 3-B-IV-12 indicates the location of sample as Tray no. 12 in Room 3, Rack B and Bay IV.

Assigning location code

- Check the inventory data file to find the next available space for the accession.
- Assign the space where the accession is to be placed.
- If the accession is stored in more than one container, keep them all together.
- Place the container in seed store in the assigned location.
- Enter the details (location, date of storage and number of containers) in the inventory data file.

5B. Safety duplication

Safety duplication means a genetically identical duplicate accession sample stored outside the country in a base collection for safety reasons. Safety duplication ensures that any given collection is securely duplicated at another institute, which can preserve the material safely. This provides insurance against loss of material. Under the Agreement with FAO (on behalf of ITPGRFA General Body), ICRISAT has the responsibility of making arrangements for the duplication of its collections. Safety duplication includes both the duplication of the material and the documentation process.

Types of duplication include:

- **Black box** — when the responsibility of the recipient's institute is to maintain the duplicates in adequate storage facilities without handling the samples. It is the originator's responsibility to monitor seed viability and, when necessary, regenerate the collection.

For black box duplication, special permissions are required to export the seeds without Phytosanitary Certification from the originating country. Similarly, the Plant Quarantine Authority in the destination country needs to permit the importation of seeds by the recipient, bypassing the routine quarantine examination.

Prepare the samples for safety duplication similar to the base collections:

- Seeds should be dried to moisture content $5 \pm 2\%$.
- Seeds should be clean and healthy.
- Percent germination should be $>85\%$.
- Vacuum sealed in laminated aluminum foil packets.
- The minimum sample size can be small, ie, approximately 25 g for sorghum and millets, and 100 g for legumes (Fig. 5B.1).
- To save time, the samples for safety duplication can be set aside at the time when the seeds for long-term conservation are processed.



Figure 5B.1. Germplasm samples packed for safety duplication at Svalbard Global Seed Vault, Norway.

5C. Storage policy of ICRISAT genebank

All FAO/ITPGRFA designated germplasm and newly acquired material, which is threatened and of value, will be conserved.

The following are conserved as base collection:

- Germplasm currently designated for FAO /ITPGRFA
- All landrace accessions collected or acquired in the future with complete passport information

ICRISAT Plant Material Identification Committee (PMIC) released the best of the breeding material received with complete pedigree information and key characterization data.

The medium-term storage conserves working collections of:

- Frequently distributed material
- Core and mini core collections
- Genetic stocks
- Undesignated stable breeding lines
- Wild species
- Emergency national holdings.

5D. Documenting inventory data

The genebank should maintain proper documentation to allow rapid accessioning of new samples, answer queries on the conserved germplasm and monitor quality and quantity of stored material to carry out regeneration and distribution. A computerized data handling system is ideal for a genebank. The genebank inventory data includes details of accessions held in storage, their location, quantity and quality. The suggested descriptors are:

ICRISAT accession identifier: Unique identifier for accession assigned when the sample is entered into the collection.

Season of harvest: Season when the crop was harvested (mm/yy).

Site of rejuvenation: Place where the accession was regenerated.

Container: Type of container used for storage, eg, plastic bottle, aluminum can and aluminum foil packet.

Number of containers: Total number of containers used for storing the sample.

Date of storage: Date on which the sample was placed in genebank.

Location in genebank: Exact location where the sample is stored in the genebank — coded for example, 05-A-VI-12, indicating room, rack, bay and tray numbers.

Seed quantity (g): Quantity of seeds currently available in storage.

Germination (%): Percent seed germination from the result of the recent germination test conducted.

Date of germination testing: Date on which seeds were tested for germination.

Remarks: Any significant observation.

Section 6

Germplasm Distribution

Distribution involves supply of representative seed samples from the genebank in response to requests from users.

- Distribute seeds only from active or working collections.
- Send the seeds in a way that they reach their destination in good condition.
- The environmental conditions during transport can be detrimental to seed quality. Therefore distribute seeds in moisture proof envelopes.

6A. Procedures for germplasm distribution within India

- Check the inventory database to see if seed quantity in genebank is sufficient for distribution.

Distribute only if a minimum of four times the number of seeds required for one regeneration cycle remain in store after meeting the request (ie, approximately 40–50 g in cereals, 100–150 g in legumes).

When seed quantity is inadequate for distribution, inform the requestor that the accessions cannot be supplied until after regeneration. Advise the crop curator to program the accessions for regeneration.

- Check the passport data to see if the requested accessions are designated to FAO and freely available for distribution.

If the requested accession is undesignated or has restrictions on distribution under the Material Acquisition Agreement with donors, inform the requestor about the nonavailability of the accession.

- If seeds are available for distribution, register the request by assigning a request number.
- Prepare the list of accessions available for distribution and obtain a SMTA signed for the selected accessions by sending:
 - SMTA for registered genebank accessions.
 - breeding Material Order Form and SMTA for ICRISAT developed varieties registered as genebank accessions but not designated to FAO (Annexure 6A.1).
- Generate labels for the selected accessions using GIMS.
- Paste the labels on seed envelopes used for distributing the seeds to requestor (Fig. 6A.1).
- Use:
 - coin envelopes for cereals, and
 - metal fold paper envelopes or aluminum foil packets for other crops.
- Check the inventory file and note the location of the container in the genebank.

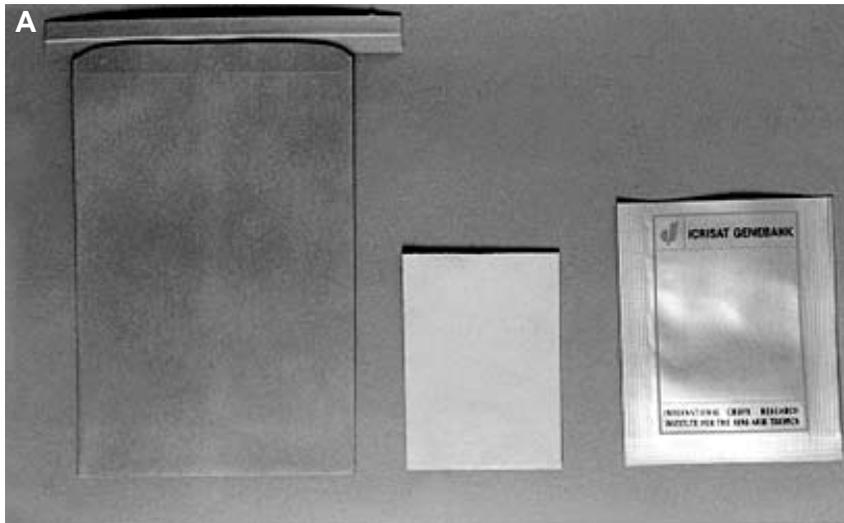


Figure 6A.1. Seed packets (A) and containers (B) used for seed distribution at ICRISAT genebank.

- Pick the containers from the genebank and move them out into a dehumidified room **the previous evening** to allow them to warm up to room temperature before opening. (If the number of samples to be distributed is small, then draw the seeds from containers in the genebank itself).
- Ensure absolute accuracy in identification of accessions while drawing the seed from the genebank.
- Open the container and quickly draw the required amount of seeds into the labeled envelopes (Table 6A.1).

Use random sampling technique so that a good representation of accession is provided.

Table 6A.1. Standard quantity of seed distributed per accession from ICRISAT genebank.

Crop	Quantity (g/nos.)
Sorghum	6 g
Millets	5 g
Chickpea	100 seeds
Pigeonpea	200 seeds
Groundnut	50-100 seeds
Wild species (any crop)	15-20 seeds

- Close the container immediately after removing the seeds for distribution to prevent moisture ingress.

Germplasm seeds are valuable, therefore they should be packed carefully for dispatch. The packing should ensure safety of the seeds and prevent contamination by insects or pathogens during transit.

- Proofread the list of accessions drawn from the genebank with the labels on the envelopes.
- Print the final list with minimum passport details (ICRISAT identification number, alternate identity, source country, location and biological status), characterization data used for verification of accessions (see Section 8 for details) and any other additional information as requested by the consignee.
- Prepare a covering letter.
- Pack the seed envelopes, covering letter and the seed list in a jiffy bag (if the number of samples is small) or a cardboard box and label it with the complete address of the consignee.

Use filling material to avoid damage to seeds during transit.

- Send the seed parcels by registered post or airmail or airfreight to avoid delay and possible loss in seed quality during transit.
- Record the shipment details into the distribution data file.
- Update inventory data deducting the seed quantity supplied in each accession.

6B. Procedures for germplasm distribution to other countries

Follow the same procedure in selecting accessions and fulfilling the SMTA requirement of FAO designated accessions. Additional requirements include:

- Check if the quarantine regulations of the country require an Import Permit (IP) for exporting the seeds to the requester (see Appendix 2).

If IP is required but not sent along with seed request, write to the requester and obtain an IP.

- Draw the seeds from the genebank as described above and prepare seed lists.
- Complete the Request for Export of Seed/Plant/Plant Products form (Annexure 6B.1).

- Check with the plant quarantine office if additional declarations are required such as need for certification that seeds are free from specific diseases and pests.
- Send the seed samples with the export request and IP to the Plant Quarantine Laboratory (PQL) for exit quarantine certification and issue of Phytosanitary Certificate (Annexure 6B.2).
- When the samples are ready, prepare a covering letter and the final list of accessions (if any accessions were detained at quarantine) along with passport data and send to the Plant Quarantine Laboratory to dispatch along with seeds.
- Record the shipment details in the distribution data file.
- Update seed inventory by deducting the quantity of seeds supplied.

6C. Procedures for germplasm distribution within ICRISAT

Seed distribution to ICRISAT staff is also subject to the provisions of the agreement between ICRISAT and FAO. Requests should be made on the Internal Germplasm Order Form (Annexure 6C.1).

Processing internal seed request

- Check the availability of seeds.
- Draw the sample from genebank.
- Prepare final list along with passport details and any other additional information requested by consignee.
- Deliver the seeds and obtain acknowledgement of receipt.
- Update the distribution and inventory databases.

Seeds from the genebank are distributed on the understanding that they will be used for ICRISAT's own research. Third party distribution is not allowed. Requests for registered germplasm accessions received by ICRISAT staff from their collaborators should be forwarded to the genebank to distribute according to the provisions of ICRISAT/FAO Agreement.



- ***Do not send designated germplasm without an SMTA.***
- ***Accessions not designated to FAO and accessions acquired after 1993 should be supplied according to the provisions of agreement with the donor institute or country.***
- ***If no agreement is made for such materials, do not supply until its status is clarified with the donors.***

Distribution of vegetatively propagated species

Distribute stem cuttings for species maintained as live plants (eg, *Arachis* and *Pennisetum* spp.):

- Cut the rhizomes into 15 cm long pieces.
- Roll them in moist paper towels and wrap them with polyethylene film.
- Pack them carefully in jiffy bags and send by the fastest means to reach destination along with instruction if any, for establishing them.

6D. Feedback on germplasm distribution

Obtain feedback on the usefulness of germplasm supplied to users at half-yearly intervals. This will help in identifying deficiencies in service, and also to know of any new traits or sources of resistance identified by the users. The form used to obtain feedback is shown as Annexure 6D.1 and 6D.2 (Annexure 6D.1 for external users and 6D.2 for users within the institute).

6E. Documenting germplasm distribution data

The genebank curator needs to keep a record of the recipients of germplasm, number of samples sent, the purpose for which the request was made, etc. The information could be better maintained in two files with a common link field. At ICRISAT, the distribution descriptors are organized into two files namely:

- A master file with details of the consignee, number of accessions sent, etc, and
- Accession details file (list of material sent).

A 'Reference number' assigned while registering the seed request serves as a link field for the two files.

The following descriptors are suggested for the distribution data files.

Master file

Reference number: Reference number assigned in sequence starting from 1 each year.

Crop: Crop name.

Consignee: Consignee's last name followed by abbreviated first and second names.

Designation of consignee: Designation of the consignee, eg, Plant Breeder, Assistant Professor, etc.

Organization: Name of the organization in full.

Address: Address of the consignee.

Location: City or town where the organization is located.

Country: Country name of consignee.

User status: Status of organization requesting germplasm (eg, commercial company, national institute, non-governmental organization, individual, etc).

Date of request: Date on which the request was received.

Date supply: Date on which seed samples were sent.

Number of samples: Number of samples sent.

Purpose: Purpose for which seeds were requested.

Remarks: Any significant observation.

Accession details file

Reference number: Reference number assigned (link filed to master file).

Crop: Crop name.

Accession number: Accession numbers distributed entered in sequence.

Quantity: Quantity of seed distributed in grams.

Remarks: Any significant observation.



- **Assess the amount of assembled diversity utilized in crop improvement.**
- **Assess the patterns of demand for types of germplasm by different users.**
- **If demand is restricted to certain accessions, select other accessions with same traits for supply.**

6F. Germplasm repatriation

National programs occasionally request repatriation of germplasm donated by them to:

- Establish or add to the genebank of their own
- Rebuild their collections lost due to inadequate facilities, natural calamities, civil disorder, etc.

Genebank personnel:

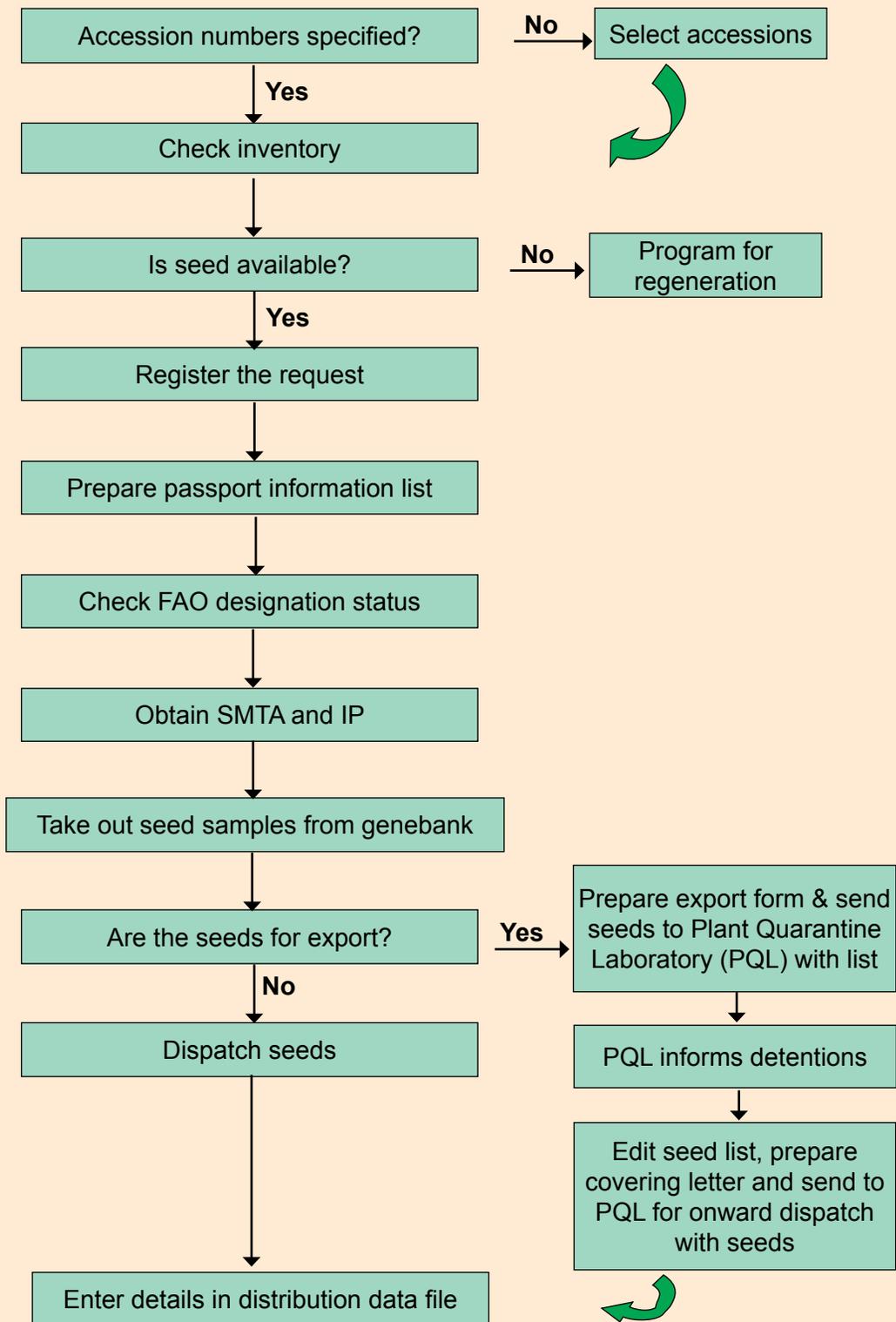
- Ask the requestor for the list of accessions required to be repatriated
- Supply seed samples if the distribution does not reduce the stocks below accepted levels for conservation.

The quantity distributed should be sufficient to conduct at least two regenerations (see Section 9).

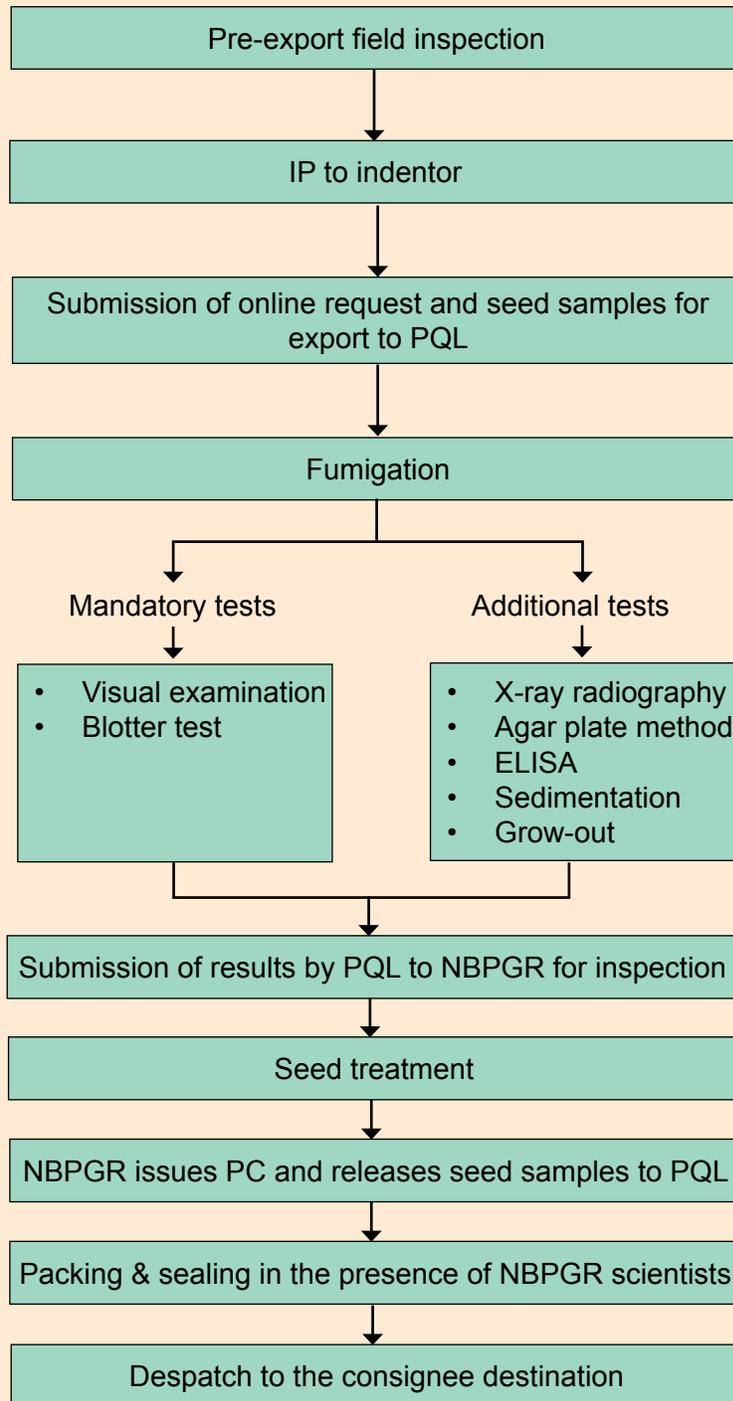
- Undertake regeneration for accessions with insufficient seed stocks.

If regeneration poses an undue burden on the technical and financial resources, ask the requestor to cover the actual cost of multiplying the accessions.

Flow chart of Germplasm Distribution



Flow chart of Seed Export



**Standard Order Form (Material Transfer Agreement) for
Genetic Material Developed at ICRISAT**

I/We order the following genetic material in the form of seed/vegetative propagules/tissue samples/DNA:

In so far as this genetic material has been developed by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) with public funds provided through the Consultative Group on International Agricultural Research (CGIAR) by donors from around the world. Hence, I/we agree the material contained herein is furnished by ICRISAT under the following conditions:

1. ICRISAT is making the material indicated above or in the attached list available as part of its policy of maximizing the utilization of genetic material for research. The material developed by ICRISAT is made freely available for any agricultural research or breeding purposes.
2. Recipients are free to commercialize ICRISAT research products in the form they are provided with due notification to ICRISAT. Prior to the application of any form of intellectual property rights (IPR) on this germplasm and related information, written permission must be obtained from ICRISAT. Moreover, while ICRISAT recognizes the validity of IPR, it reserves the right to distribute all material in accordance with paragraph (1) above.
3. The recipient agrees that any subsequent person or institution to which they provide samples of this material is bound by these same provisions.
4. Although the material and associated information being supplied by ICRISAT were developed following careful and comprehensive research, ICRISAT makes no warranties as to the safety or title of the material nor as to the accuracy or correctness of any passport or other data provided with the material. Neither does it make any warranties as to the quality, viability, or purity (genetic or mechanical) of the material being furnished. The phytosanitary condition of the material is warranted only as described in the attached phytosanitary certificate. The recipient assumes full responsibility for complying with the recipient nation's quarantine or biosafety regulations and rules as to import or release of genetic material.
5. The recipients agree to furnish ICRISAT performance data collected during evaluations. Recipients should give due acknowledgement to ICRISAT in their reports for having provided the source material used for their research or to derive a process or product.

Place and date: Indentor's signature:

Name and institutional affiliation of the person requesting the genetic material:

Address:

Shipping address (if different from the above):.....

International Crops Research Institute for the Semi–Arid Tropics (Plant Quarantine Laboratory)

Request for Export of: seed/cuttings

Crop/Other material:	Center Project Number:
Year of harvest/Collection:	Season:
Field(s)	Location:
Number of samples:	Total weight:
Crop inspection by Pathologist/Entomologist/Virologist/ Nematologist:	
Description of material:	List
E-mail to: PQL@ICRISAT.EXCH.CGIAR.ORG	
Objective:	Budget Code:
Consignor:	
Program:	Discipline: Breeding
Consignee Details:	Sector NARS
Consignee:	
Address:	
Country:	Telephone:
E-mail:	Fax:
Dispatch instructions: Air mail	Nearest airport
Special instructions:	Preferred airline:
SMTA attached: No Reason:	Soft copy approach
Import permit no:	
(Attach original, along with additional declaration certificate if any)	
Date:	Chief Plant Quarantine Officer:

Seeds treated with chemicals are not acceptable.

Seed should be cleaned and pest free.

<p style="text-align: center;">GOVERNMENT OF INDIA MINISTRY OF AGRICULTURE DEPARTMENT OF AGRICULTURAL RESEARCH & EDUCATION INDIAN COUNCIL OF AGRICULTURAL RESEARCH NATIONAL BUREAU OF PLANT GENETIC RESOURCES</p> <p style="text-align: center;">PHYTOSANITARY CERTIFICATE</p>		PCS NO
		E.Reg. No/Date:
1. FROM : NATIONAL BUREAU OF PLANT GENETIC RESOURCES, Regional Station, Rajendra Nagar, Hyderabad-500 030 AP (INDIA)		2 To: THE PLANT PROTECTION ORGANISATION OF
DESCRIPTION OF CONSIGNMENT		
3. Name & address of exporter		4. Declared name & address of consignee
5. Declared means of conveyance		6. Place of origin
		7. Declared point of entry
8. Distinguishing marks		9. Number & description of packages
10. Name of produce/botanical name of plants		11. Quantity declared
This is to certify that the plants or plant products described above have been inspected according to appropriate procedures and are considered to be free from quarantine pests and practically free from other injurious pests and they are considered to conform with the current phytosanitary regulations of the importing country.		
DISINFESTATION AND/OR DISINFECTION TREATMENT		
12. Date		13. Treatment
14. Chemical (active ingredients)		15. Duration & temperature
16. Concentration		17. Additional information
18. Additional declaration		
19. Date	Stamp of organization	Name of authorized officer
20. Place of issue		(Signature & stamp of Officer – in-charge)
21. Code No.		

No financial liability with respect to this certificate shall attach to the Ministry of Agriculture (Department of Agriculture and Co-operation), Government of India or to any of its officers or representatives.

For internal distribution only

**ICRISAT
Germplasm Order Form**

I/We order the following material from genebank*

*Attach list if space is insufficient

Purpose: _____

Special requirements, if any: _____

Name of person: _____ Division/Project: _____ Experiment _____

Location: _____

For Project use For onward transfer

Address of consignee: _____

Authorized signature: _____ Date: _____

For internal use only

Ref. No. _____ Crop: _____ Date: _____

No. of accessions requested: _____ No. of accessions supplied: _____

Quantity: _____

Remarks: _____

Received above material

Authorized signature: _____ Date: _____

Feedback on germplasm received from ICRISAT Genebank
(for external use)

We received germplasm of the following crop(s) from ICRISAT Genebank:

Crop	Purpose requested	Comments on appropriateness of the material received
Sorghum		
Pearl millet		
Chickpea		
Pigeonpea		
Groundnut		
Small Millets		

- The samples were received in satisfactory condition.
- The samples were received in unsatisfactory condition. The unsatisfactory condition was due to:
- Inferior packaging Poor germinability Other reasons
(please specify below)

The following samples performed well under our environmental conditions/contributed significantly to our research objective.

ICRISAT Accession No.	Requester's identification number	Special characters observed

General advice or comment to improve services (please use additional sheet if necessary):

Name of respondent:

Address:

Signature:

Date:

Feedback on germplasm received from genebank (for internal use)

Germplasm evaluation- Entomologists/Pathologists/ Physiologists/Others*	Number of accessions received	No. of accessions identified with specific traits
Traits screened Pests Diseases Drought Salinity Agronomic traits Nutritional traits Any other traits		
Breeders/Molecular Biologists		
Genetic diversity studies Germplasm accessions used as parents Crosses made Populations developed Breeding lines developed Varieties released by NARS partners Agronomic traits Nutritional traits Any other traits		

*Data available. Please send a soft copy to Head, Genetic Resources Unit (h.upadhyaya@cgiar.org)

Name of the scientist:

Signature:

Date:

Section 7

Germplasm Utilization

Although there is an increase in the number of germplasm accessions in genebanks, there is no corresponding increase in their use by the crop improvement scientists, indicating that the collections were not being used to their full potential. Thus, a very large gap exists between availability and actual utilization of the materials. For example, very few of the 15,445 groundnut and 20,267 chickpea accessions conserved in the genebank have been utilized in cultivar development of these two crops at ICRISAT. Similarly, in the national programs, the number of germplasm lines used in breeding programs is very limited. Not only is the limited use of germplasm a worrisome issue, but also the large-scale deployment of a very few genotypes complicates the whole situation even more. Extensive use of fewer and closely related parents in crop improvement is contrary to the purpose of collecting a 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 during 1970 (resulting in huge economic loss) and late blight of potato (that resulted in the famine in Europe during 1840s) due to a narrow genetic base of crop cultivars looms large even today.

Reasons for low use of germplasm are:

- Difficulty of crop improvement in handling large collections,
- Lack of information on a large number of accessions, particularly for traits of economic importance, which display large genotype × environment interactions and require multilocation and replicated evaluation,
- Limited capacity of breeding programs to absorb new material,
- Restricted access to the germplasm collections due to insufficient seed quantities, and
- Inadequate linkage between genebanks and the users of germplasm.

7A. Enhancing germplasm utilization

Utilization of germplasm can be enhanced by:

- Developing representative core and mini core collections to overcome the size related problems of collections,
- Identifying trait-specific germplasm for use in crop improvement programs,
- Multilocation evaluation of germplasm, including core and mini core collections, and organizing field days facilitating the selection of germplasm by crop improvement scientists (see section 10C),
- Developing trait-specific genebanks in case of cross pollinating crops to provide partially converted populations to the breeders, and
- Ease of accessibility to all accessions of the collection by the users of germplasm.

7B. Core collections

The establishment of *ex situ* germplasm collections has been the result of several decades of efforts to conserve plant biodiversity. As collections rapidly grew beyond easily-manageable sizes, the task of quantifying diversity became daunting. Also, with the increase in size of collections, the realization that they are little used by breeders also grew.

7B.1. Concept of core collection

Frankel (1984) proposed sampling of the collections to a manageable sample or 'core collection'. A core collection consists of a limited set of accessions derived from a germplasm collection, which would 'represent, with a minimum of repetitiveness, the genetic diversity of a crop species and its relatives'. The accessions excluded from the core collection are retained as the reserve collection. Due to its reduced size, the core collection can be studied extensively and the information derived can be used to guide more efficient utilization of the much larger reserve collection.

Basic elements of core collection concept are:

- the original collection is large in size in view of management or use, but has taxonomic integrity;
- the core collection from this large original collection has a small size;
- the core collection is a representative sample of the collection; and
- the core collection retains sufficient diversity.

7B.2. Steps in setting up the core collection:

- Defining the collection to be represented in the core collection, and assembling all relevant data on these accessions
- Deciding the size of the core collection
- Grouping of accessions into groups that reflect the major genetic and ecological categories within the entire collection
- Selecting the core entries – how many from each group and which ones
- Representativeness of the core collection (in terms of diversity and inclusiveness)
- Managing the core collection for supply to potential users.

Defining the core collection and assembling data on accessions

It is important to ascertain what collection is to be used for developing a core collection. The core collection should serve as many users and uses as possible, and it should be comprehensive. The passport data on taxonomy, geographical origin, and ecological adaptation for each accession in the collection should be assembled. Characterization data on morphological traits, genetic markers and evaluation data (if available) should be assembled. All the available information should be used to develop the most representative core collection.

Size of core collection

The first decision to make in setting up a core collection is regarding its size. The size of the core (number of core entries) may be set by the resources available. In general, 10% of the parent collection is accepted as core collection. This proportion, in theory, should retain more than 70% of the alleles in the parent collection. For a very large collection, a core of 10% might still amount to a very large number of accessions to deal with, and hence suggest 3,000 as an upper limit for the size of a core collection. The reduced collection size will also help in reducing expenses for the genebank management.

Grouping of accessions

The grouping of accessions into categories of genetic similarity or commonality among accessions and determining groups in the entire collection is one of the most crucial steps. The hierarchy of grouping begins with the groupings suggested by taxonomy (species, subspecies, races), followed by assigning accessions to major geographic groups, climatic or agroecological regions. The accessions from larger countries can be divided into ecological regions; and those from small and adjacent countries can be grouped together. Clustering could be done within the broad geographical group to sort accessions into clusters using hierarchical clustering methods. At ICRISAT, we have used Ward (1963) method for clustering. This method optimizes an objective function because it minimizes the sums of squares within groups and maximizes the sums of squares between the groups.

Selecting the core entries

The number of accessions in different groups is likely to vary greatly. The accessions allocated to a cluster will share genetic affinity. Once the decision on size of the core is taken, the decision on the number of accessions from each cluster will depend on the strategy to be adopted. The following three strategies have been suggested to decide on the number of accessions from each cluster:

- *Constant strategy (C)* – equal number of accessions are sampled from each cluster into the core irrespective of the total number of accessions in different groups.
- *Proportional strategy (P)* – a fixed proportion of each group is selected for inclusion into the core collection, so that the group is represented in proportion to its frequency in the entire collection.
- *Logarithmic strategy (L)* – The number of accessions included into core are in proportion to the logarithm of the number of accessions in that cluster.

Representativeness of core collection

It is important that the core collection is representative of the entire collection, and its diversity needs to be assessed while setting up the core collection. Various parametric and non-parametric statistical methods can be used to compare the adequacy of core as a representative sample of the entire collection in terms of means, variances, frequency distributions, etc, between core and entire collections.

Managing the core collection

Managing the accessions included in a core collection is important so that it truly becomes a point of entry to the proper exploitation of genetic resources for crop improvement. The core accessions may be multiplied, conserved, evaluated and kept ready for dispatch to the researchers on short notice.

7B.3. Types of core collections

It has been generalized that a core collection is about 10% of the parent collection. However, the spectrum of germplasm diversity and relevance of the proposed core collection could vary depending on its relevance. Accordingly, these could be of three types: Global core collection, Regional core collection and Demand driven core collection.

Global Core Collection: The international agricultural research centers (IARCs) working under the CGIAR have the wider responsibility to assist the researchers globally, and therefore conserve germplasm from all geographical regions and countries. A core collection developed from these global germplasm collections could be termed as *Global core collection*. Sets of seven core collections of ICRISAT mandate crops (chickpea, groundnut, pigeonpea, sorghum and pearl millet) and two small millets (finger millet and foxtail millet) have already been developed. These core collections represent >80% diversity of the respective entire collections.

Regional Core Collection: Ecological environment differs from location to location, and so will differ adaptation of the germplasm accessions. For example, groundnut is a crop of worldwide importance and cultivated in over 90 countries. Considering its importance in Asia, it was decided to develop a core collection based on accessions of Asian origin, presuming that all accessions will carry higher level of adaptation and provide better options to scientists in the Asia region.

Demand Driven Core Collection: Some research programs may have special focus on developing a collection of particular biological/market type cultivars such as large seeded kabuli chickpea, Valencia type groundnut, etc, since researchers will find a specific collection to be more useful. Scientists at New Mexico State University (NMSU), USA and ICRISAT have developed a core collection of Valencia market type groundnut having 77 accessions representing germplasm conserved at NMSU and facilitating better focus in research on this type of groundnut in New Mexico and West Texas regions. Similarly, a *Guinea* core collection of 293 accessions was selected from 3,907 accessions using data on nine quantitative traits at ICRISAT.

7B.4. Global core collections developed at ICRISAT (Table 7A.1)

Chickpea: From the collection of 16,991 accessions (as on June 2000), a core collection was developed using data on country of origin and 13 quantitative traits through clustering by Ward's method and 10% of the accessions or a minimum of one accession were randomly selected from each cluster to constitute a core collection of 1,956 accessions. The validity of core accessions was tested using standard statistical parameters and the core collection was found to be a good representative of the entire collection.

Groundnut: A core collection was developed from the collection of 14,310 accessions using data on taxonomic affiliation, geographical origin and 14 morphological traits. Similar to the case of chickpea, clusters were formed and 10% of the accessions were picked from each cluster to constitute a core collection of 1,704 accessions which was 11.9% of the parent collection. On testing the validity of core accessions, it was found to be adequate.

Pigeonpea: The pigeonpea core collection was constituted based on the data on geographical origin and 14 qualitative traits and comprised 1,290 accessions. The core accessions were derived from the entire collection of 12,153 pigeonpea accessions in the ICRISAT genebank representing 56 countries. The statistical estimates on core as well as the entire collection indicated that the core developed is a good representative of the parent collection.

Sorghum: To develop a sorghum core collection from 22,473 landraces in the ICRISAT genebank, accessions were stratified in four clusters based on four classes of photoperiod sensitivity: photoperiod insensitive, mildly sensitive, sensitive and highly sensitive. The landrace accessions were classified into 60 groups as results of the combinations of 15 basic and intermediate races and four photoperiod groups. From each group following a logarithm strategy of sampling, a core collection of 2,247 accessions was constituted.

Pearl millet: The pearl millet core collection was developed based on the data on geographical origin and 11 quantitative traits. This was derived from the entire collection of 16,063 representing 25 countries. The core collection contained 1,600 accessions, which is 10% of the entire collection. The estimates of various statistical parameters revealed that the core collection was a fairly good representative of the entire collection.

Finger millet: A core collection of finger millet was developed from 5,940 accessions held in the ICRISAT genebank. These accessions represented 23 countries. The core collection was constituted using the data on geographic origin and 14 quantitative traits and contained 622 accessions, which was 10.47% of the entire collection.

Foxtail millet: A core collection of foxtail millet was developed from 1,474 accessions held in ICRISAT genebank from 23 countries. The core collection was constituted using data on geographic origin and 12 qualitative traits and contained 155 accessions, which accounted for 10.52% of the entire collection.

7B.5. Augmenting the core collection

Core collections are dynamic and need updating/augmenting when new accessions or information becomes available. For example, a core collection of pearl millet (1,600 accessions) was developed in 1998 using available data for 11 agronomic traits on 16,063 accessions. Meanwhile, by the year 2007, a total of 4,717 germplasm accessions were assembled additionally and characterized. This necessitated augmenting the core collection. For this, a phenotypic distance matrix was created for 4,717 accessions by calculating differences between each pair of accessions for each of the 22 (10 morphological and 12 quantitative) traits. The diversity index was calculated by averaging all the differences in the phenotypic values for each trait divided by the respective range. This distance matrix

was subjected to hierarchical cluster algorithm of Ward (1963) at an R^2 (squared multiple correlation value) of 0.75. 10% of the accessions or a minimum of one from each cluster was randomly selected to form a representative sample of 4,717 accessions. Various statistical analyses indicated that the selected sample not only represented the 4,717 accessions, but when added to the core collection, the augmented core collection (2,094 accessions) represented the entire collection (20,844 accessions).

7C. Mini core collections

Most often, the germplasm collections at IARC genebanks are very large. Thus, the number of core accessions will be too high for meaningful replicated evaluations and evaluation at different locations. To overcome this, Upadhyaya and Ortiz (2001) postulated the concept of mini core collection. A mini core collection consists of 10% accessions of the core collection, and hence only 1% of the entire collection. This mini core collection still represents the diversity of the entire collection. In fact, constituting a mini core collection is a two-stage strategy. The first stage involves developing a representative core collection (about 10%) from the entire collection using available information on origin, characterization and evaluation data of the accessions. The second stage involves evaluation of the core collection for various morphological, agronomic, and grain quality traits; and then selecting a further core of about 10% accessions. At both the stages, standard clustering procedures are used to cluster groups of similar accessions. Following this strategy, mini core collections of chickpea, groundnut, pigeonpea and sorghum have been developed; and development of mini core of pearl millet and finger millet is in progress.

Global mini core collections developed at ICRISAT (Table 7A.1)

Chickpea: The chickpea core collection of 1,956 accessions was evaluated in the 1999-2000 post-rainy season at ICRISAT, Patancheru, India. The data was recorded on 16 quantitative and qualitative traits. A phenotypic distance matrix was created by calculating differences between each pair of accessions for each of 22 traits following Johns et al. (1997). The distance matrix was subjected to the hierarchical cluster algorithm of Ward (1963) and 28 clusters were formed. From each of the 28 clusters, 10% accessions were randomly selected, which resulted in the formation of global mini core collection of chickpea germplasm of 211 accessions, which was 10.8% of the core collection and 1.24% of the entire collection.

Groundnut: To constitute a mini core collection of groundnut, the core collection comprising 1,704 accessions was evaluated during the 1999 rainy season for 13 qualitative and 16 quantitative traits and in the 1999-2000 post-rainy season for 18 quantitative traits at ICRISAT, Patancheru, India. A phenotypic distance matrix was created by calculating differences between each pair of accessions for each trait following Johns et al. (1997). The distance matrix was subjected to the hierarchical cluster algorithm of Ward (1963) and 77 clusters were formed. From each cluster, 10% accessions were randomly selected, which resulted in the formation of global mini core collection of groundnut germplasm of 184 accessions, which was 10.8% of the core collection and 1.29% of the entire collection.

Pigeonpea: The core collection of 1,290 accessions was evaluated at ICRISAT, Patancheru, India for 18 qualitative and 16 quantitative traits. A phenotypic distance matrix was created by calculating differences between each pair of accessions for each of 34 traits following Johns et al. (1997). The distance matrix was subjected to the hierarchical cluster algorithm of Ward (1963) and 79 clusters were formed. From each cluster, 10% accessions were randomly selected, which resulted in the formation of global mini core collection of pigeonpea containing 146 accessions, which was 11.3% of the core collection or 1.20% of the entire collection.

Sorghum: To develop a mini core collection of sorghum, the core collection of 2,246 accessions was evaluated in 2004-05 post-rainy season (October-April) at ICRISAT, Patancheru, India, for 11 qualitative and 10 quantitative traits. As was the practice for the other crops, a phenotypic distance matrix of 2,246 accessions for each of the 21 traits was created. The distance matrix was subjected to the hierarchical cluster algorithm of Ward (1963) and 21 clusters were formed. From each cluster, 10% accessions or a minimum of one were randomly selected, which resulted in the formation of global mini core collection of sorghum, which had 242 accessions. The mini core strength was 11.3% of the core collection or 1.20% of the entire collection.

Table 7A.1. Core and mini core collections of ICRISAT mandate crops.

Crop	Number of accessions used	Number of traits involved	Number of accessions in subset	Reference
Core collections				
Sorghum	33,100	7	3,475	Prasada Rao and Ramanatha Rao 1995
	22,473	2 ¹	2,246 ²	Grenier et al. 2001a, b
Pearl millet	16,063	11	1,600	Bhattacharjee et al. 2007
	20,766	12	2,094	Upadhyaya et al. 2009a
Chickpea	16,991	13	1,956	Upadhyaya et al. 2001a
Pigeonpea	12,153	14	1,290	Reddy et al. 2005
Groundnut	14,310	14	1,704	Upadhyaya et al. 2003
Groundnut (Asia)	4,738	15	504	Upadhyaya et al. 2001c
Finger millet	5,940	14	622	Upadhyaya et al. 2006c
Foxtail millet	1,474	12	155	Upadhyaya et al. 2008
Mini core collections				
Sorghum	2,246 ²	21	242	Upadhyaya et al. 2009b
Pearl millet	2,094	18	238	ICRISAT unpublished data
Chickpea	1,956	22	211	Upadhyaya and Ortiz 2001b
Pigeonpea	1,290	34	146	Upadhyaya et al. 2006d
Groundnut	1,704	31	184	Upadhyaya et al. 2002

¹ Photoperiod response as measured by flowering and plant height was used to group the accessions and 19 traits used to validate core.

² One accession has been denotified, therefore, the core is of 2,246 accessions.

7D. Identification of new sources

The core and mini core collections of various crops were evaluated to identify trait-specific diverse parents. Due to the reduced size, the core and the mini core sets have been evaluated and characterized precisely and useful trait-specific accessions have been identified for use in breeding programs to develop cultivars with a broad genetic base.

- *Drought tolerance*: 18 new sources of drought tolerance have been identified in groundnut and chickpea. These new sources are similar or better than the known sources for drought resistance, and are superior or similar for the agronomic traits.
- *Salinity tolerance*: 12 tolerant sources in chickpea and 16 in pigeonpea were identified.
- *Diseases resistance in chickpea*: 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.
- *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 in groundnut. 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 23% more yield.
- *Productivity traits in groundnut, chickpea and pigeonpea*: A number of high-yielding sources from the Asia region core collection in different botanical varieties (20 Spanish, 15 Valencia and 25 Virginia). Similarly, high-yielding and diverse sources have been identified in chickpea and pigeonpea.
- *Large-seeded kabuli chickpea*: Sixteen diverse germplasm lines, which have 100-seed weight up to 55 g compared to 20 g of the popular Indian cultivar L 550 have been identified. Scientists at Indian Institute of Pulses Research, Kanpur, India have identified 12 accessions for large scale evaluation and five accessions for breeding large seeded kabuli cultivars.
- *Agronomic traits in Thailand and China*: Thai scientists have identified five 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.

7E. Composite collections

The revolution in molecular biology, bioinformatics and information technology has provided the scientific community with tremendous opportunities to address some of the world's most serious agricultural and food security issues. ICRISAT in collaboration with the Generation Challenge Program (GCP) on "Unlocking Genetic Diversity in Crops for the Resource-Poor (www.generationcp.org)" has constituted composite collections of chickpea, sorghum, groundnut, pigeonpea, finger millet and foxtail millet that encompass the crop diversity. Phenotypic and genotypic characterization of these sets will provide opportunities and scope of identifying useful and unique germplasm resources for utilization in crop improvement. The composite collections have been genotyped using SSR markers to study genetic diversity, population structure and select a reference set of 200-400 most diverse accessions for research use (see section 10B).

Section 8

Monitoring

The viability of seeds stored in the genebank decrease slowly during storage. Removal of seeds for distribution and germination testing results in a decrease of seed quantity over time. Hence, the genebank accessions should be continuously monitored both for viability and seed quantity during storage to avoid excessive deterioration or reduction in seed quantity.

8A. Monitoring seed viability

- Monitor the viability of seeds at regular intervals.
- Monitoring interval depends on the species, seed viability at the beginning of storage or in the previous test, and conditions of storage, as shown below:

Germination (%)	Monitoring interval (years)			
	Active collection (4°C)		Base collection (-20°C)	
	Sorghum, millets, chickpea and pigeonpea	Groundnut	Sorghum, millets, chickpea and pigeonpea	Groundnut
<85	5	3	10	5
85-95	8	5	15	8
>95	10	8	20	10

- *Active collections* of sorghum, pearl millet, chickpea and pigeonpea and *base collections* of groundnut with initial viability >95% are monitored every 10 years. Accessions with the initial viability between 85% and 95% are monitored every eight years and those with <85% every five years.
- *Base collections* of non oily crops with >95% viability are monitored every 20 years, those with viability between 85 and 95% every 15 years, and accessions with viability <85% every 10 years.
- *Active collections* of groundnut with >95% viability can be monitored every 8 years, accessions with 85–95% viability every 5 years and those with <85% viability every 3 years.

Seed viability is monitored by conducting germination test on a fixed sample size as described in section 4D.

- Identify and prepare a list of the accessions requiring viability testing on a monthly basis, using genebank documentation system.
- Find the location of the containers in storage from inventory database.
- Remove the containers from storage and leave them overnight at room temperature.
- Open the container and draw a sample of seeds needed for the test and close the containers.

- Update the seed quantity in inventory database, deducting the quantity of seeds drawn.
- Conduct the germination tests as described in section 4D.
- Update the germination data in the inventory database.

8B. Monitoring seed quantity

- Seed quantity is best monitored through a computerized inventory.
- Record the weight of the seeds initially transferred to genebank.
- Record all subsequent seed withdrawals for distribution, regeneration and germination testing.
- Update seed stock immediately adjusting all seed withdrawals.



- ***The genebank documentation system can be designed to issue a warning as seed quantity reaches critical level. The genebank curator can stop further distribution of such accessions until they are regenerated.***
- ***The system can generate reports of accessions at regular intervals when the quantity or viability of seeds falls below minimum so that the genebank curator can program the accessions for regeneration.***

Section 9

Germplasm Regeneration

Regeneration is renewal of germplasm accessions by sowing and harvesting seeds, which will possess the same characteristics as the original population. Germplasm regeneration is the most critical operation in genebank management, because it involves risks to genetic integrity of germplasm accessions due to selection pressures, outcrossing and mechanical mixtures, among other factors. Seed regeneration should be undertaken only in the postrainy season. Due to the low ambient relative humidity and absence of rains in the postrainy season, incidences of diseases and pests are low, and consequently the quality of the seed produced is high. The short days during postrainy season also induce flowering in photosensitive germplasm accessions, enabling their seed production. ICRISAT genetic resources scientists have developed regeneration guidelines for sorghum, pearl millet, pigeonpea and finger millet in collaboration with Bioversity International (BI) (formerly IPGRI).

9A. Reasons for regeneration

Germplasm is regenerated for the following purposes:

Initial seed increase

In case of new collections or materials received as donations, the quantity of seeds received by the genebank is often insufficient for direct conservation. It is also possible that the seeds are of poor quality due to low viability or infections. All such materials need multiplication for the first time.

Long-term conservation

Seed accessions that are not in base collection as well as in backup collection.

Replenish seed stocks in active and base collections

Seed increase of accessions that have

- Low viability (percent germination <75%), identified during periodical monitoring, and
- Insufficient stocks (<50 g for cereals and <100 g in legumes) for either distribution or conservation.



- ***The FAO/IPGRI (now, Bioversity International) genebank standards recommend that the initial germination value should exceed 85% for most seeds and regeneration should be undertaken when viability falls below 85% of the initial value.***
- ***The FAO/IPGRI genebank standards recommend that regeneration should be undertaken when the number of seeds in base collection falls below the number required for at least three cycles of regeneration.***

- Active collections should preferably be regenerated from original seeds in the base collection. This is particularly important for outbreeding species such as pearl millet, pigeonpea and sorghum. However, using seeds from the active collection **for up to three regeneration cycles** before returning to original seeds (base collection) is also acceptable (Genebank Standards, FAO/IPGRI 1994).
- Base collections should normally be regenerated using the residual seed in that same sample.

Meet special requirement

Special requirement for seed multiplication may arise for accessions that are often requested or with special traits that breeders and researchers frequently use (high yielding, pest and disease resistant accessions, genetic stocks, etc) or accessions required for safety duplication and repatriation.



Newly acquired germplasm of foreign origin should be first grown in the Post-Entry Quarantine Isolation Area (PEQIA) under the supervision of the National Plant Quarantine Services.

Consider the following factors when regenerating germplasm accessions:

- Suitability of environment to minimize natural selection.
- Special requirements if any to break dormancy and stimulate germination (eg, scarification).
- Correct spacing for optimum seed set.
- Breeding system of the species and need for controlled pollination.

9B. Procedures for regeneration

- If possible, regenerate germplasm in ecological region of its origin. Alternatively, seek a location that does not selectively eliminate some genotypes in preference to others in a population.
- If no suitable site is found, seek collaboration with an institute that can provide a suitable site or regenerate in a controlled environment.
- Examine the biotic environment in the context of prior information about the plants and past experience. An inappropriate biotic environment due to its differential effect can be detrimental to plants, seed quality and genetic integrity of an accession.

Selection of accessions

- Regenerating accessions that have inadequate quality (low viability) should take priority over accessions with inadequate number of seeds.

- Regenerating accessions in base collections should take priority over accessions in active collections.

Preparation of field

- The regeneration plot should be as uniform as possible.
- The field should have good drainage.
- Consider the need for soil analysis and apply treatments appropriate for the crop and site (eg, fertilizers, soil amendments, irrigation, etc).
- If possible, solarize the field to eliminate soil borne pathogens.
- Prepare the regeneration field considering:
 - number of accessions to be regenerated,
 - number of plants per accession,
 - spacing between rows and between plants, and
 - mechanical accesses for weeding.
- Method of preparation depends on:
 - soil structure,
 - species to be sown or transplanted and its cultural requirement, and
 - where there is need for plant supports, eg, for climbers such as *Cajanus. albicans* or *Cajanus. volubilis*.

Solarization

Solarization refers to heating the soil by covering it with polyethylene sheets during hot summer to control soilborne diseases (Fig. 9B.1). It is particularly useful to control fusarium wilt in chickpea and pigeonpea, which is a major limitation during regeneration, as accessions/plants that do not have resistance get killed and eliminated. Solarization is conducted for at least 6 weeks during the hottest part of the year.

- Thoroughly cultivate the land and level it to minimize protrusions.
- Give 50 mm irrigation before laying of the polythene sheets.
- Use clear transparent polythene sheet, 25–100 µm thick.
- Insert two edges of the polythene sheet in the furrows, and bury the edges in the soil tightly.
- Place weights on the sheet to prevent flapping and tearing of polythene sheets in the wind.
- When planting, leave a buffer zone of at least 0.5 m around the edges of solarized area due to dilution of heat near edges.
- Do not allow irrigation water to flow in from other non-solarized areas and during crop growth.



Figure 9B.1. Covering the field with polythene sheet to solarize soil for chickpea germplasm regeneration at ICRISAT.

Cleanliness

- Identify the problem weeds, pests and pathogens, by inspection and prior experience.
- Consider reducing such problems during land preparation by application of appropriate treatment (such as weedicides).
- Keep the plots absolutely clean from alien seed and plants by
 - herbicide spray,
 - sterilizing soil,
 - ploughing to encourage weed germination followed by herbicide spraying, and
 - deep ploughing to kill emerging seedlings.
- Consider the risk of contamination with alien pollen and take appropriate measures to reduce it. Ensure that field preparation is appropriate for the chosen method of establishing plants, eg, ridges and flat beds.

Fertilizers

- Fertilizer requirement varies by crop and location of regeneration.
- If possible, arrange for soil testing and apply fertilizers as per the recommendation.

Weeds

- Arrange for 2-3 manual weedings depending on the weed populations.

Preparation of seed

- Dry, thresh and clean the seed if the samples are newly acquired.
- Those in storage,
 - identify the candidate accessions that require regeneration using the genebank documentation system,
 - remove the containers from the genebank the previous day.
 - draw seed samples keeping in mind the minimum sample size required for regeneration and current level of germination.

Ensure absolute accuracy in identification of accessions while drawing the seeds from the genebank, packaging and labeling the seed. Use the genebank documentation system to print labels.



If limited number of seeds are available, raise seedlings under carefully controlled conditions, transplant them into pots with sterilized soil and grow them in a screen house under close supervision.

Seed pretreatments

Specific pretreatment may be necessary to improve seed germination and establishment.

- Break dormancy for species or accessions (eg, stratification, scarification).
- Apply proprietary seed dressings to reduce soil borne disease and insect damage.
- Inoculate with appropriate symbionts (*Rhizobium* treatment for legumes).
- For wild species and accessions with limited seeds, pregerminate in controlled conditions, eg, incubator, agar, etc, and transplant the seedlings.

Sowing and crop management

Crop management for regeneration differs from normal commercial practices where interplant variation is not of primary consideration.

To maximize seed yield and avoid losses of alleles:

- Use 100 or more plants in cross-pollinating species and 25-50 plants in self pollinating species.
- Provide suitable conditions for growth to trigger abundant flowering.
- Eliminate competition by weeds.
- Ensure maximum survival.

Regular inspection of plants is mandatory to achieve these objectives.

Sowing

- Sow at an optimum time so that maturity and harvesting coincide with the most favorable weather conditions.
- If there is variation between accessions at flowering time, sort on maturity (eg, early and late) based on previous documentation and adjust the planting dates so that all accessions mature under uniform favorable environment.
- Sow in uniformly spaced rows and with uniform spacing between plants within rows.
- Avoid competition for light and nutrients by sowing at wide spacing.
- Ensure complete control of weeds, pathogens and pests.
- Ensure continued absence of alien plants in the vicinity throughout the regeneration cycle by hand weeding or intercultivation.

Irrigation

- Irrigate the field when necessary.
- Never subject the crop to water stress.
- Ensure adequate drainage and no water logging.

Flowering stage is sensitive in plant development. Care must be taken to avoid any stresses such as high temperature (see sowing date) and drought.

Verifying accession identity

- Accession identity should be verified while the plants are growing by comparing:
 - morphological data in documentation system, or
 - reference material such as original herbarium specimens or seed.
- Roguing must be undertaken with caution and only when it is absolutely clear that the rogue plants are genuine mixtures.

Unless the species is an obligate inbreeder, appropriate pollination control should be implemented.

Elimination of alien pollen can be achieved through:

- Bagging selected inflorescence with pollen-proof or pollinator-proof cloth bags.
- Erecting temporary pollinator-proof cages over the plots (pigeonpea).

Pollination of male-sterile lines depends on the genetic control of male-sterility. In case of genetic male-sterility, pollen is collected manually from the maintainer line and applied to the stigmas of the male sterile line.

Harvesting and post-harvest management

- Harvest at optimum maturity:
 - when maximum number of seeds are ripe,

- seeds become tolerant to desiccation,
- before deterioration sets in, and
- before natural dispersal occurs, eg, through shattering.
- Stagger the harvest if there are differences in maturity of the accessions.
- Harvest individual plants within an accession when there are differences in flowering and maturity between plants.
- Mix equal proportion of seeds from different mother plants.
- Bags to hold harvested seeds or heads should be made of porous material enabling good air circulation for drying.
- Options for harvesting depend on crop:
 - harvest plants individually, preferably by hand. If machine harvested, use custom-built machinery because commercial machinery cannot be cleaned adequately between regeneration plots.
 - harvest infructescences individually by hand. If bags are used for controlling pollination, they can be left in place until harvest. However, this procedure requires caution in relation to infestations of pathogens and pests inside the bags.
- Initiate seed drying immediately after harvesting to prevent seed deterioration.
- If seeds cannot be processed quickly, they should be placed in a temporary holding area under controlled environment (eg, short-term storage, at 20°C and 30–40% RH).

Seed drying and processing

- Drying should be in two stages:
 - initial drying to reduce the moisture content low enough for effective threshing without damaging the seed, and
 - final drying for conservation in genebank (refer to Section 4C for more details).

Initial drying

Generally refers to drying of plants, panicles, pods, etc.

Options for initial drying include:

- Outside in shade, if the climate is suitable,
 - requires additional control measures against birds, insects and dew,
- Passive drying in a room with good ventilation and air circulation,
 - not feasible in hot and humid climates of moist tropics,
- Active drying under forced ventilation.

Threshing and cleaning

- Threshing should be done at optimum moisture (<15%) to avoid damage to seeds.
- Seeds may be threshed preferably by hand.
- Use purpose-built equipment that can be cleaned adequately between accessions.

Final drying

The moisture content to which seeds should be dried depends on species, seed characteristics and intended storage duration (medium-term or long-term). Drying to low moisture content improves longevity of some species, while it can damage other species. Seeds dried to low moisture content can be brittle, and therefore, should be handled carefully.

Options for final drying include:

- Drying in artificially dehumidified conditions,
 - with self-indicating silica gel, which is cheaper and least expensive, or
 - in controlled environment of seed-drying cabinet or room.

Dry the seeds to recommended moisture levels depending on storage requirements using methods described in Section 4C.

Seed health

To ensure the production and conservation of high quality seeds with maximum potential longevity, organize:

- Periodic field inspection by pathologists and virologists during the growing season, and
- Seed health tests of representative sample of the harvested seeds.

Initial viability testing

Test the germination of the seeds after drying and before packing them for storage following methods described in Section 4D.

For species with dormancy, apply appropriate dormancy breaking treatments when testing.

Seed packing and storage

Dried seed with adequate germination should be immediately packed for storage using methods described in Section 4F.

Check with reference sample for genetic integrity of regenerated sample before packing and transfer to storage room.

9C. Sorghum regeneration

Season

Regeneration of sorghum germplasm is undertaken in postrainy season. All tropical photoperiod sensitive materials flower during the postrainy season, facilitating seed production.

Sowing time

Sorghum is sown for regeneration between 15 September and 15 October at Patancheru, India.

Field

Vertisols (black soil) are used for seed regeneration.

- Select fields in which sorghum was not grown in the previous two years.
- Prepare the land to a fine tilth by deep ploughing followed by 3–4 harrowings.
- Level the field and make ridges spaced 75 cm apart.
- Field should have good drainage. It should be free from weeds at the time of sowing.

Fertilization

Apply diammonium phosphate @ 100 kg ha⁻¹ as basal dose, and 100 kg ha⁻¹ urea as top dressing thirty days after sowing.

Field layout

Mark the field into tiers of 9 m, leaving 1-m walking space (alley-way) between tiers. Assign plot numbers in a serpentine pattern.

Sowing method

Use one row of 9 m for regenerating each accession. Use the four-cone planter for seeding. It requires about 8 g of seeds per row.

Irrigation

Irrigate the field after sowing to save the crop. Do not allow the leaves to wilt at any stage. Ensure enough moisture in soil at the time of flowering. See that the field has proper drainage, as water logging damages the crop.

Labeling

Label each row with a tag fastened to a stake about knee high. The tags should be of strong paper to withstand weathering.

Thinning

Thin when the seedlings are 2 weeks old. Maintain a plant-to-plant distance of 10 cm, which provides a stand of at least 90 plants per row.

Cultural practices

Interculture 2-3 times at early stages of crop growth to destroy weeds and to loosen the soil for good aeration. Earth up by pushing the soil at the base of plants to minimize lodging.

Weed management

Apply a pre-emergence herbicide. One or two hand weedings are required.

Pest and disease control

Follow normal cultural practices to control diseases and pests.

Pollination control

Genetic integrity of sorghum accessions is maintained by selfing.

- Trim flag leaves of emerging panicles.
- Cover the panicles in paper bags marked with the Julian date.
- Staple or put a paper clip holding the corners together so that the bag will not be blown off the panicle.
- Remove the bags after 21 days (ie, at dough stage) and clasp them around the peduncles to identify selfed panicles when harvesting (Fig. 9C.1).
- Employ bird scarer since birds easily damage the exposed panicles during daytime.



Figure 9C.1. Clasp paper bags around peduncles to identify selfed panicles.

Verification of accession identity

- Eliminate off-types and plants growing off-row.
- Verify accession identity as the plants grow by comparing the following key traits in existing characterization data:
 - panicle exertion,
 - panicle compactness and shape,
 - glume color,
 - glume covering (race), and
 - grain color

Rogue the plants that are genuine mixtures.

Harvesting

Seed maturity can be identified by black layer formation on seeds. Optimum time to harvest seed with maximum longevity is **7 weeks after anthesis**. Harvesting and threshing are done manually. Seeds from **at least 50 selfed plants** are bulked to maintain the accession.

- Cut the panicles (select only selfed panicles - one from each plant, identified by clipped flag leaf and selfing bag clasped around peduncles) just below the base with a secateur.
- Collect the panicles from each row (accession) into a gunny bag (45×30 cm) labeled both within and outside with the accession number and row number.

Use tear-off tags for labeling. Label inside accompanies the sample when it is threshed and cleaned and the label outside helps in sorting of the samples.

- Dry the panicles in shade for a week until the seed moisture content is reduced to 12%.
- Thresh individual panicles, and clean the seeds of debris by winnowing.
- Take equal quantity of seeds from each panicle (plant) and bulk them together to reconstitute the accession.
- Prevent spill over and contamination of accessions during threshing and subsequent handling.
- Move the seeds to short-term storage area for further drying and storage.

Seed health

- Coordinate periodic field inspection by pathologists and virologists during the growing season.
- Send a representative sample of the harvest for standard seed health testing.
- Process the material for storage if the infection level is within allowable limits.
- Materials with high infection are enlisted for next multiplication.

Wild species

- Grow the wild species in a field genebank to avoid possible outcrossing of germplasm with related species and avoid introduction of new.
- Maintain the perennial wild species that do not produce seeds as living plants in the field genebank.
- Prepare plots of appropriate size depending on the species.
- Germinate the wild species in paper cups and transplant them at a distance of 50 cm.
- Follow all crop husbandry practices of cultivated sorghums.
- Cover the panicles in parchment paper bags before stigma emergence to prevent outcrossing weeds (Fig. 9C.2).
- Harvest the panicles individually as they mature, ie, before shattering.
- Collect the seeds from each plant into a labeled paper envelope.
- Dry the seeds under shade and clean them by gentle blowing or winnowing.
- Take equal quantity of seed from each plant and reconstitute the accession for further drying and subsequent storage.



Figure 9C.2. Bagging panicles of sorghum wild relatives during regeneration.

9D. Pearl millet regeneration

Season

Pearl millet regeneration is conducted in the postrainy season to facilitate flowering and seed production in photoperiod sensitive material.

Sowing time

Sow the seeds between 1 and 15 of November at Patancheru.

Field

- Alfisols (red soils) are best suited for seed multiplication.
- Choose a field that was not under millet cultivation in the previous two years to reduce risk of volunteer plants.
- The field should have good drainage.
- The field should be free from weeds at the time of sowing.
- Prepare the land to a fine tilth by deep ploughing followed by 3–4 harrowings.
- Level the field and make ridges spaced 75 cm apart.

Fertilization

Apply diammonium phosphate @ 150 kg ha⁻¹ as basal dressing and urea @ 100 kg ha⁻¹ as top dressing.

Field layout

Mark the field of 4-m tiers, leaving 1 m walking space (alley way) in between tiers. Assign plot/row numbers in a serpentine pattern.

Sowing method

Grow each accession in four rows, each of 4 m length. Sowing is done using a four-cone planter. About 3 g of seeds is used per row.

Irrigation

Irrigate the field after sowing, and when needed subsequently. Do not allow the leaves to wilt at any stage. Ensure sufficient moisture in soil at the time of flowering.

Labeling

Label each plot/row with a tag fastened to a stake about knee high. The tags should be of strong paper to withstand weathering.

Thinning

Thin 2 weeks after sowing to maintain a distance of 10 cm between plants within the row and to provide about 160 plants per accession.

Cultural practices

Intercultivate 2-3 times during early stages of crop growth and earth up once after intercultivation to minimize lodging.

Weed management

Apply a preemergence herbicide. One to two hand weedings are undertaken to keep the crop weed free.

Pest and disease control

Follow normal cultural practices to control diseases and pests.

Pollination control

Out-crossing in pearl millet germplasm is controlled by cluster bagging, selfing and sibbing. Landraces are maintained by cluster bagging, genetic stocks by selfing, and male sterile lines by sibbing (Fig. 9D.1).



Figure 9D.1. Field view of pearl millet germplasm regeneration.

Cluster bagging

- Cover one panicle each from two to four adjacent plants in a row with one parchment paper bag before stigma emergence (Fig. 9D.2).

- Staple corners together or use a paper clip, so that bags are not blown off the panicle.
- In cluster bagging, cross-pollination takes place among the diverse plants covered in one bag, thereby, reducing the inbreeding depression.

Selfing

- Cover individual panicles in parchment paper bags before stigma emergence.
- Mark the date of covering on the bag.

Sibbing

- Cover the individual panicles in parchment paper bags before stigma emergence. Staple or put a paper clip holding the corners together so that the bags are not blown off the panicle.
- As anthers begin to dehisce, remove the bags from panicles, collect the pollen into a common paper bag by gently tapping the panicles and then cover the panicles immediately with bags after collecting the pollen.
- Remove the bags from each panicle with emerged stigmas and dust the collected pollen on to the stigmas and cover the panicles immediately with paper bags.
- Mark the date of pollination on the bags.
- Continue the process of pollen collection and dusting for 4–5 days in each accession, depending on panicle length and flowering duration.
- Self the plants that flower very early by covering the panicles in parchment paper bags. If the plants flower very late, pollinate them with pollen collected from tillers of the early flowering plants. If no tillers are available, self the late flowering plants too.



Figure 9D.2. Cluster bagging method of pollination control in pearl millet.

Ensure that all plants within the accession are either sibbed or selfed.

- Remove the bags two weeks after flowering (at dough stage) and clasp them around the panicles to identify sibbed panicles while harvesting.

Verification of accession identity

- Eliminate off-types and plants growing off-row.
- Verify accession identity as the plants grow by comparing the following key traits from characterization data:
 - panicle shape,
 - seed shape, and
 - seed color.
- Rogue the plants that are genuine mixtures.

Harvesting

- The optimum time to harvest seeds with maximum quality is 5 to 6 weeks after anthesis.
- Cut the bagged or selfed panicles just below the base — one from each plant, from **at least 120 plants** per accession.
- Collect the panicles within the row (accession) into a gunny bag labeled both within and outside using tear-off tags.
- Dry the panicles under shade for about a week to reduce the moisture content to about 12%.
- Thresh the panicles individually by gently beating with sticks.
- Clean the seed by winnowing.
- Take equal quantity of seeds from each plant to reconstitute the accession.
- Prevent seed mixtures during threshing and seed handling.

Seed health

- Coordinate periodic field inspection by pathologists and virologists during the growing season.
- Send a representative sample of the harvested seed for health testing.
- Process the material for storage if the level of infection is within limits.
- Materials with heavy infection are enlisted for next multiplication.

Wild species

- Maintain the perennial species that do not produce or produce few seeds, as living plants in the field genebank.
- Regenerate the wild species in the field genebank to avoid possible outcrossing of germplasm with related species and the introduction of new weedy species (Fig. 9D.3 and 9D.4).
- Prepare plots of appropriate size depending on the species.
- Germinate the wild species in paper cups and transplant them at a distance of 50 cm.



Figure 9D.3. Maintenance of perennial, non seed producing *Pennisetum* species at ICRISAT, Patancheru.



Figure 9D.4. *Pennisetum polystachion* grown for regeneration and characterization at ICRISAT, Patancheru.

- Cover the panicles in parchment paper bags before stigma emergence to prevent out-crossing and to prevent seed loss due to shattering.
- Harvest the panicles individually, 5-6 weeks after anthesis.
- Separate the seeds by crushing the florets between hands.
- Clean the seeds and take equal quantity of seed from each plant to reconstitute the accession.
- Prune the perennial and rhizomatous species up to 30 cm from the ground level during the rainy season to avoid mixing with adjacent accessions.

9E. Chickpea regeneration

Season

Postrainy season is best suited to chickpea.

Sowing time

Sow the seeds in the middle of October at ICRISAT, Patancheru.

Field

- Chickpea regeneration is done in Vertisols (black soils).
- Select a field in which chickpea was not grown in the last three years.
- The field should have good drainage.
- The field should be free from weeds at the time of sowing.
- Prepare the land by deep ploughing followed by 2-3 harrowings.
- Level the field and make ridges spaced 60 cm apart.
- Solarize the field during summer to minimize soil borne fungi or select fields that are disease free.

Fertilization

Apply a basal dose of Diammonium Phosphate (DAP) @ 100 kg ha⁻¹.

Field layout

Mark the field into 4-m tiers, leaving a 1 m path in between.

Sowing method

Sowing is done by hand. Dibble 2-3 seeds at a distance of 10 cm on the ridge. Use two rows of 4 m, providing *at least 80 plants* for regenerating an accession.

Irrigation

Irrigate the field after sowing. Subsequently irrigate when necessary.

Labeling

Label each row with a tag fastened to a stake about knee high. The tags should be of strong paper to withstand weathering.

Thinning

Thinning is done 2 weeks after sowing to maintain a distance of 10 cm between plants within the row to provide about 80 plants per accession.

Weed management

Apply a preemergence herbicide. Intercultivation is done twice during early stages of crop growth. If required, hand weeding is undertaken at later stages.

Pest and disease control

Follow normal cultural practices to control diseases and pests.

Verification of accession identity

- Eliminate off-types and plants growing off-row.
- Verify accession identity by comparing the following traits in characterization data:
 - growth habit,
 - flower color,
 - seed color, and
 - seed shape.
- Rogue the plants that are genuine mixtures.

Harvesting

- Harvest when the pods are dry. Dryness can be judged by rattling sound of pods when shaken. Older leaves become yellow and drop indicating maturity. Harvesting is done by hand.
- Hold the stem at the base and pull out the plants from the soil.
- Tie the uprooted plants from a row into small bundles and label them with accession number and field plot number.
- Thresh the pods from individual plants and collect the seeds into paper packets.
- Ensure that spillover and seed mixing do not occur during threshing.
- If limited number of pods are available, separate the seeds manually.
- Clean the seeds of debris.
- Take equal quantity of seeds from each plant and place them in muslin cloth bags labeled within and outside with tear-off tags.
- Move the bags into temporary storage area for further drying.

Seed health

- Coordinate periodic field inspection by pathologists and virologists during the growing season.
- Send a representative sample of the harvested seed for health testing.
- Process the material for storage, if the level of infection is within allowable limits.
- Enlist the materials with heavy infection for next multiplication.

Wild species

Chickpea wild relatives are regenerated in a glasshouse (Fig. 9E.1). Raise seedlings in small pots and then transfer them to big pots or to the field. Pasteurize the soil mixture to protect plants from soil borne diseases such as wilt and collar rot.



Figure 9E.1. Regeneration of chickpea wild relatives in plastic pots kept in a glasshouse.

Raising seedlings

- Fill small pots (earthen or plastic pots, 10×10 cm with a hole at the bottom) with the pasteurized mixture of 3:1 soil and farmyard manure mixture.
- Scarify the seeds by making a small cut to the seed coat to improve water absorption and germination.
- Dress the seeds with Benlate®.
- Put two seeds in each pot at about 2 cm depth.
- Water the pots every day using rose cans.

Transplanting

- Transplanting should be done in the evening. Transplant seedlings when they have 3-4 leaves or are 2-5 cm in height. Do not water the small pot the day before transplanting.

- Use big pots (size 30×30 cm) with a hole at the bottom for transplanting. Use at least 5-10 pots for each accession.
- Cover the hole with a piece of rubble and fill the pot with a pasteurized mixture of 3:1 soil and farmyard manure mixture.
- Turn the small pot upside down holding both sides of the plant with your fingers.
- Tap gently until the seedling with all the soil comes out into your palm.
- Fix the seedling in the desired pot or field and water it with a rose can.
- Keep the new pots in shade for 2 days, providing optimum moisture. If transplanted in the field, arrange shade for 2 days.
- Collect the ripe pods from each plant within the row into paper envelopes before they shatter.
- Dry the pods in shade and thresh them by hand.
- Mix equal quantity of seeds from each plant to reconstitute the accession.

9F. Pigeonpea regeneration

Season

Pigeonpea is a long duration crop and grown for regeneration during rainy season. Late sowing in July/August results in reduced plant height, and thus allows whole plants to be conveniently covered using either muslin cloth bags or insect proof cages, to control out-crossing.

Sowing time

Accessions are sown during the last week of July at Patancheru, India.

Field

- Pigeonpea regeneration is done in Vertisols (black soils) as well as in Alfisols (red soils).
- Select a field in which pigeonpea was not grown in the last three years.
- The field should have good drainage.
- The field should be free from weeds at the time of sowing.
- Prepare the land by deep ploughing followed by 2-3 harrowings.
- Level the field and make ridges spaced 75 cm apart.

Fertilization

Apply a basal dose of Diammonium Phosphate (DAP) @ 100 kg ha⁻¹.

Field layout

Mark the field into tiers of 9 m with 1 m path between tiers. Use one row of 9 m, providing a minimum of 70 plants for regenerating each accession.

Sowing method

Sowing is done by hand. Dibble 3-4 seeds per hill at a distance of 25 cm along the ridge, and cover with soil.

Irrigation

Irrigate the field after sowing (if soil moisture is not sufficient) and to save the crop subsequently.

Labeling

Label each row with a tag fastened to a stake about knee high. The tags should be of strong paper to withstand weathering.

Thinning

Reduce the plant stand by thinning after 15 days to two plants per hill with a distance of 25 cm between plants within the row providing a minimum of 70 plants per accession.

Cultural practices

Intercultivation is done twice during early stages of crop growth. Earthing up is done once after interculturing.

Weed management

Apply 1-2% Glycel as a preemergence herbicide. If required, hand weeding is undertaken.

Pest and disease control

Follow normal cultural practices to control diseases and pests.

Pollination control

- Pigeonpea is cross-pollinating (0-40%, depending on genotype and insect pollinator populations). Seed increase must preclude cross pollination.
- Cover the plants using muslin cloth bags before flowering (Fig. 9F.1) or grow them under insect proof cages (Fig. 9F.2).
- Spray thiodon (@ 2 mL⁻¹) before covering the plants with muslin cloth bags.
- Growing accessions under cages is cost-effective and allows harvest of more seed.



Figure 9F.1. Pigeonpea plants bagged to avoid cross-pollination during regeneration at ICRISAT, Patancheru, India.



Figure 9F.2. Roguing in pigeonpea germplasm grown for regeneration under insect proof cages at ICRISAT, Patancheru, India.

Verification of accession identity

- Eliminate off-types and plants growing off-row.
- Verify accession identity as the plants grow by comparing the following traits in characterization data:
 - flowering pattern,
 - flower color,
 - pod color, and
 - primary seed color.
- Rogue the plants that are genuine mixtures.

Harvesting

Harvesting is done when the pods become dry. Dryness can be judged by rattling sound of pods when shaken. Hand pick the selfed pods (pods inside cages or muslin cloth bags) from each plant and place them in labeled paper bags. Keep the bags of a plot (accession) together inside a labelled jute sack (63×33 cm).

- Dry the pods under shade for 2-3 days to reduce the seed moisture content to about 12%.
- Thresh the pods and collect the seeds into paper packets.
- Ensure that spillover and seed mixing do not occur during threshing.
- If limited number of pods are available, separate out seeds manually.
- Clean the seeds of debris.
- Take equal quantity of seeds from each plant and put them in a muslin cloth bag labeled within and outside with tear-off tags.
- Move the bags into temporary storage area for further drying.

Seed health

- Coordinate periodic field inspection by pathologists and virologists during the growing season.
- Send a representative sample of the harvested seed for health testing.
- Process the material for storage if the level of infection is within allowable limits.
- Materials with infection beyond the allowable limit are enlisted for next multiplication.

Wild species

Pigeonpea wild relatives are regenerated in the field genebank (Fig. 9F.3). Raise seedlings in small pots and then transfer them to big pots or to the field. Pasteurize the soil mixture to protect plants from soil borne diseases such as wilt and collar rot.



Figure 9F.3. Wild relatives of pigeonpea grown for regeneration in field genebank at ICRISAT, Patancheru, India.

Raising seedlings

- Fill small plastic/paper cups or small pots (10×10 cm with a hole at the bottom) with pasteurized mixture of 3:1 soil and farmyard manure mixture.
- Scarify the seeds by making a small cut to the seed coat to improve water absorption and germination.
- Dress the seeds with Benlate®.
- Put two seeds in each pot at about 2 cm depth.
- Water the pots every day using rose cans.

Transplanting

- Transplant in the evening.
- Transplant seedlings when they have 3-4 leaves or are 2-5 cm in height. Do not water the plastic/paper cups or small pot the day before transplanting.
- Large pots (30×30 cm) filled with a pasteurized mixture of 3:1 soil and farmyard manure mixture are used for transplanting creeping herbs such as *C. platycarpus*, *C. scarabaeoides* and *Rhyncosia* species. Perennial shrubs and creepers such as *C. albicans*, *C. crassus*, *C. goensis*, *C. heynei* and *C. mollis* should be transplanted and grown in a field or botanical garden.
- Turn the plastic cup upside down holding the plant with your fingers.
- Tap gently until the seedling with all the soil comes out into the palm.
- Fix the seedling in the desired pot or field and water it with a rose can.
- Keep the new pots in the shade for 2 days, providing optimum moisture.
- If transplanted in the field, transplant in rows of 4 m length at a distance of 25 cm or more depending on growth habit, and arrange shade for 2 days.
- Use a sample size of 8-10 plants for each accession.
- Provide bamboo stakes to support climbers such as *C. albicans*, *C. crassus*, *C. goensis*, *C. heynei* and *C. mollis*.
- Collect the ripe pods from individual plants into paper envelopes before they shatter.
- Bulk equal quantity of seeds from each plant to reconstitute the accession.

9G. Groundnut regeneration

Heavy soils or wet conditions are not suited for groundnut seed multiplication because the seed matures below ground. Well-drained Alfisols (red soil) with good status of calcium are most suited. The field should not have been under groundnut cultivation in the previous 2 years.

Season

Groundnut is day-neutral, and seeds can be regenerated both during rainy and postrainy seasons (preferable).

Sowing time

During rainy season, groundnut is sown in June whereas during postrainy season, they are sown in November at Patancheru.

Field

- Groundnut regeneration is done in well drained Alfisols (red soils).
- Select a field in which groundnut was not grown in the last two years.
- Field should be free from weeds at the time of sowing.
- Prepare the land by deep ploughing followed by 2-3 harrowings.
- Level the field and make ridges spaced 75 cm apart.

Fertilization

Apply single super phosphate @ 375 kg ha⁻¹ as a basal dose and gypsum (calcium sulphate, dihydrate) @ 400 kg ha⁻¹ 40 days after sowing.

Field layout

Mark the field into tiers of 4 m with 1 m path between tiers. Use four rows of 4 m, providing at least 160 plants for regenerating each accession.

Sowing method

Sow 1-2 seeds with a spacing of 10 cm between plants.

Labeling

Label each plot/row with a tag fastened to a peg. The tags should be of strong paper to withstand weathering.

Irrigation

Irrigate the field after sowing. Give protective irrigation, as soon as wilting is noticed subsequently.

Thinning

Maintain 10 cm plant-to-plant distance in Spanish and Valencia types and 15 cm in *Hypogaea* bunch and runner-type accessions.

Weed management

Apply a preemergence herbicide. Intercultivation is done twice during early stages of crop growth. Hand weeding is undertaken if required at later stages.

Verification of accession identity

- Eliminate off-types and plants growing off-row.
- Verify accession identity as the plants grow by comparing characterization data on:
 - branching pattern,
 - leaflet shape,
 - flower color,
 - pod constriction, and
 - primary seed color.
- Rogue the plants that are genuine mixtures.

Harvesting

Check harvest-maturity by digging samples from below ground. Prominent symptoms of maturity are yellowing of leaves and dropping of old leaves. The pods become hard and tough with a dark tannin discoloration inside the shell. The testa develops color characteristic of genotype.

- Harvest when 75% of the pods are mature.
- Irrigate the field 1 day before harvesting (in postrainy season).

Pod moisture plays an important role in determining seed viability. Harvesting is done at seed moisture content of 30-40%.

Groundnut harvesting consists of two operations:

- Lifting the vines from the soil with pods intact, and
- Separating the pods from the vines.

Lifting of vines is done manually.

Pods of Spanish, Valencia and Virginia-bunch types are confined to the base of plant and lifting plants from soil brings out most of the pods. In Virginia-runner type, however, pod formation takes place all along the creeping branches. Therefore plants are lifted from the soil by digging with a spade.

Stripping pods from vines

- Leave the harvested plants to dry in the field with pods turned upwards in windrows for 2-3 days. Alternatively, tie the plants into small bundles, label, and dry them under shade.
- Strip the dry pods from the plant by hand and collect them into paper bags.
- Clean the pods of the soil and dry them further to about 8-9% moisture content by slow drying.
- Bulk equal number of pods from each plant to make up the accession.
- Shell the seeds manually for long-term storage.



The dryness of pods can be judged by the following tests:

- **Pods should give a rattling sound when shaken.**
- **When the kernel is pressed, it should easily split into two cotyledons.**
- **When the surface of the kernel is rubbed hard, a portion of the testa should come off.**

Seed health

- Coordinate periodic field inspection by pathologists and virologists during the growing season.
- Send a representative sample of the harvested seed for health testing.
- Process the material for storage if the level of infection is within allowable limits.
- Materials beyond the allowable limit of infestation are stored temporarily and enlisted for next multiplication.

Wild species

Seed propagated species

Use pots (earthen or plastic pots 38×28 cm with a hole at the bottom) or concrete rings (90 cm diameter × 83.3 cm height × 5.5 cm thick) to grow wild *Arachis* species (Fig. 9G.1).

- Cover the hole at the bottom of the pots with pieces of rubble.
- Fill the pot or concrete rings with 3 red soil :2 sand:1 farmyard manure mixture, pasteurized (3 cycles of 1 h each) at 82.2°C (180°F) and 5 psi.
- Apply a basal dose of urea and diammonium phosphate (25 g pot⁻¹) at a depth of 7.5 cm.



Figure 9G.1. Groundnut wild relative grown in cement ring in a special facility for regeneration (A) and harvested pods (B).

- Dress the seeds in a 2:3 mixture of Bavistin® and Thiram® and sow them at a depth of about 3.75 cm.
- Apply 2-3 drops of 0.2% Etherel (2-chloroethylphosphonic acid, 39%) solution (3 ml in 1 l) on seeds before covering them with soil.
- Top the soil in concrete rings with 5-7.5 cm sand.
- Water the pots after seeding and twice a week, subsequently.
- Apply Gypsum @ 10 gm pot⁻¹ 50 days after sowing.
- *Yellowing of foliage and formation of dark lining inside the shell indicate maturity.*
- Stop watering the pots 2 days prior to harvesting.
- Sift the soil through a sieve and strip the pods.
- Dry the pods in shade.
- Transfer them to a temporary holding room for further drying and processing.

Rhizomatous species

- Use rhizomes of 20 cm length, cut from mother plant.
- Soak the rhizomes in Bavistin® suspension (@ 3 g l⁻¹ water) for 5 min.
- Plant the rhizomes in a potting mixture consisting of 3 parts of red soil, 2 parts of sand and 1 part of farmyard manure.
- Plant the rhizomes 5 cm deep preferably in plastic or earthen pots, or on a raised nursery bed.
- Maintain the rhizomes in a greenhouse at 25 ± 2°C until they are established. If greenhouse facility is not available, maintain them in shade avoiding exposure to direct sunlight.



- ***It is important that the rhizomes are maintained under alternating dry and wet conditions until they are established by avoiding continuous watering.***
- ***It takes about one month for the rhizomes to be established after which they can be transferred to the field.***
- ***Transplantation should be done in the evening.***

9H. Small millets regeneration

Small millets are self pollinating. The field used for regeneration should not have grown the same crops in the previous 2 years.

- Apply diammonium phosphate @ 100 kg ha⁻¹ as a basal dose prior to sowing and urea @ 100 kg ha⁻¹ as top dressing.
- Prepare the field as fine tilth as the species will not tolerate a seedbed that is not properly compacted.
- Grow the crop along 4 m rows.

- Hand weeding is done when seedlings are about 5 cm high.
- Thin the plants so that they are 10 cm apart.
- Cultivars vary in their ability to resist shattering, so harvest before there is any great loss of seed.
- Harvest the panicles by hand.
- Dry the seed heads to about 12% under shade.
- Thresh the panicles by hand.
- Clean the seeds by winnowing.
- Bulk equal amount of seeds from each plant to make up the accession.

9I. Documentation on regeneration

Regeneration data includes information on grow-out conducted to restore viability or multiply seed stocks. The following descriptors are used to store the information on generation.

Field plot number: Plot number assigned in the field when regenerating the accession.

Date of sowing: Date on which the accession is sown.

Date of flowering: Date on which anthesis occurs in 50% of the plants in the plot.

Pollination method: Method of pollination used to preserve genetic integrity of the accession.

Date of harvest: Day, month and year on which the accession was harvested.

Plants harvested: Number of plants from which seed was harvested.

Seed moisture content at harvest (%): Seed moisture content at the time of harvest.

Method of drying: Methods used for drying seed samples are

- Ambient or natural drying.
- Controlled environment.
- Both above.

Method of threshing: Method used for threshing the seed samples.

Seed moisture content after drying (%): Seed moisture content after drying.

Seed quantity after drying (g): Quantity of seeds available for storage.

Section 10

Characterization and Preliminary Evaluation

Characterization and preliminary evaluation of germplasm are the prerequisites for utilization in crop improvement.

10A. Phenotypic characterization and evaluation

- Characterization involves recording characters, which are
 - highly heritable,
 - easily seen by the eye, and
 - are expressed in all environments.
- Preliminary evaluation consists of recording a limited number of additional agronomic traits considered to be desirable by users of the crop.

Follow the same sowing and cultural practices for the field grow-out, as described under regeneration (see section 9). Grow the accessions in 1-3 rows of 4 m each. Maintain the row to row distance at 60 cm (chickpea) or 75 cm (other crops) and plant-to-plant distance at 10 cm (50 cm in pigeonpea). Evaluate the accessions in an augmented block design. Plant standard check cultivars at every 10 or 20 accessions. Use the descriptors developed by ICRISAT and IBPGR (now Bioversity International) for characterization and preliminary evaluation (ICRISAT/IBPGR 1992a,b and 1993a,b; ICRISAT/IBPGR/ICARDA 1993).

10A.1. Descriptors for characterization of sorghum

Vegetative phase

Plant height (cm): Height of the main axis from ground to the top of inflorescence at 50% flowering. Mean of 5 randomly selected plants (Fig. 10A.1.1).

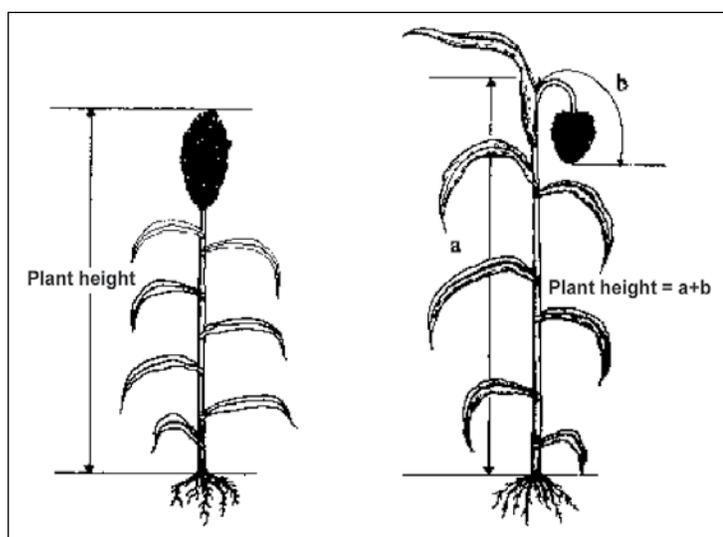


Figure 10A.1.1. Plant height in sorghum.

Plant pigmentation: Stem and plant pigmentation at maturity.

P Pigmented

T Tan

Basal tillers number: Number of basal tillers, main plant as 1.

Nodal tillers number: Presence or absence of nodal tillers.

P Present

A Absent

Midrib color: Color of the midrib.

W White

D Dull green

Y Yellow

B Brown

Reproductive phase

Days to flowering: Number of days from the day of first irrigation to the date when 50% of plants started flowering within an accession.

Panicle exertion: Length of peduncle from ligule flag leaf to base of inflorescence (Fig. 10A.1.2).

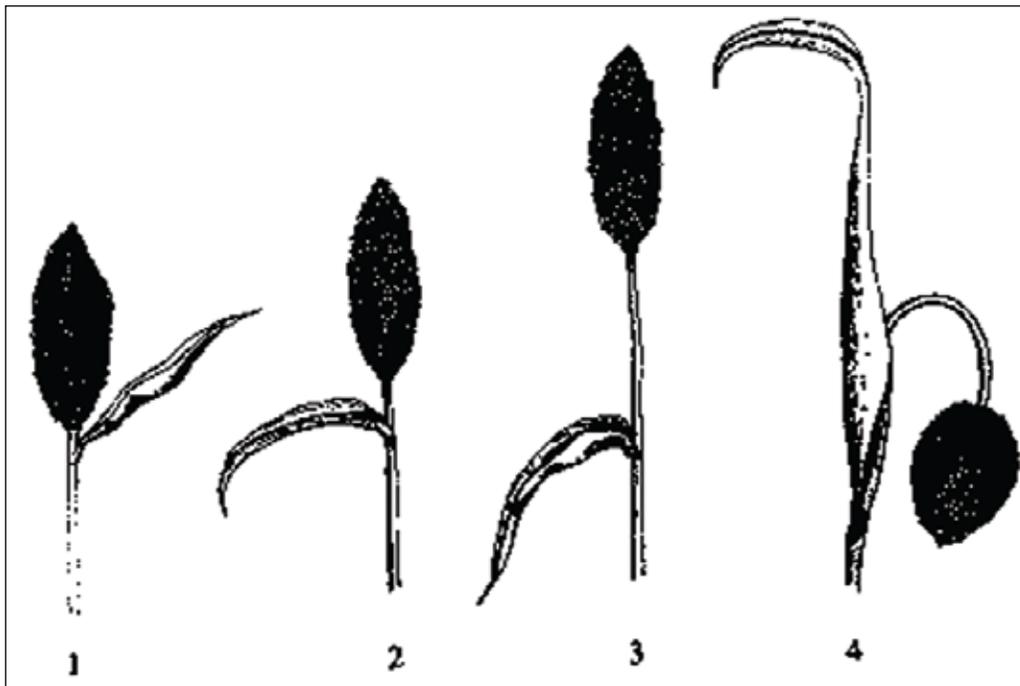


Figure 10A.1.2. Panicle exertion in sorghum.

- 1 Slightly exerted
- 2 Exserted
- 3 Well-exserted
- 4 Peduncle recurved

Panicle length (cm): Length of panicle from base to the tip. Mean from five representative plants.

Panicle width (cm): In natural position at the widest portion. Mean from five representative plants.

Panicle compactness and shape (Fig. 10A.1.3 and 1.4)

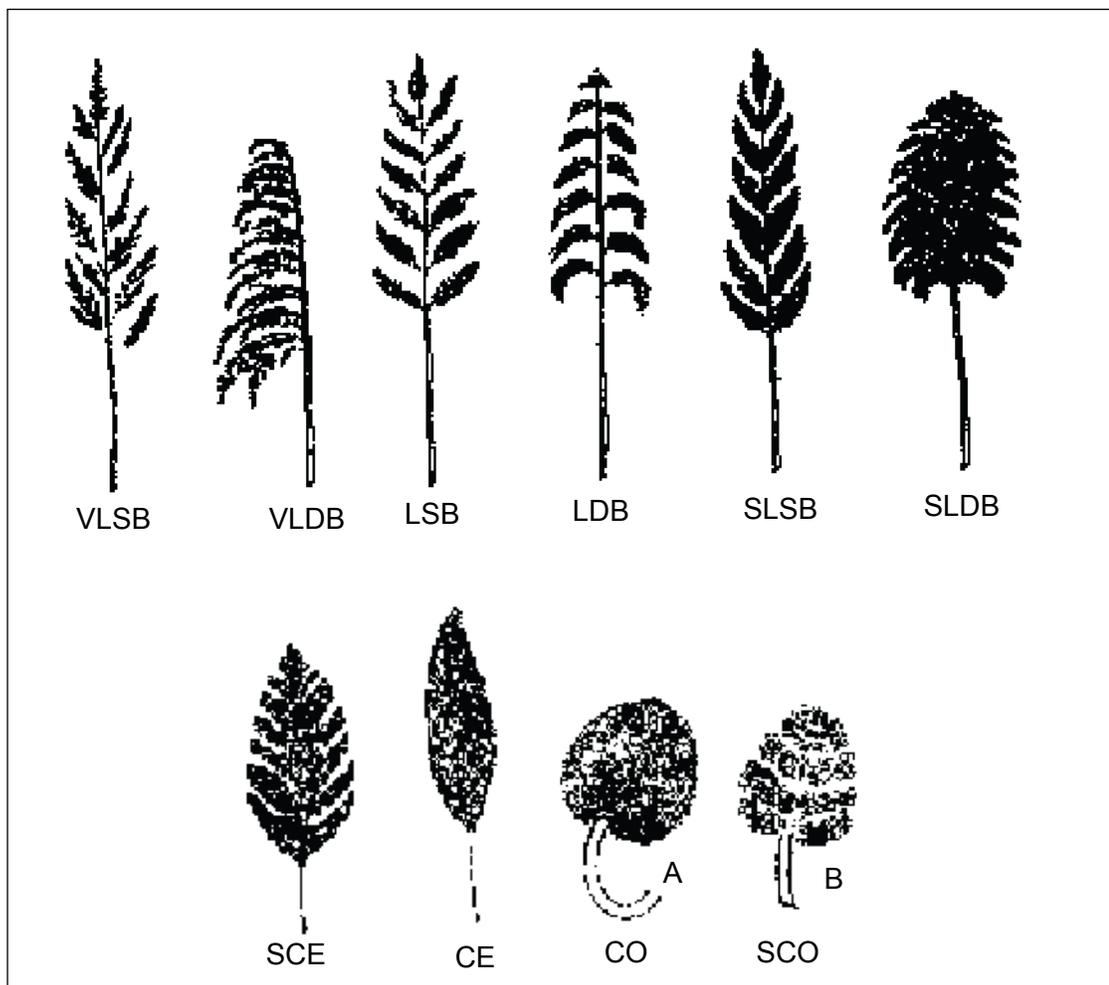


Figure 10A.1.3 Inflorescence compactness and shape in sorghum.

- VLSB Very loose stiff branches
- VLDB Very loose drooping branches
- LSB Loose stiff branches
- LDB Loose drooping branches

SLSB	Semi-loose stiff branches
SLDB	Semi-loose drooping branches
SCE	Semi-compact elliptic
CE	Compact elliptic
CO	Compact oval
SCO	Semi-compact oval

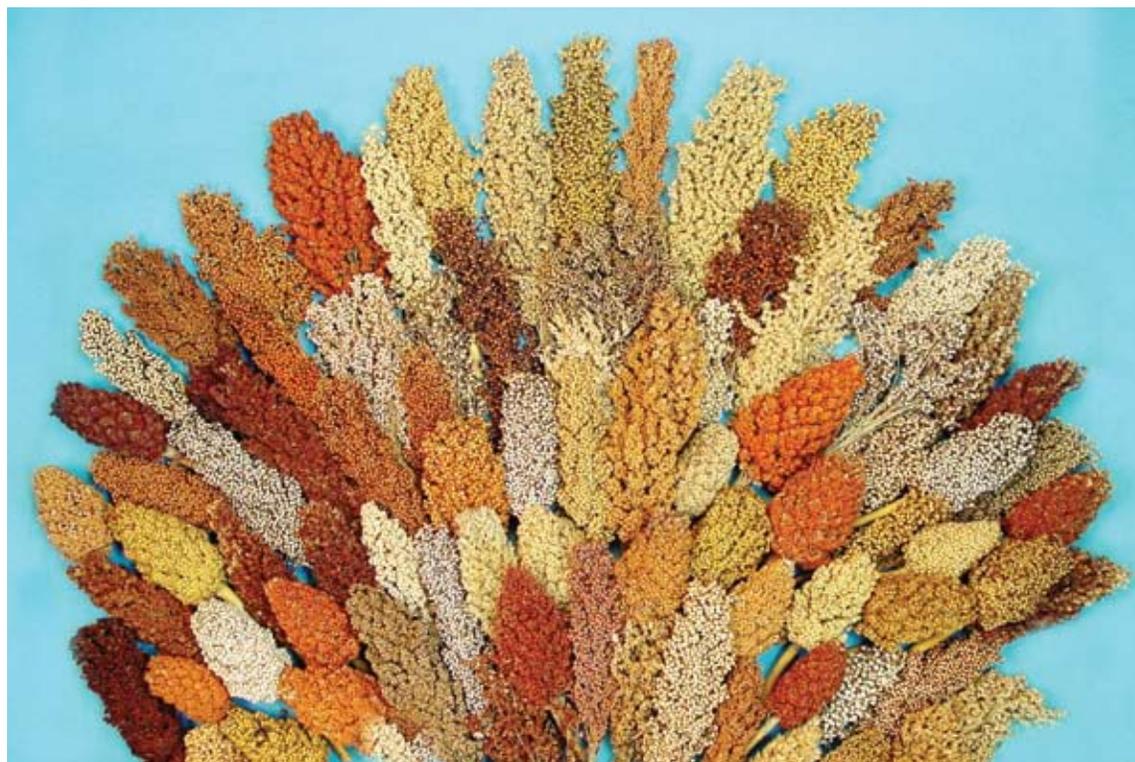


Figure 10A.1.4. Diversity for panicle traits in sorghum germplasm assembled at ICRISAT.

Glume color: Color of the seed covering structures.

W	White	R	Red
S	Straw	DR	Dark red
Y	Yellow	P	Purple
LB	Light brown	B	Black
B	Brown	G	Grey
RB	Reddish brown	PSB	Partly straw and brown
LR	Light red	PSP	Partly straw and purple

Glume covering: Extent of seed covered by glumes at maturity (Fig. 10A.1.5).

1	25% seed covered
2	50% seed covered

- 3 75% seed covered
- 4 Seed fully covered
- 5 Glumes longer than seed

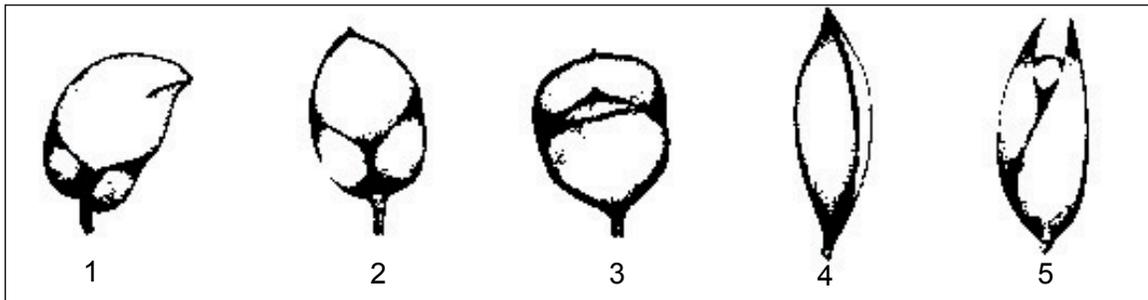


Figure 10A.1.5. Seed covering in sorghum.

Seed color: Color of freshly harvested seeds.

CW	Chalky white	RB	Reddish brown
W	White	LR	Light red
S	Straw	R	Red
Y	Yellow	G	Grey
LB	Light brown	P	Purple
B	Brown	WR	White and red mixed

Seed lustre: Shininess of seed.

L	Lustrous
NL	Nonlustrous

Seed sub-coat: Presence or absence of black layer below the testa.

P	Present
A	Absent

Seed size (mm): Width of the seed at the broadest point.

Seed weight (g): Weight of 100 seeds at 12% moisture content.

Endosperm texture: Nature of endosperm (Fig. 10A.1.6).

- 1 Completely corneous
- 2 Almost corneous
- 3 Partly corneous
- 4 Almost starchy
- 5 Completely starchy

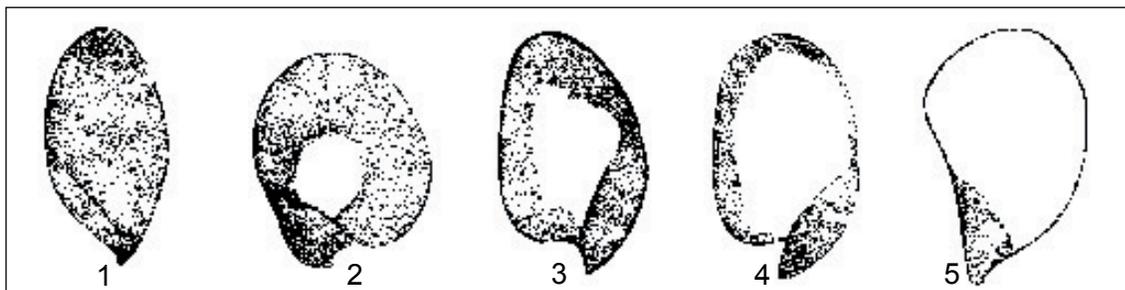


Figure 10A.1.6. Endosperm texture in sorghum seed.

Threshability: Ease with which seeds can be separated from the panicle.

FT Freely threshable

PT Partly threshable

DT Difficult to thresh

10A.2. Descriptors for characterization of pearl millet

Vegetative phase

Plant height (cm): Mean height of five plants measured from ground level to the tip of the panicle at dough stage (Fig.10A.2.1).

Productive tillers number: Number of tillers bearing panicles, counted at dough stage. Recorded as mean of five plants.

Nodal tillers: Visual score on 1–9 scale for number of nodal tillers at dough stage.

- 3 Few
- 5 Intermediate
- 7 Many

Total tillers number: Total number of tillers including main stem, counted at dough stage. Recorded as mean of five plants.

Photoperiod sensitivity: Visual score on 1–9 scale for sensitivity to photoperiod.

- 3 Insensitive
- 5 Partly sensitive
- 7 Highly sensitive



Figure 10A.2.1. Recording plant height in pearl millet germplasm.

Fodder yield potential: Visual score on 1-9 scale for green fodder yield potential considering tillering, leafiness and bulk at flowering.

- 3 Poor
- 5 Intermediate
- 7 Good

Reproductive phase

Days to 50% flowering: Number of days from first irrigation after sowing to when 50% of plants flower in the accession. Stigma emergence on the main panicle is considered as flowering.

Panicle exertion (cm): Distance between ligule of the flag leaf and the base of the panicle on main plant.

Panicle length (cm): Mean length of five panicles on main axis of five representative plants, measured at dough stage.

Panicle thickness (mm): Mean thickness of five panicles at widest portion on main tiller of five plants, measured at dough stage.

Panicle shape: Shape of panicle at dough stage (Fig. 10A.2.2 and 3).

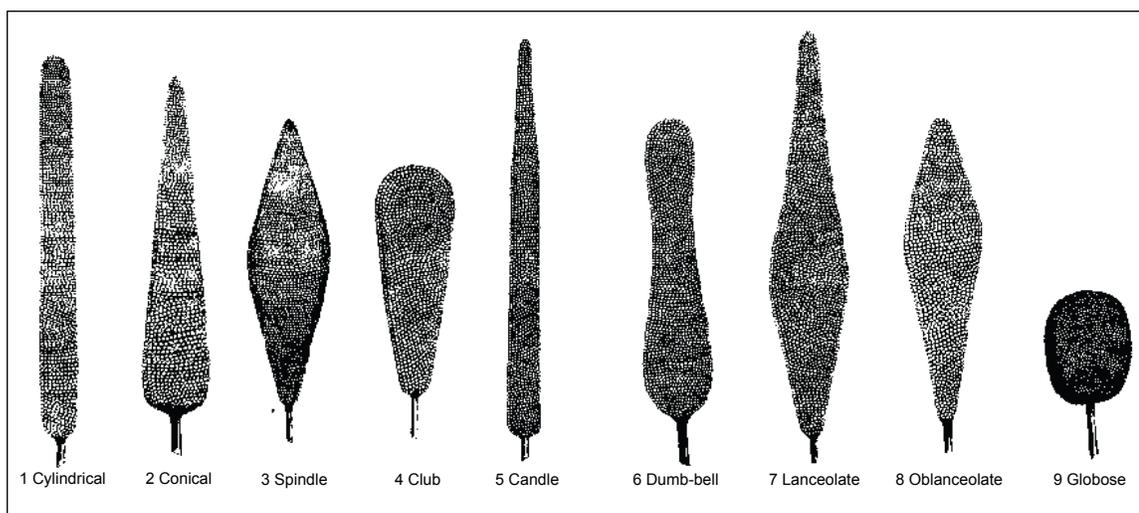


Figure 10A.2.2. Panicle shapes in pearl millet.



Figure 10A.2.3 Diversity for panicle traits in pearl millet germplasm.

Spikelet density: Density of spikelets, visually scored on 1–9 scale at maturity. Also referred to as compactness of panicle.

- 3 Loose
- 5 Intermediate
- 7 Compact

Synchrony of panicle maturity: Uniformity for maturity, visually scored on 1–9 scale at dough stage.

- 3 Non-synchronous
- 5 Intermediate
- 7 Synchronous

Bristle length: Length of bristles, visually scored on 1–9 scale at dough stage.

- 3 Short (bristles below the level of apex of the seed)
- 5 Medium (bristle length between 0 and 2 cm above the seed)
- 7 Long (bristles longer than 2 cm above the seed)

Seed color: Color of freshly harvested seeds recorded after threshing.

- | | |
|-------------|------------------------------|
| 1 Ivory | 6 Grey brown |
| 2 Cream | 7 Brown |
| 3 Yellow | 8 Purple |
| 4 Grey | 9 Purplish black |
| 5 Deep grey | 10 Mixture of white and grey |

Seed weight (g): Weight of 1,000 seeds drawn randomly from plot yield, at 12% moisture content.

Seed shape: Shape of seed after drying (Fig. 10A.2.4).

Seed yield potential: Seed yield potential of the accession, visually scored on 1 – 9 scale considering panicle number, size and density.

- 3 Low
- 5 Intermediate
- 7 High

Endosperm texture: Texture of endosperm visually scored on 1–9 scale.

- 3 Mostly corneous
- 5 Partly corneous
- 7 Mostly starchy

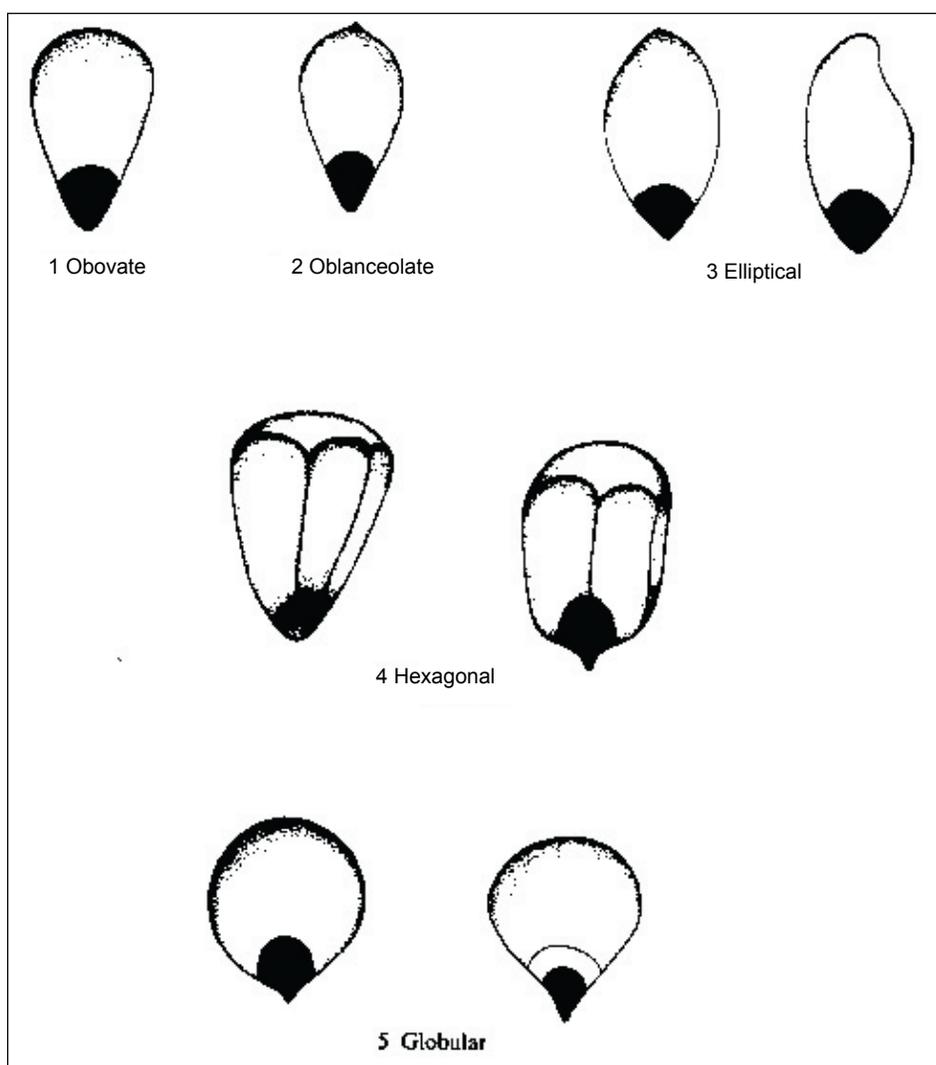


Figure. 10A.2.4. Seed shapes in pearl millet.

10A.3. Descriptors for characterization of chickpea

Vegetative phase

Growth habit: Angle of primary branches, recorded at mid-pod filling stage (Fig. 10A.3.1).

- E Erect; 0–15° from vertical
- SE Semi-erect; 16–25° from vertical
- SS Semi-spreading; 26–60° from vertical
- S Spreading; 61–80° from vertical
- P Prostrate, branches flat on the ground

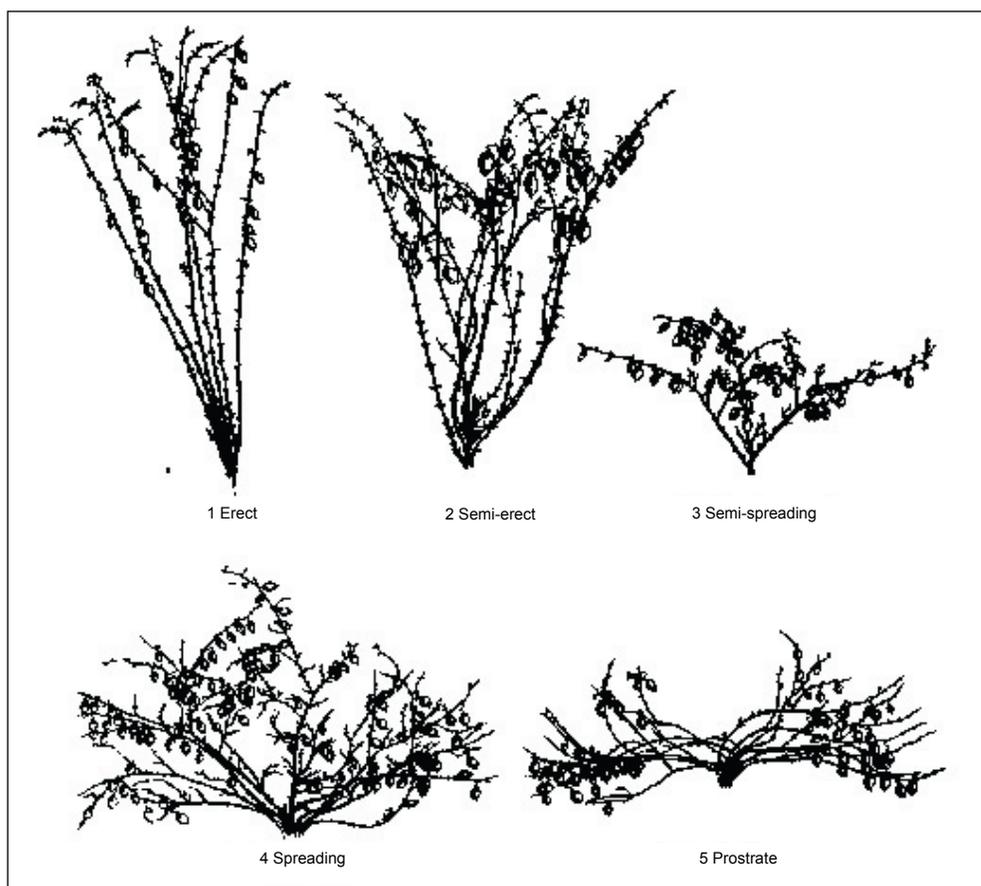


Figure 10A.3.1. Growth habit in chickpea.

Plant height (cm): Mean canopy height of five representative plants, measured from soil surface at the end of flowering.

Plant width (cm): Mean canopy spread of five representative plants, measured at the time of flower ending.

Plant pigmentation: Presence of anthocyanin pigment in plant parts.

- NA No anthocyanin
- LA Low anthocyanin
- HA High anthocyanin

Basal primary branches number: Branches emerging from the axils on the lower half of the main stem, average of five representative plants from each accession at the time of harvest.

Apical primary branches number: Number of branches emerging from the leaf axils on the upper half of the main stem, average of 5 representative plants from each accession at the time of harvest.

Basal secondary branches number: Number of branches emerging from the leaf axils of basal primary branches, average of 5 representative plants from each accession at the time of harvest.

Apical secondary branches number: Number of branches emerging from the leaf axils of apical primary branches, average of 5 representative plants from each accession at the time of harvest.

Tertiary branches number: Number of branches emerging from the leaf axils of basal and apical secondary branches, average of 3–5 representative plants from each accession at the time of harvest.

Reproductive phase

Days to 50% flowering: Number of days from sowing (first irrigation) to the stage when 50% of plants have begun to flower in an accession.

Flowering duration: Number of days from 50% flowering to the date when 50% of the plants of an accession stops flowering.

Flower color: Color of standard petal.

B	Blue	VLP	Very light pink
LB	Light blue	W	White
DP	Dark pink	WBS	White with blue streaks
P	Pink	WPS	White with pink streaks
LP	Light pink		

Days to maturity: Number of days from sowing (first irrigation) to the stage when 90% of pods have matured and turned yellow in an accession.

Pods per plant: Average number of fully formed pods per plant from 5 representative plants at maturity.

Seeds per pod: Number of seeds per pod estimated by dividing the total number of seeds by the total number of pods harvested from 5 representative plants.

Seed color: Color of mature seeds stored not longer than 5 months.

BL	Black	YB	Yellow brown
B	Brown	OY	Orange yellow
LB	Light brown	O	Orange
DB	Dark brown	YE	Yellow beige
RB	Reddish brown	I	Ivory
GB	Greyish brown	G	Green
SB	Salmon brown	LG	Light green
OB	Orange brown	BR	Brown reddish
GR	Grey	M	Variegated
BB	Brown beige	BM	Black brown mosaic
Y	Yellow	LO	Light orange
LY	Light yellow		

Dots on seed coat: Presence or absence of minute black dots on the seed coat.

A	Absent
P	Present

Seed shape: Shape of mature seeds (Fig. 10A.3.2).

ANG	Angular, ram's head
OWL	Irregular round, owl's head
PEA	Pea-shaped, smooth round

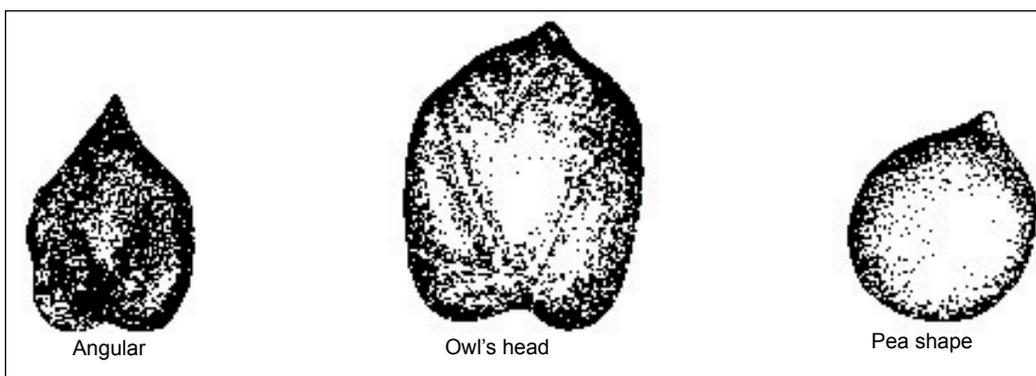


Figure 10A.3.2. Seed shape in chickpea.

Seed surface: Seed surface observed from dry mature seed (Fig. 10A.3.3).

R	Rough — wrinkled with uneven surface.
T	Tuberculated — sticky because of tiny projections.
S	Smooth.

Seed weight (g): Weight of 100 seeds at 10% moisture content.

Seed yield (kg ha⁻¹): Seed yield from all the plants of the plot. Plant stand is also counted. If the plant stand is at least 60% of the optimum number, then plot yield is converted to seed yield in kg ha⁻¹.

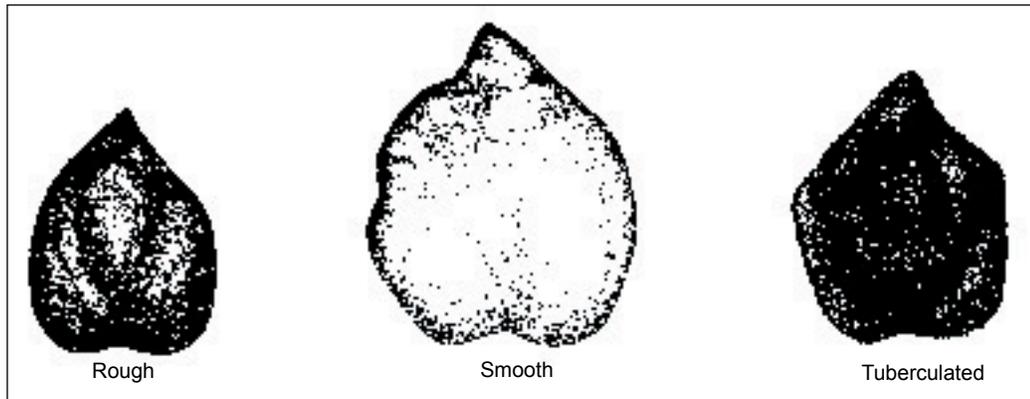


Figure 10A.3.3. Testa texture in chickpea.

Protein content (%): The percentage of crude protein in the freshly harvested seeds, estimated using dye-binding method or automatic protein analyzer.

Diseases

Wilt: Scoring for fusarium wilt (causal organism: *Fusarium oxysporum*) resistance. Accessions sown in wilt-sick plots, plant mortality counted at the end of the season and converted into percentage.

- R Resistant: <10% mortality
- M Moderately resistant: 10-20% mortality
- S Susceptible: >20% mortality

Ascochyta blight: Scoring for ascochyta blight (causal organism: *Ascochyta rabiei*) resistance. Ten day-old seedlings are inoculated in a plant propagator and disease severity scored after 15-day incubation on a 1–9 scale.

- 1 No damage
- 9 Severe damage

Colletotrichum blight: Scored for colletotrichum blight, caused by *Colletotrichum dematium*. Screening done by artificial inoculation with the pathogen twice and scored on a 1–9 scale.

- 1 No damage
- 9 Severe damage

Botrytis grey mold: Screening done using isolation plant propagator. Ten-day old seedlings inoculated and disease severity scored 15 days after inoculation on a 1–9 scale.

- 1 No damage
- 9 Severe damage

10A.4. Descriptors for characterization of pigeonpea

Vegetative phase

Growth habit: Pattern of growth and plant habit.

- C Compact — having relatively few branches, borne at narrow angles to the stem
- S Spreading — having relatively many branches, resulting in a broad canopy
- SS Semi-spreading — intermediate between the above two types

Plant height (cm): Average height of three randomly chosen plants measured at maturity.

Primary branches number: Average number of branches borne on the main stem, recorded from three plants at the time of harvest.

Secondary branches number: Average number of branches borne on the primary branches, recorded from three plants at the time of harvest.

Plant pigmentation: Color of the stem at the time of 50% flowering.

- D Dark purple
- G Green
- P Purple
- R Sun red

Reproductive phase

Days to 50% flowering: Days from effective sowing date to when 50% of the plants in the plot have at least one open flower.

Flowering Pattern: The pattern of flowering habit (Fig. 10A.4.1).

- DT (Determinate): Apical buds of the main shoots develop into inflorescence, the sequence of inflorescence production is basipetal.
- NDT (Indeterminate): Inflorescences develop as axillary racemes from all over the branches, flowering proceeds acropetally from base to apex both within the racemes and on the branches.
- SDT (Semi-determinate): Flowering starts at nodes behind the apex and proceeds both acropetally and basipetally.

Flower color: The main color of the standard petal recorded from the plot.

- I Ivory
- L Light yellow
- OY Orange yellow
- Y Yellow

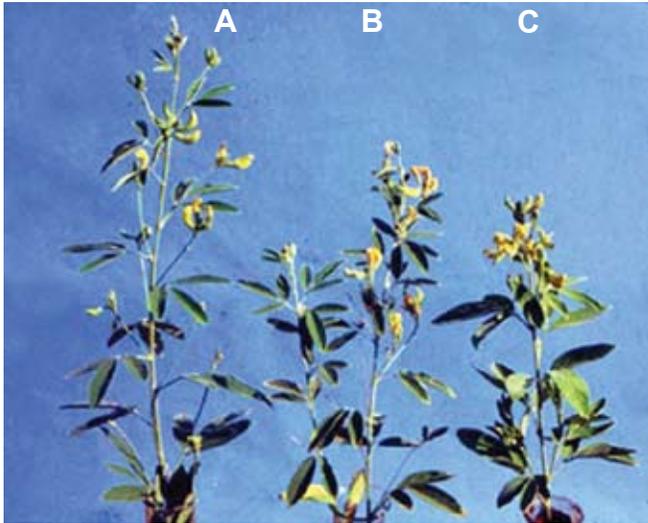


Figure 10A.4.1. Flowering pattern in pigeonpea – Indeterminate (A), semi-determinate (B) and determinate (C).

Streak color: Color of streaks on the dorsal side of the standard petal.

- NO None
- Pu Purple
- R Red

Streak pattern: Pattern of streaks on the dorsal side of the standard petal (Fig. 10A.4.2).

- FS Few streaks
- MS Medium streaks
- DS Dense streaks
- P Plain, uniform coverage
- NO None

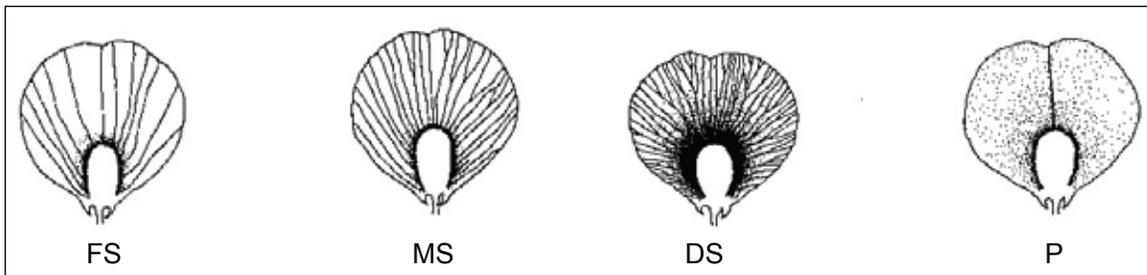


Figure 10A.4.2. Pattern of streaks on standard petal in pigeonpea.

Raceme number: Average number of racemes per plant, recorded from three plants at the time of 50% flowering.

Days to 75% maturity: Number of days taken from effective sowing date to when 75% of the plants in the plot reach maturity.

Pod color: Main color of the pod (Fig.10A.4.3)

- DP Dark purple
- G Green
- M Mixed green and purple
- P Purple



Figure 10A.4.3. Diversity for pod color in pigeonpea germplasm at ICRISAT genebank.

Seeds per pod: Number of seeds per pod, determined from 10 pods randomly picked from three plants at harvest maturity.

Seed color pattern: Color pattern of seed coat recorded after drying (Fig. 10A.4.4).

- P Plain
- M Mottled
- S Speckled
- MS Mottled and speckled
- R Ringed

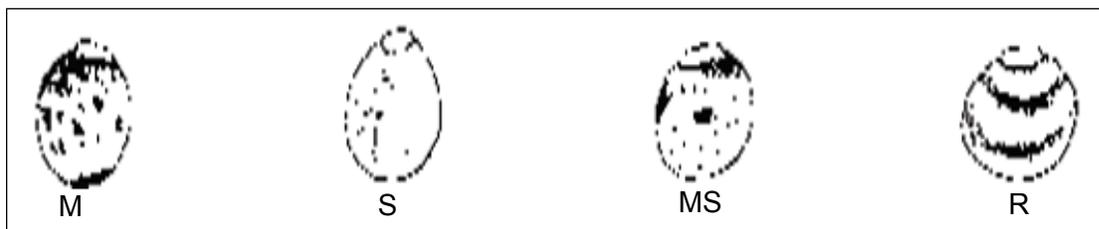


Figure 10A.4.4. Seed color pattern in pigeonpea.

Primary seed color: Main color of the seed coat recorded after drying (Fig.10A.4.5).

W	White	DP	Dark purple
BL	Black	LB	Light brown
C	Cream	LC	Light cream
O	Orange	LG	Light grey
G	Grey	RB	Reddish brown
P	Purple		

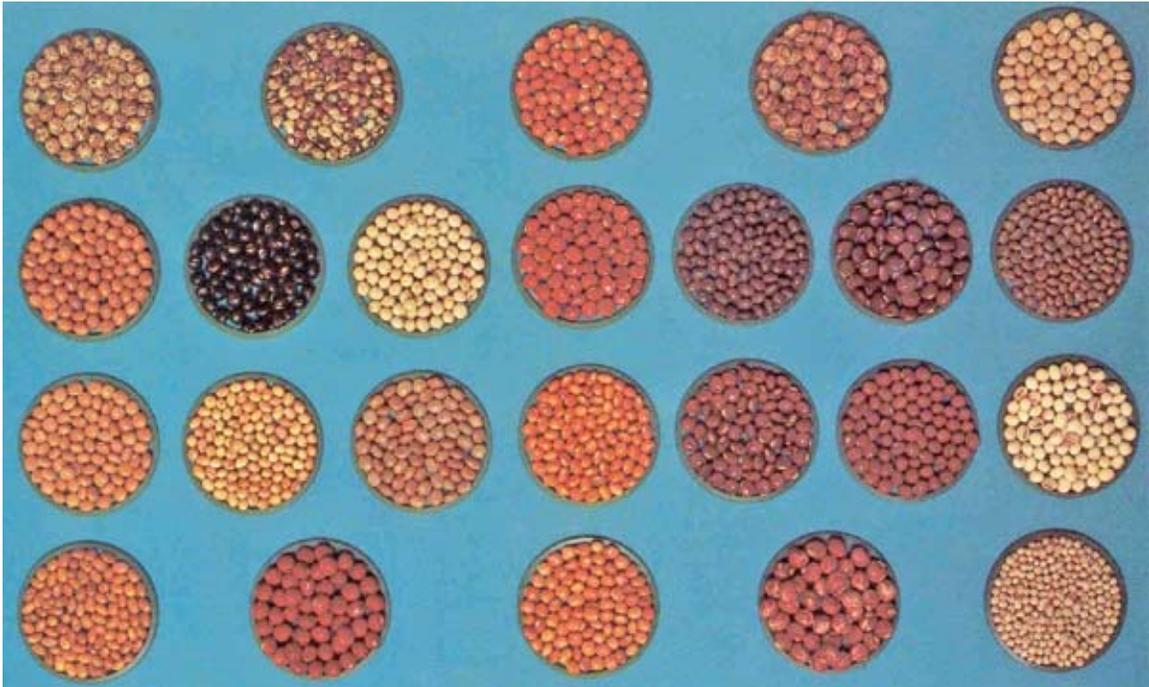


Figure 10A.4.5. Diversity for seed color in pigeonpea germplasm.

Secondary seed color: Eventual other color on the seed coat, coded as in primary seed color.

Seed eye color: Color around hilum, recorded after drying, coded as in primary seed color.

Seed eye color width: Width of color around hilum, recorded after drying.

N	Narrow
M	Medium
W	Wide

Seed shape: Shape of the seed recorded after drying (Fig. 10A.4.6).

O	Oval
P	Pea (globular)
S	Square
E	Elongate



Figure 10A.4.6. Seed shape in pigeonpea.

Seed hilum: Presence or absence of strophiole.

A Absent

P Present

Seed weight (g): Weight of 100 seeds, from a random sample taken from the whole plot, recorded after the seed is sun dried.

Shelling percentage: Seed:pod ratio expressed as percentage based on weight from three randomly selected plants after harvesting and drying.

Protein content (%): Crude protein percentage of seed on dry weight basis.

Seed yield per plant (g): Average seed yield from three randomly selected plants.

Harvest index (%): Ratio of total seed yield to the total biological yield expressed as percentage from three plants.

10A.5. Descriptors for characterization of groundnut

Vegetative Phase

Days to emergence: Number of days to 75% seedling emergence from the day of sowing or first irrigation.

Growth habit: Recorded at podding stage for plants at 10–15 cm interplant spacing (Fig. 10A.5.1).

- 1 Procumbent-1
- 2 Procumbent-2
- 3 Decumbent-1
- 4 Decumbent-2
- 5 Decumbent-3
- 6 Erect
- 7 Others

Plant height (cm): Height of main axis, measured from cotyledonary axil up to terminal bud, mean of 5 plants recorded 60–85 days after emergence.

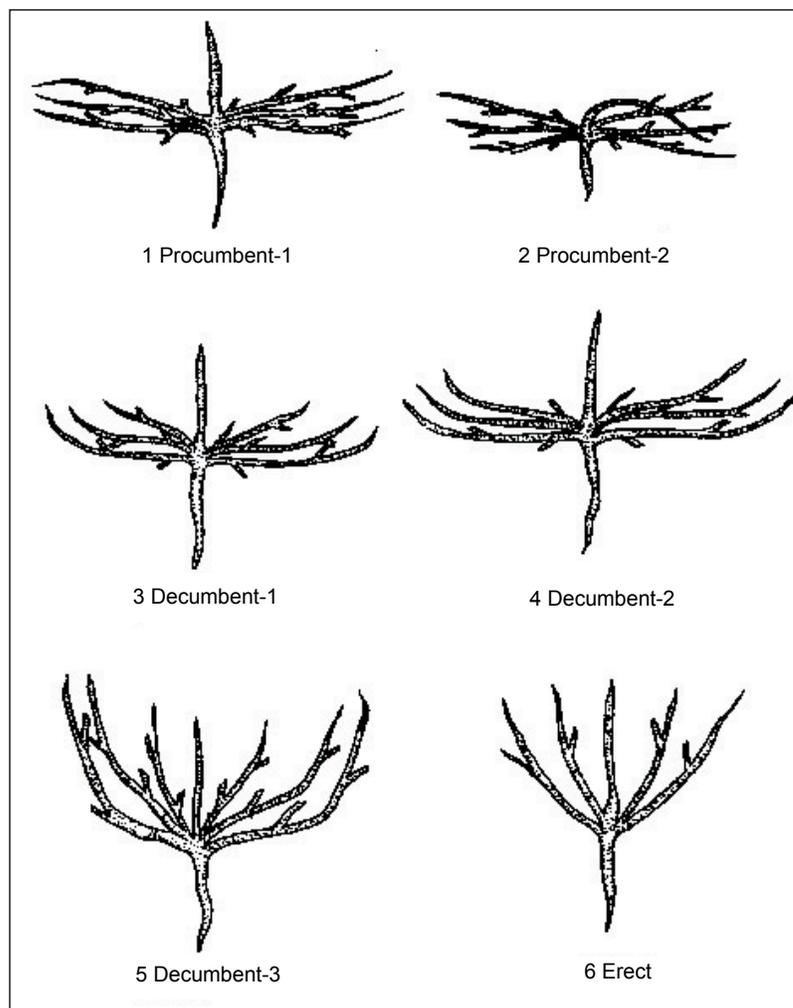


Figure 10.A.5.1. Growth habit in groundnut.

Plant pigmentation: Presence of anthocyanin pigmentation in mature plants.

- 0 Absent
- + Present

Stem hairiness: Hairiness, observed on main axis.

- 1 Glabrous
- 3 Sub-glabrous, hairs in one or two rows along main stem
- 5 Moderately hairy, three or four rows along the main axis
- 7 Very hairy, most of the stem surface covered with hairs
- 9 Woolly, most of the stem surface covered with long hairs

Branching pattern: Pattern of cotyledonary branching (Fig. 10A.5.2).

- 1 Alternate
- 2 Sequential
- 3 Irregular with flowers on main stem
- 4 Irregular without flowers on main stem
- 5 Others

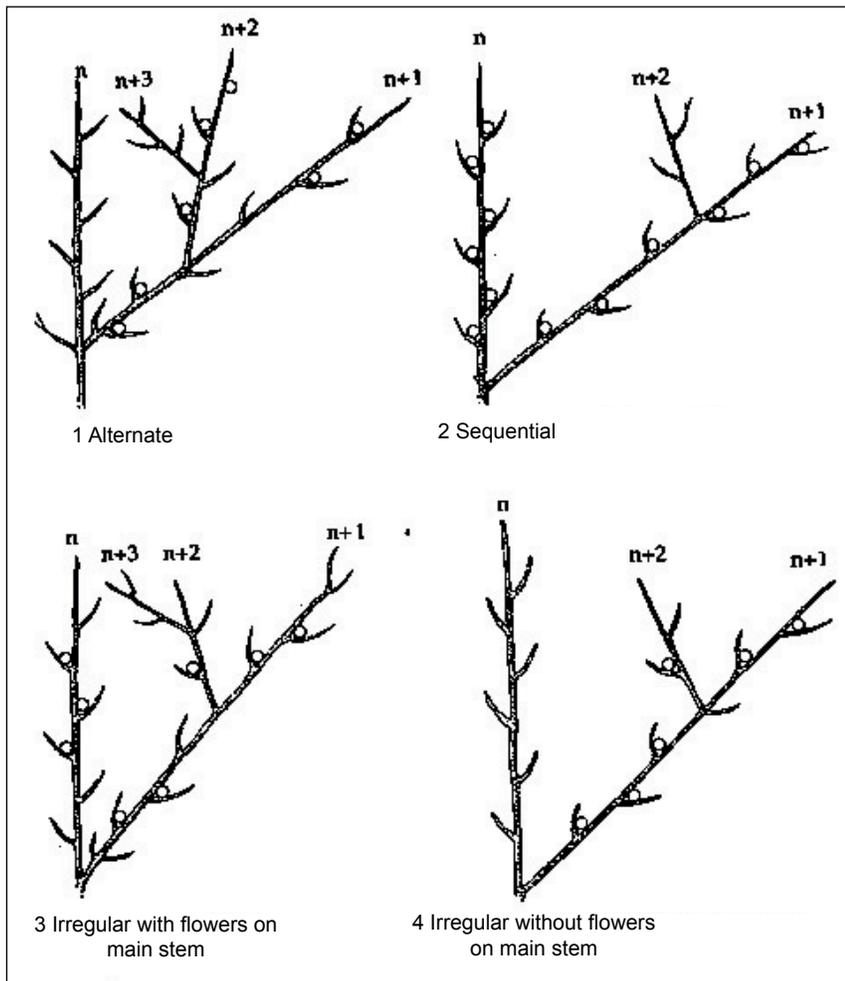


Figure 10A.5.2. Branching pattern in groundnut.

Primary branches number: Number of primary branches.

Leaflet color: Color of fully expanded leaf.

- 1 Yellow or yellow-green
- 2 Light green
- 3 Green
- 4 Dark green
- 5 Bluish green
- 6 Other

Leaflet length (mm): Length of apical leaflet of the fully expanded third leaf on the main stem. Mean of 5 leaflets recorded from different plants.

Leaflet width (mm): Width of fully expanded apical leaflet of the third leaf on the main stem, measured at its widest portion. Mean of 5 leaflets recorded from different plants.

Leaflet shape: Shape of fully expanded apical leaflet of the third leaf on the main stem (Fig. 10A.5.3).

1	Cuneate	9	Ovate
2	Obcuneate	10	Obovate
3	Elliptic	11	Oblong
4	Oblong-elliptic	12	Oblong-lanceolate
5	Narrow-elliptic	13	Lanceolate
6	Wide-elliptic	14	Linear-lanceolate
7	Suborbicular	15	Others
8	Orbicular		

Leaflet hairiness: Hairiness on both surfaces, recorded from leaflets at the third node of the main stem.

- 1 Almost glabrous on both surfaces
- 2 Almost glabrous above, hairs below
- 3 Almost glabrous above, hairs and/or bristles below
- 4 Almost glabrous below, hairs above
- 5 Almost glabrous below, hairs and bristles above
- 6 Hairs on both surfaces, without bristles
- 7 Hairs on both surfaces, with bristles at least on one surface
- 8 Woolly without bristles
- 9 Woolly with bristles on one surface
- 10 Others

Reproductive Phase

Days to 50% flowering: Number of days from emergence to the day on which 50% plants of an accession have flowered.

Flower color: Color of front face of the standard petal excluding the crescent portion of fresh and fully opened flowers.

- 1 White
- 2 Lemon
- 3 Yellow
- 4 Orange-yellow
- 5 Orange

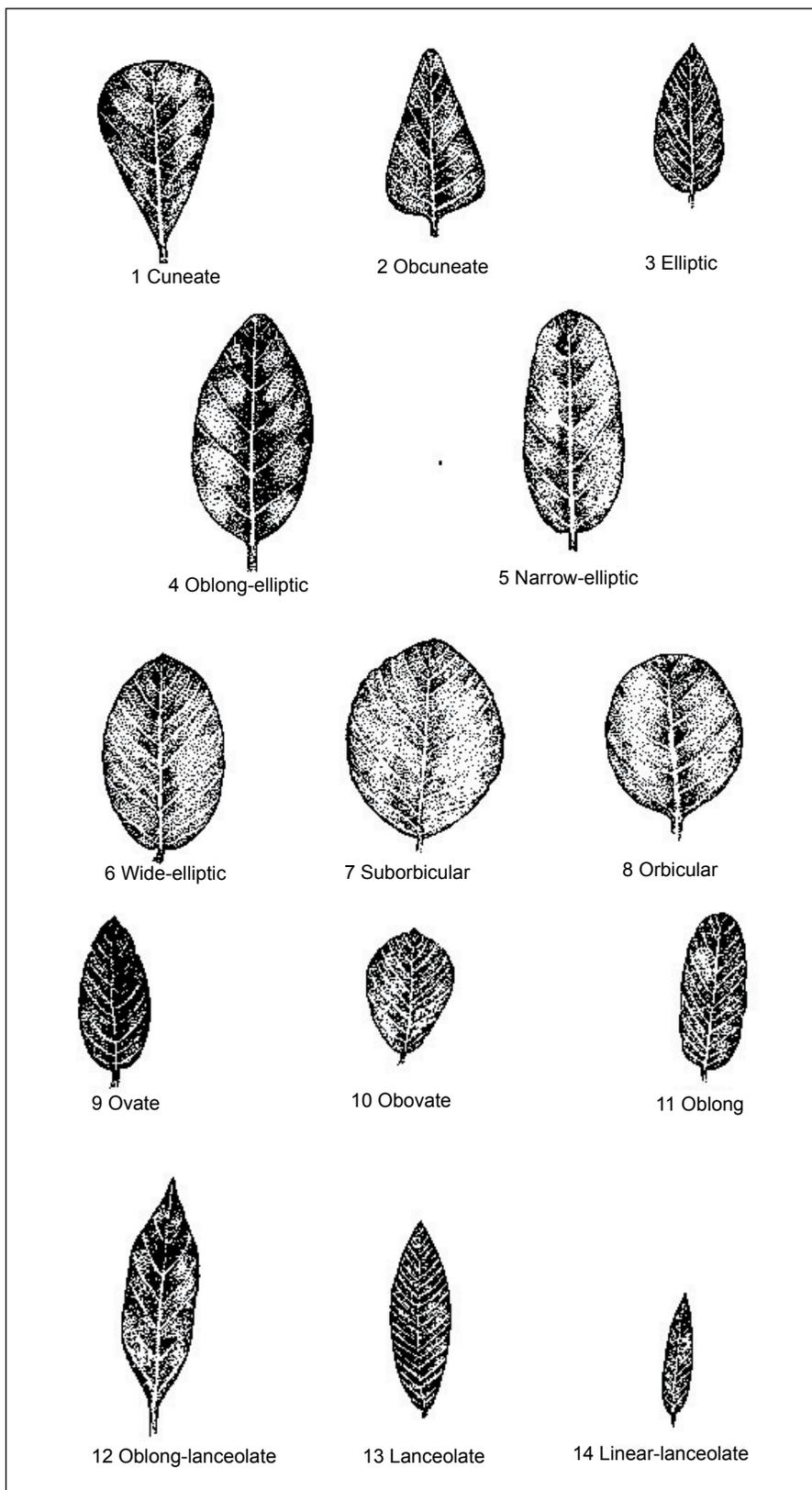


Figure 10A.5.3. Leaflet shape in groundnut.

- 6 Dark orange
- 7 Garnet/brick red
- 8 Others

Streak color: Color of the markings (crescent) on the front face of the standard petal.

- 1 White
- 2 Lemon
- 3 Yellow
- 4 Orange-yellow
- 5 Orange
- 6 Dark orange
- 7 Garnet or brick red
- 8 Others

Peg color: Pigmentation on peg.

- 0 Absent
- + Present

Days to maturity: Number of days from emergence to maturity.

- 1 <90
- 2 91-100
- 3 101-110
- 4 111-120
- 5 121-130
- 6 131-140
- 7 141-150
- 8 151-160
- 9 >160

Pod beak: Tip of the indehiscent fruit (Fig. 10A.5.4).

- 0 Absent
- 3 Slight
- 5 Moderate
- 7 Prominent
- 9 Very prominent

Pod constriction: Degree of pod constriction (Fig. 10A.5.5).

- 0 None
- 3 Slight
- 5 Moderate

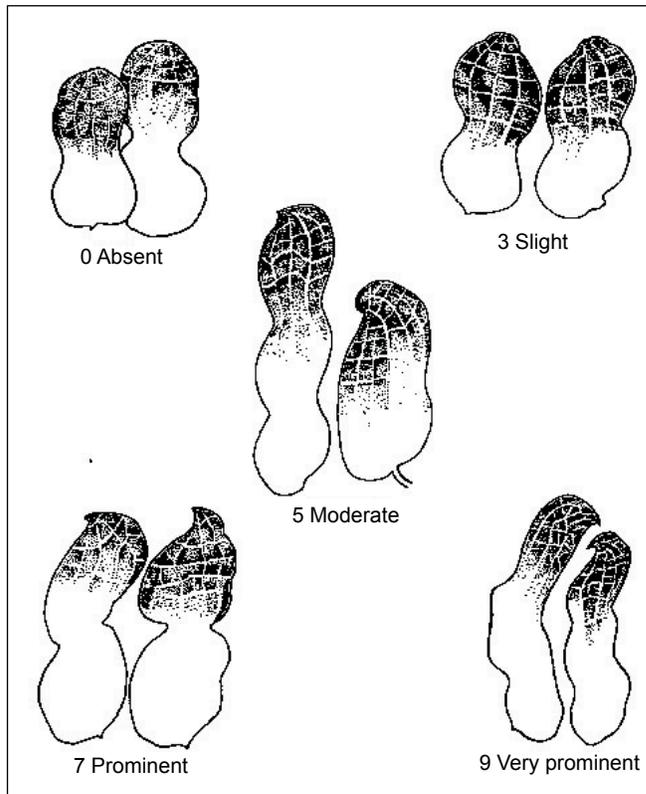


Figure 10A.5.4. Pod beak in groundnut.

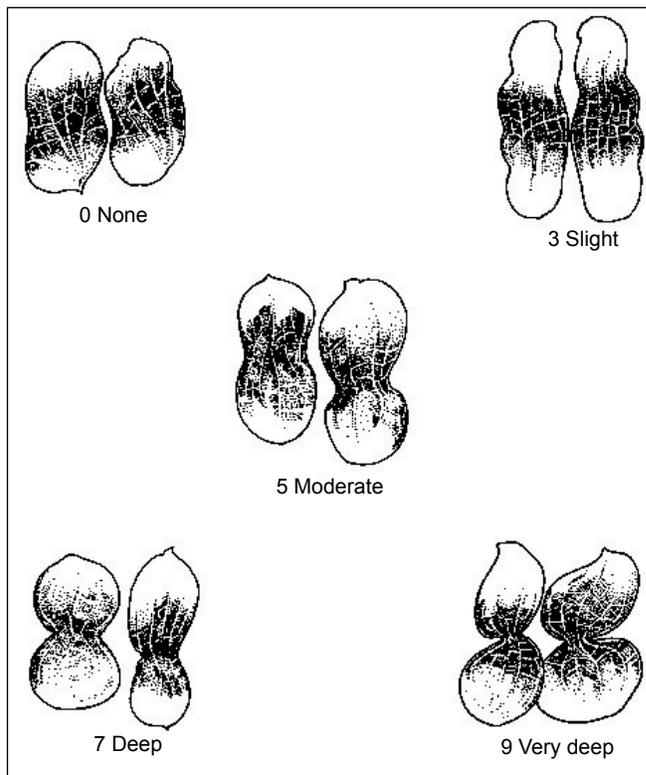


Figure 10A.5.5. Pod constriction in groundnut.

- 7 Deep
- 9 Very deep

Pod reticulation: Reticulation (venation, ribbing, ridging) on the shell of the pod.

- 0 None
- 3 Slight
- 5 Moderate
- 7 Prominent
- 9 Very prominent

Pod length (mm): Mean length of the pod, recorded from 10 mature pods (Fig. 10A.5.6).



Figure 10A.5.6. Diversity for pod and seed traits in groundnut.

Pod width (mm): Mean width of pod at widest point, recorded from 10 mature pods.

Seeds per pod: Number of seeds per pod. First number indicating most frequent number of seeds per pod, second indicating second most frequent number and so on.

- 1 2-1
- 2 2-3-1/2-1-3
- 3 3-2-1/3-1-2
- 4 2-3-4-1/2-4-3-1/2-3-1-4/2-4-1-3/2-1-3-4/2/1/4/3
- 5 3-2-4-1/3-2-1-4
- 6 3-4-2-1/3-4-1-2
- 7 4-3-2-1/4-2-3-1

- 8 4-3-1-2/4-2-1-3
 9 3 or 4 seeded with occasional 5 seeded pods

Seed color pattern: Pattern of seed color, recorded within a month of harvest after complete drying (Fig. 10A.5.6)

- 1 One color
 2 Variegated

Primary seed color: Major color of seeds recorded within one month of harvest after complete drying of mature, wrinkle free seeds (Fig.10A.5.6)

- | | | | |
|----|---------------|----|-----------------------------|
| 1 | White | 11 | Salmon |
| 2 | Off-white | 12 | Light red |
| 3 | Yellow | 13 | Red |
| 4 | Very pale tan | 14 | Dark red |
| 5 | Pale tan | 15 | Purplish red/reddish purple |
| 6 | Light tan | 16 | Light purple |
| 7 | Tan | 17 | Purple |
| 8 | Dark tan | 18 | Dark purple |
| 9 | Greyed orange | 19 | Very dark purple |
| 10 | Rose | 20 | Other |

Secondary seed color: Minor color of variegated seeds (Fig. 10A.5.6)

- 1 Blotched
 2 Flecks of color
 3 Striped
 4 Tipped at the embryo end
 5 Obscure or hazy
 6 Others

Seed length (mm): Length of seed, recorded from an average of 10 mature seeds (Fig. 10A.5.6).

Seed width (mm): Width of seeds measured at mid point.

Shelling percentage: Shelling percentage recorded with seeds at about 8% moisture as

$$(\text{Mass of mature seeds} \times 100) / (\text{Mass of mature pods}).$$

Fresh seed dormancy (%): Germination immediately after harvest and number of days to achieve 70% germination, eg, 65/12 indicates that 65% seed can germinate immediately after harvest, and seeds require 12 days to reach 70% germination.

10B. Molecular characterization

The objective of molecular characterization of germplasm collections is to discern the diversity and population structure at DNA level and identify genetically diverse parents for mapping and use in breeding programs. As it is costly to characterize the entire collection, only selected sets such as core and mini core collections and trait-specific germplasm accessions are characterized to identify genetically diverse parents for use in crop improvement.

- Develop a composite collection of germplasm accessions from the entire collection for diversity, in such a way that it includes core or mini core collection and also trait specific germplasm.
- Genotype the accessions using available Simple Sequence Repeats (SSR) markers or Microsatellites.
- Analyze the genotypic data using Powermarker V3.0 and DARwin 5.0.
- Choose a reference set of 200-400 most diverse accessions using 'Max length sub tree' option of DARwin 5.0, which creates the subset of units minimizing the redundancy between units and limiting the loss of diversity (Fig. 10B.1-2).
- Assess the reference set for allelic richness and diversity.
- Reference set should capture maximum diversity of the composite collection.
- For example, as part of the Generation Challenge Program (GCP), scientists at ICRISAT in collaboration with partners such as ICARDA, Syria; CIRAD, France; EMBRAPA, Brazil; and CAAS, China have developed the composite collections of sorghum, pearl millet, chickpea, pigeonpea, groundnut, finger millet and foxtail millet (500-3,000 accessions) for molecular characterization.
- Reference sets of 200-400 accessions developed at ICRISAT had captured 78% alleles of composite collection in chickpea and sorghum, 95% in groundnut and pearl millet, 83% in pigeonpea, 89% in finger millet and 87% in foxtail millet.

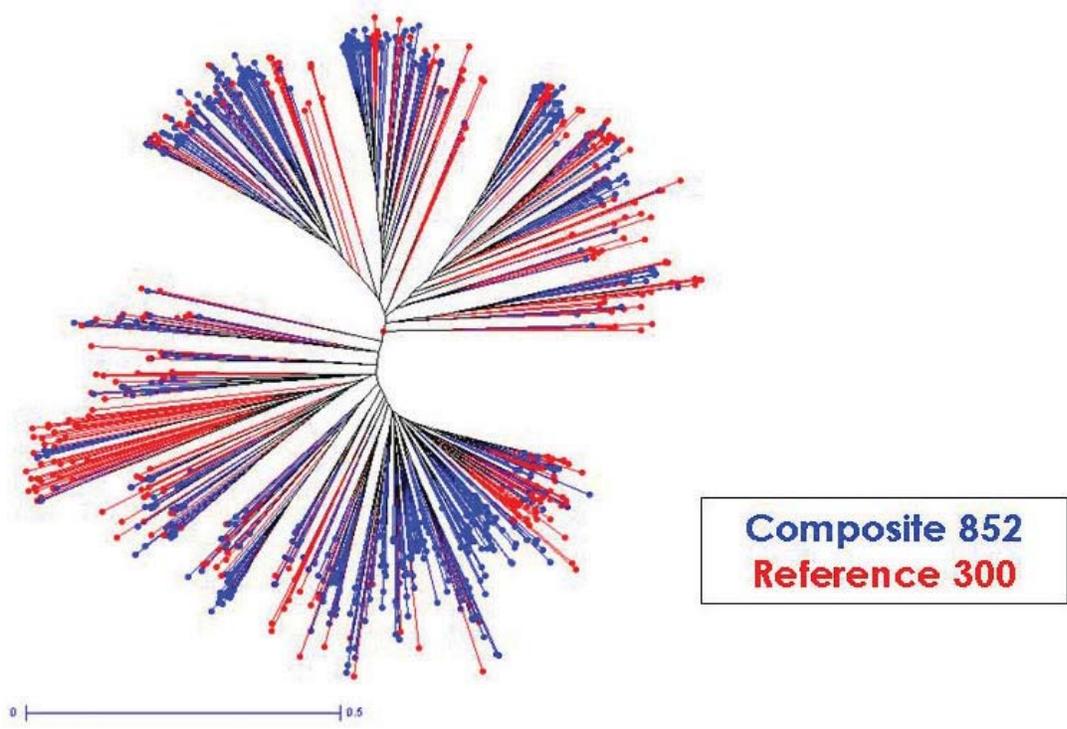


Figure 10B.1. Tree of groundnut composite collection and reference set based on SSR markers.

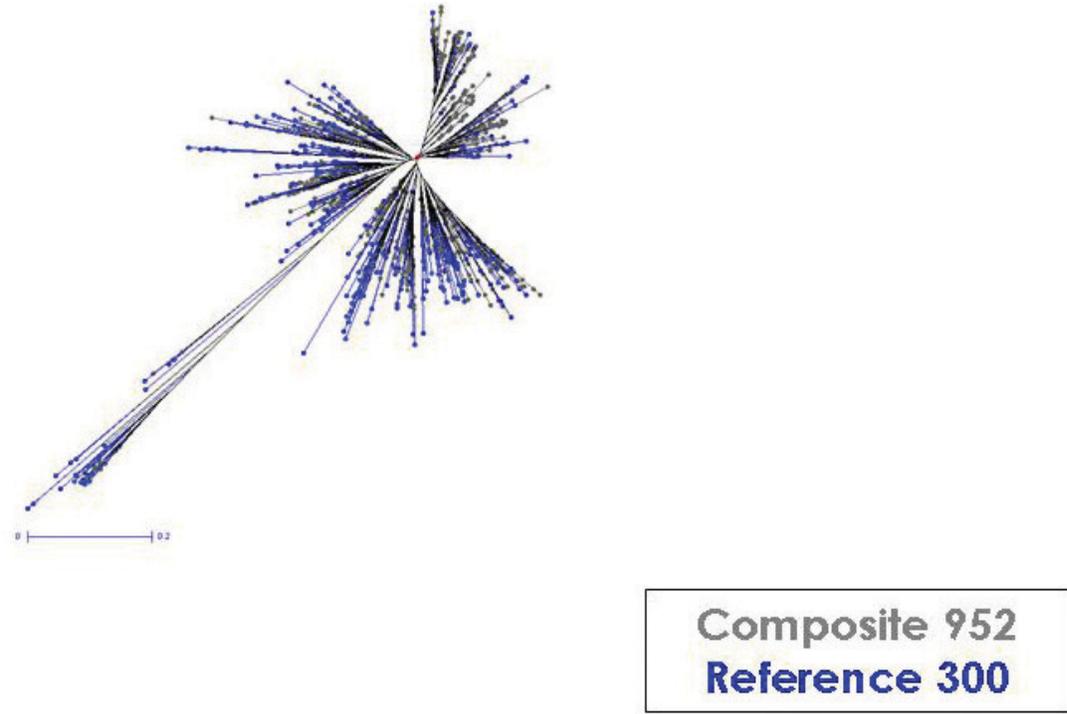


Figure 10B.2. Tree of pigeonpea composite collection and reference set based on SSR markers.

10C. Multilocation evaluation

There is a lack of reliable information on the performance of a large number of accessions, particularly, for traits of economic importance, which display large genotype×environment interactions and require multilocation evaluation. Multilocation evaluation of germplasm sets such as mini core collections, which can be handled easily and economically, for important agronomic traits in different countries, preferably at or near its place of origin, will provide the most reliable data (Fig. 10C.1).



Figure 10C.1. Evaluation of chickpea mini core collection at Patancheru location, India.

Procedure for multilocation evaluation

- Plan well in advance, atleast a year ahead.
- Select sets of germplasm accessions.
- Select locations suitable for the selected accessions.
- Identify appropriate design for evaluation.
- Correspond with NARS, universities, NGOs, etc, in selected countries for collaborative evaluation.
- Arrange for the export of seed material well in advance by obtaining SMTA, IP and other necessary documents.
- Send the seed, list of material, characters to be recorded along with the procedures and planting plans, to all locations.
- Visit each location preferably at the time of planting to entrust the job and train the local staff to record the observations.
- If possible, visit the locations at the peak period of recording observations, preferably at the time of harvesting.
- Compile data from all locations and update the databases.

- Analyze the data using appropriate procedures and softwares. At ICRISAT, we use Residual Maximum Likelihood (REML) procedure for analysis of multilocational data.
- Identify promising accessions as sources for utilization.
- Publish the results in the form of catalogs, journal articles, etc.
- Assess the impact of multilocation evaluation on utilization of germplasm by crop improvement scientists.

10D. Diversity assessment

Diversity in the germplasm collections could be mainly due to natural and human selection. Ecology, climatic, geographic location (latitude and longitude), and elevation of the collection site are the important factors that determine the patterns of diversity in the collections. Therefore, the collection team should remember to collect the above information at the time of collecting germplasm.

Assessment of diversity in the germplasm collections is essential to:

- Identify trait specific germplasm and its areas of adaptation
- Identify suitable locations for characterization and regeneration
- Classify the germplasm
- Select appropriate germplasm for distribution to scientists in different regions
- Develop representative core and mini core collection.

Diversity can be:

- Phenotypic diversity based on qualitative and quantitative traits
- Molecular diversity based on molecular markers such as Amplified Fragment Length Polymorphism (AFLP), Restriction Fragment Length Polymorphism (RFLP), Single Nucleotide Polymorphism (SNP) and Simple Sequence Repeats (SSR).

Phenotypic diversity can be assessed by estimating the following statistical parameters for characters under study:

- Frequency distribution
- Range
- Mean
- Variance
- Diversity indices

Molecular diversity can be assessed by using different molecular markers such as AFLP, RFLP, SSR and SNP. Simple matching allele frequency-based distance matrix in DARwin 5.0 can be used to dissect the genetic structure and diversity, while Power Marker V 3.0 can be used to estimate basic statistics such as PIC, allelic richness, gene diversity, heterozygosity and occurrences of unique, rare, common and most frequent alleles in the population.

Section 11

Taxonomic Classification

11A. Key to the identification of *Sorghum* species

Sorghum, which belongs to the tribe Andropogoneae of family Poaceae, is the fifth important cereal of the world. It is widely grown in the semi-arid areas of the tropics and subtropics.

The genus sorghum is divided into five sections: *Parasorghum*, *Stiposorghum*, *Heterosorghum*, *Chaetosorghum* and *Sorghum*.

Parasorghum: Sheath-nodes densely bearded, pedicellate spikelets staminate, panicle branches simple, awn and callus well developed, chromosomes large, $n=5$.

Stiposorghum: As *Parasorghum* except awn and callus better developed, $n=5$.

Heterosorghum: Pedicellate spikelets reduced to glumes, panicle branches divided, awn and callus poorly developed, chromosomes medium, $n=10$ or 20 .

Chaetosorghum: Pedicellate spikelets reduced to glumes, panicle branch simple, awn well developed, callus poorly developed, chromosomes medium, $n=10$ or 20 .

Sorghum: Sheath-nodes glabrous or finely pubescent, not bearded, pedicellate spikelet staminate, panicle branches divided, awn and callus poorly developed, chromosomes medium, $n=10$.

Section *Sorghum* includes annual cultivated forms from Asia and Africa and perennial taxa from southern Europe and Asia.

Three species are recognized:

- *S. halepense* (L.) Pers. ($2n=40$): A rhizomatous perennial species with creeping rhizomes, pedicellate spikelets deciduous when mature, and up to 25 cm long.
- *S. propinquum* (Kunth) Hitchc ($2n=20$): A rhizomatous perennial species with creeping rhizomes, pedicelled spikelets deciduous when mature, sessile spikelets acute to acuminate, lower glume with the keels ending without or with only obscure teeth, leaf blades broad, panicles large, and 20–60 cm long.
- *S. bicolor* (L.) Moench: annual taxa cultivated.

Species *S. bicolor* comprises three subspecies: *bicolor*, *drummondii* and *verticilliflorum*.

- ***Subsp. bicolor***: Plants annuals, with stout culms, often branched, frequently tillering, leaf blades up to 90 cm long and 12 cm wide, inflorescence open or contracted panicle, branches often several from lower nodes, obliquely ascending or spreading, racemes tough.
- ***Subsp. drummondii***: Plants annual with relatively stout culms, leaf blades lanceolate up to 50 cm long and 6 cm wide, panicles rather contracted, branches somewhat pendulous, racemes more or less crowded, mostly 3-5 noded, tardily disarticulating at maturity.

- *Subsp. verticilliflorum*: Plants tufted annuals or weak biannual, leaf blades linear-lanceolate up to 75 cm long and 6 cm wide, panicles usually large, somewhat contracted to loose with branches obliquely ascending or spreading, racemes 1–5 noded, fragile.

The cultivated sorghums (*Sorghum bicolor* Subsp. *bicolor*) were classified into five basic races (Fig. 11A.1) (Harlan and de Wet 1972):

- **Bicolor**: Grain elongate, sometimes slightly obovate, nearly symmetrical dorso-ventrally, glumes clasping the grain, which may be completely covered or exposed as much as $\frac{1}{4}$ of its length at the tip, spikelets persistent.
- **Guinea**: Grain flattened dorso-ventrally, sublenticular in outline, twisting at maturity nearly 90 degrees between gaping involute glumes that are from nearly as long to longer than the grain.
- **Caudatum**: Grain markedly symmetrical, the side next to the lower glume flat or in extreme cases somewhat concave, the opposite side rounded and bulging, the persistent style often at the tip of a beak pointing towards the lower glume, glumes $\frac{1}{2}$ the length of the grain or less.
- **Kafir**: Grain approximately symmetrical more or less spherical, glumes clasping and variable in length.
- **Durra**: Grain rounded obovate, wedge-shaped at the base and broadest slightly above the middle; glumes very wide, the tip of a different texture from the base and often with a transverse crease across the middle.

These five basic races and ten intermediate combinations can account for all the variation in the cultivated forms (Fig.11A.1).

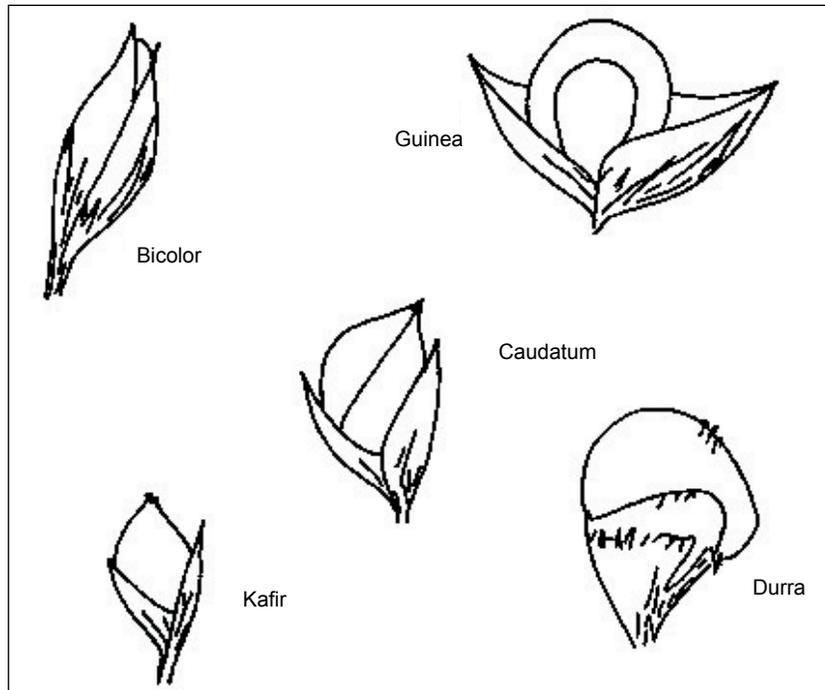


Figure 11A.1. Glume coverage and racial classification in sorghum.

Key to indigenous Australian Sorghum

(Source: Lazarides et al. 1991)

1. Pedicelled spikelet reduced or wanting, neuter, linear, markedly dissimilar to the sessile spikelet
 2. Callus elongated, pungent; articulation joint linear, acutely oblique; pedicelled spikelet rudimentary and minute, or absent *S. angustum*
 2. Callus minute, obtuse; articulation joint cupular, horizontal or slightly oblique; pedicelled spikelet reduced to the glumes, 4–13.5-mm long
 3. Sessile spikelet 5.3–6.6-mm long; racemes 8–12-mm long; racemes 8–12-mm long; 1–3-jointed; plant 1–2-m high; primary branches of panicle up to 3-cm long, branched; glumes of pedicelled spikelet unequal, the upper mostly enveloped in the lower and about half its length *S. laxiflorum*
 3. Sessile spikelet 10–11-mm long; racemes 40–120-mm long, 9–21-jointed; plant 1.8–3.6-m high; primary branches to 8-cm long; simple or the lower ones branched; glumes of pedicelled spikelet subequal, the upper free of the lower *S. macrospermum*
1. Pedicelled spikelet well developed, often staminate, usually lanceolate or lanceolate-oblong, somewhat similar to the sessile spikelet
 4. Awn on sessile spikelet to 3-cm long or absent; callus minute, obtuse; articulation joint usually cupular, horizontal
 5. Primary branches of panicle usually very many, whorled, branched, bearing lateral and terminal racemes; sessile and pedicelled spikelets sometimes persistent; culm nodes often glabrous or pubescent; often annuals *S. spp.* (cultivated)
 5. Primary branches of panicle usually 1–6-nate and simple, bearing only terminal racemes; sessile and pedicelled spikelets deciduous; culm nodes bearded; perennials
 6. Panicle 14.5–19-cm long; racemes 1.8–5.0-cm long. Sessile spikelet: lower lemma 5.5–7.0-m long; upper lemma 2.5–4.0-mm long; awn 15–20-mm long. Pedicelled spikelet 5–8-mm long, often imperfectly awned. Blades 10–70-cm long; ligule a glabrous membrane 1.0–1.3-mm long *S. leiocladum*
 6. Panicle 16–26-cm long; racemes 1.5–2.2-cm long. Sessile spikelet: lower lemma 4.3–5.3-mm long; upper lemma 1.3–3.0-mm long; awn usually 10–15-mm long or absent. Pedicelled spikelet 4.3–6.0-mm long, awnless. Blades 30-cm long; ligule a (usually ciliate) membrane 1.5–2.0 mm long *S. nitidum*
 4. Awn on sessile spikelet 3–15-cm long; callus often elongated and pungent; articulation joint usually longer than wide and oblique
 7. Ovary and caryopsis with an apical tuft of hairs
 8. Sessile spikelet subulate or elliptic; lower glume narrowed towards the apex and beak-like, its apical keels acute or ribbed, without wings; often plants glaucous or pruinose and culm nodes pubescent to bearded *S. intrans*
 8. Sessile spikelet usually obovate; lower glume not or scarcely narrowed in the upper part, truncate or broadly obtuse, its apical keels thickened in the lower part, narrowly winged on the inner edge upwards, and terminating in a tooth or acute lobe; usually plants green and culm nodes glabrous *S. exstans*
 7. Ovary and caryopsis glabrous
 9. Sessile spikelet usually 9.5–18.0-mm long (including callus); the callus 3–10-mm long, long-pungent; articulation joint linear, distinctly longer than wide
 10. Sessile spikelet elliptic or subulate, narrowed to a beak-like apex; lower glume usually hirsute
 11. Perennial; panicle branches usually branched; racemes usually 2–4-jointed *S. interjectum*
 11. Annual; panicle branches simple; racemes usually 1-jointed *S. stipoides*
 10. Sessile spikelet obovate or oblanceolate, not or scarcely narrowed to the apex; lower glume glabrous and glossy or (in *S. ecarinatum*) hirsute
 12. Lower glume of sessile spikelet inflated, bulbous, abruptly constricted near the apex into a prolonged beak, entire or notched, usually black and glossy; apical keels ribbed and wingless, absent.
 13. Apical keels present on the apical beak; ligule 1.3–2.3-mm long, ciliate or scaberulous on the apex; culm nodes bearded; lodicules often long-ciliate; pedicelled spikelet subsistent *S. bulbosum*
 13. Apical keels poorly or not developed; ligule 2.5–5.8-mm long, glabrous or scaberulous on the apex; lodicules and culm nodes often glabrous; pedicelled spikelet caduceous *S. ecarinatum*

12. Lower glume of sessile spikelet neither inflated nor bulbous, not prolonged and usually flattened near the apex, 3-lobed or 3-toothed, yellowish (when young) to reddish brown or partly black; apical keels thickened and rounded in the lower part, flattened and winged in the upper part
 *S. brachypodium*
- Sessile spikelet usually 4.5–10.0-mm long (including callus); the callus usually 0.3–2.0-mm long, shortly pungent to obtuse; articulation joint oblong or elliptic or obovate, relatively wide and sometimes almost as wide as long
14. Callus shortly pungent, slender and curved; articulation joint longer than wide
15. Perennials; racemes usually 2–4-jointed; awns 5–7-cm long
16. Blades flat or loosely folded, 4–12-mm wide, herbaceous..... *S. plumosum* var. *plumosum*
16. Blades terete (with flattened apex), 1–2-mm wide, indulated *S. plumosum* var. *teretifolium*
15. Robust annual; racemes 1-jointed; awns 6.5–9.0-cm long..... *S. amplum*
14. Callus subacute to obtuse, thickened, straight; articulation joint almost as wide as long
17. Sessile spikelet 4.5–6.0-mm long (including callus); panicle-branches branched
 *S. matarankense*
17. Sessile spikelet 6–9-mm long (including callus); panicle-branches usually simple
18. Perennial; sessile spikelet 8–9-mm long; awn with ciliate column; articulation joint elliptic to obovate *S. grande*
18. Annual; sessile spikelet 6–7-mm long; awn with scabrous column; articulation joint broadly elliptic to ± circular *S. timorensis*

11B. Key to the identification of *Pennisetum* species

(Adapted from Clayton and Renvoize 1982)

- Inflorescence reduced to a cluster of 2–4 subsessile spikelets enclosed in the uppermost leaf-sheath, protruding with long filaments and stigmas *P. clandestinum*
- Inflorescence a spiciform panicle, conspicuously exerted:
- Clusters persistent, usually stipulate, the bristles plumose or glabrous; lemma usually pubescent on the margins; cultivated *P. glaucum*
 - Clusters readily deciduous; lemmas glabrous or almost so; spontaneous:
 - Bristles of the involucre, or at least the inner, ciliate to plumose:
 - Involucre borne upon a terete pubescent stipe 1–3-mm. long, falling with it at maturity *P. setaceum*
 - Involucre without a stipe:
 - Rachis cylindrical or with rounded ribs:
 - Spikelets all pedicelled. *P. quamulatum*
 - Spikelets, or at least one in each involucre, sessile:
 - Plants robust, 1–6-m high, with broad flat leaf-blades *P. purpureum*
 - Plants densely caespitose, 0.3–1.5-m high, with narrow convolute leaf-blades *P. phacelatum*
 - Rachis angular, with sharp-edged decurrent wings below the scars of the fallen involucres:
 - Spikelets solitary and sessile within the involucre *P. polystachion*
 - Spikelets in clusters of 1–5 within the involucre, at least one of them on a pedicel 0.5–3.5-mm. long; involucres fluffy, ovate *P. pedicellatum*
- Bristles of the involucre glabrous:
- Panicles terminal and also axillary:
 - Bristles solitary below each spikelet; panicles gathered into a leafy false inflorescence:
 - Leaf-blades (5–)10–30-mm wide; spikelets 2–3-mm long *P. unisetum*
 - Leaf-blades 1–4-mm wide; spikelets 3–4-mm long. *P. procerum*
 - Bristles several below each spikelet:
 - Involucre sparse, of 2–5(–15) bristles, all but the longest shorter than the spikelet; panicles forming a scanty false inflorescence *P. trisetum*
 - Involucre well developed; axillary panicles few; nodes black *P. achyphyllum*
- Panicles terminal on the culms and branches:
 - Rachis with sharp decurrent wings below the scars of the fallen involucres:
 - Spikelets 2.5–4.0-mm long; upper floret readily deciduous, shining, obtuse; perennial *P. polystachion*
 - Spikelets 5.0–6.5-mm long; upper floret not deciduous, acuminate; involucre stiffly bristly, truncate at the base; annual *P. ramoum*
 - Rachis cylindrical or with rounded ribs, some times ± angular but scarcely winged:
 - Ligule membranous, 0.5–1.5-mm long; culms wiry, much branched *P. stramineum*
 - Ligule represented by a line of hairs:
 - Rachis pubescent; spikelets in groups of 1–5, 1 sessile the others pedicelled; upper lemma subcoriaceous and shining in the lower half; anther tips minutely hairy; robust plant with stout culms and broad leaf-blades *P. purpureum*
 - Rachis scaberulous, occasionally pubescent but then the spikelets solitary and the lemmas scarcely different in texture:
 - Spikelets 6–12-mm long:
 - Plant forming large tussocks; leaf-blades rigid, harsh, smoothly ridged above; involucral scars cupular *P. hohenackeri*
 - Plants rhizomatous, forming low mats:
 - Lower lemma as long as the spikelet or almost so *P. riparium*
 - Lower lemma 1/3–2/3 as long as the spikelet *P. dowsonii*
 - Spikelets 2–6-mm long:
 - Upper glume 2/3 as long as the spikelet or more;

- plants shrubby, much branched:
- Culm smooth below the ovate to oblong panicle *P. mezianum*
 - Culm scaberulous below the oblong to linear panicle *P. massaicum*
 - Upper glume up to 1/2 as long as the spikelet, sometimes more but then the culm pubescent below the panicle:
 - Lower lemma less than 1/4 the length of the spikelet:
 - Tip of lower lemma usually cuspidatearistate; basal sheaths glabrous; anther-tips or culm-summit sometimes hairy; base of involucre truncate; loosely tufted, rhizomatous *P. thunbergii*
 - Tip of lower lemma acute to acuminate; basal sheaths ± pilose; anther-tips and culm summit glabrous; densely tufted *P. mildbraedii*
 - Lower lemma more than 3/4 the length of the spikelet:
 - Plant densely tufted, with narrow convolute leaf-blades; hairy below panicle *P. sphacelatum*
 - Plant reed like from a creeping rhizome, often robust with an elongated panicle; rarely hairy below the panicle *P. macrourum*

11C. Key to the identification of *Cicer* species

(Source: van der Maesen 1987)

Annual species

1. Leaves with end leaflet, plant prostrate or erect2
 - Leaves ending in a tendril, plant climbing *C. cuneatum*
2. Leaflets 3, cuneate-flabellate; stipules small, 1 mm; flowers 5–6 mm; plant up to 15 cm with prostrate branches *C. chorassanicum*
 - Leaflets more than 33
3. Leaflets in 2–3 pairs with end leaflet4
 - Leaflets more numerous5
1. Leaflets oblong-obovate; stipules ovate-lanceolate, 2–5 mm; flowers ca 9 mm; arista 0–3 mm; plant up to 30 cm (Turkey, Syria) *C. bijugum*
 - Leaflets cuneate-elliptic or lanceolate; stipules bidentate, ca 2 mm; flowers ca 7 mm; arista very long, 5–20 mm; plant up to 20 cm, sometimes 30 cm (Afghanistan) *C. yamashitae*
5. Leaflets generally small, 4–7(–10) mm; flowers 5–8 mm; seeds 3–6 mm6
 - Leaflets larger, 5–12 mm; flowers 8–12 mm; seeds 5–12 mm7
- Leaflets in 3–4 pairs, simply serrate, 4–10 mm; leaf petiole long, 10–17 mm; stipules ovate to flabellate, 5(–7) mm, 2–3 teeth; flowers 6–8 mm; plant 10–30(–40) cm; seeds 4–6 mm diameter *C. pinnatifidum*
 - Leaflets in 3–6 pairs, often double-serrate at the top, 4–7 mm; leaf petiole short, 5–12 mm; stipules 2–3 mm, 2–5 teeth; flower 5–6 mm; plant 15–40 cm; seeds 3–4 mm diameter *C. judaicum*
- Stems erect to semi-spreading, rarely prostrate; leaflets in 3–7 pairs, elliptical, serrate; seeds large, 5–12 mm, smooth, rugose or tuberculate; plant cultivated *C. arietinum*
 - Stems prostrate at first, then ascending; leaflets in 3–5 pairs, elliptical to elongate, serrate; seeds large, up to 8 mm, seeds echinate or reticulate8
- Seeds echinate *C. echinospermum*
 - Seeds reticulate *C. reticulatum*

Perennial species

1. Leaf rachis ending in a tendril or leaflet2
 - Leaf rachis ending in a sturdy spine (Sect. *Acanthocicer*)24
2. Flowers small, 8–10 mm; leaves imparipinnate, 5–7 leaflets; rootstocks slender; creeper of 5–15 cm *C. incisum*
 - Flowers larger3
3. Flowers medium large, ca 15 mm; leaves imparipinnate, 5–16 leaflets; rootstocks slender; habit sturdy, erect, 4–10 cm (Morocco) *C. atlanticum*
 - Flowers medium large or large, plants taller, rootstocks woody (Sect. *Polycicer*)4
4. Flowers medium large, ca 15 mm5
 - Flowers large, ca 20–27 mm8
5. Leaflets spine-shaped, plant glabrous *C. subaphyllum*
 - Leaflets normal, flat; plant pubescent6
6. Leaflets rounded, 5–15-mm long, 5–17-mm wide, with 10 or more teeth, in 3–5 pairs; tendril often ramified *C. oxyodon*
 - Leaflets fan-shaped, base cuneate, very remote; tendril sturdy, curled7
7. Leaflets 3–7-mm long, 3–9-mm wide, with 5–7(–10) teeth, in 3–8 pairs *C. spiroceras*
 - Leaflets 5–9-mm long, 5–15-mm wide, with 5–9(–10) teeth, in (3–) 6–12 pairs *C. kermanense*
8. Flowers 1–2 per peduncle, rarely more; bracts minute9
 - Flowers (1) 2–5 per peduncle10
9. Stipules flabellate-rounded, about as large as or larger than the leaflets, toothed11
 - Stipules obliquely ovate or triangular, small or half as long as the leaflets, at some nodes sometimes nearly as large incised12
10. Leaflets in 20–30 linear leaflets of 15–30-mm long, ca 1-mm wide; bracts absent *C. canariense*
 - Leaflets in fewer pairs, more rounded-serrate or reduced to spines; bracts more or less foliolate16
11. Plant 18–35 cm, sticky, intensely glandular-pubescent; leaves imparipinnate, with 4–7 pairs of leaflets, obovate, 5–13-mm long, 4–7-mm wide; arista ending in a small leaflet, 1–5 mm *C. fedtschenkoi*
 - Plant 25–40 cm, less densely pubescent; leaves ending in a tendril or tendrillous leaflet; leaflets 5–7 pairs, flagellate, 4–12-mm long, 2–8-mm wide; arista rarely ending in a small foliole *C. songaricum*
- Leaflets small, up to 10(–17) mm13
 - Leaflets larger, up to 25(–27) mm, sometimes smaller20
13. Leaflets in 2–4(–5) pairs, rachis with end leaflet or ending in a slender spine *C. paucijugum*
 - Leaflets more numerous14

14. Plant densely pubescent, 10–30 cm; leaves imparipinnate; leaves in 9–18 pairs, ovate to oblonglanceolate, top toothed, flower 1 per peduncle	<i>C. multijugum</i>	
- Plant less pubescent, 20–70 cm; leaflets less numerous; rachis ending in a tendril or an end leaflet at the lower leaves, flowers 1–2 per peduncle		15
15. Plant more or less glandular pubescent; tendril always simple; leaflets narrowly cuneate to cuneateobovate, upper half of margin toothed; stipules triangular-incised, up to nearly as large as the leaflets, 2–12 mm	<i>C. microphyllum</i>	
- Plant thinly pubescent		16
16. Leaflets not very close, obovate or obovate-elliptic, 5–15 mm, in 8–13 pairs, margin toothed except near the base; tendril simple, stipules small, triangular-incised, 2–4 (–7) mm	<i>C. nuristanicum</i>	
- Leaflets very remote, broadly cuneate-flabellate, 3–7 mm, in 8–11 pairs, top truncate-toothed; tendril often ramified, stipules small, triangular-incised, 2–4 mm	<i>C. mogoltavicum</i>	
17. Hairs very long, 1–2 mm		18
- Hairs shorter, up to 1 mm		19
18. Leaves ending in a leaflet; leaflets elliptical; flowers white	<i>C. montbretii</i>	
- Leaves ending in a tendril, ramified or not, at lower leaves a top leaflet; flowers blue or purple; plant climbing.	<i>C. graecum</i>	
19. Leaflets oblong-obovate, spiny toothed, 7–24-mm long, 5–15-mm wide; bracts 1–2 mm; flowers white	<i>C. isauricum</i>	
- Leaflets oblong-elliptical, finely toothed, 8–15 mm, 3–8-mm wide; bracts 2–3 mm; flowers blue-violet	<i>C. qoribundum</i>	
20. Leaflets in 4–7 pairs, rather close, cuneate-obovate, elliptic or subrotundate, 7–15 (–18)-mm long; stipules generally half as long as the leaflets (Asia Minor, Iran, Caucasus)	<i>C. anatolicum</i>	
- Leaflets in 4–8 pairs, rather close or more remote, ovate to subrotundate, 5–22-mm long. (Central Asia)		21
21. Plant densely glandular pubescent, 30–40 cm; stems flexuous; leaflets in 5–8 pairs, cuneate-obovate, up to 15 mm long, 12 mm wide; rachis ending in a ramified or simple tendril	<i>C. flexuosum</i>	
- Plant less glandular pubescent, stems straight or slightly flexuous		22
22. Plant sparsely mainly eglandular pubescent, 30–40 cm; leaflets in 4–8 pairs, rounded to cuneate-truncate, 5–18-mm long, 4–15-mm wide; rachis ending in a simple tendril.	<i>C. baldshuanicum</i>	
- Plant glandular pubescent, 20–80 cm		23
23. Plant 20–50 cm; leaves ending in a simple or ramified tendril; 4–6 pairs of leaflets, elliptic, 10–25-mm long, 6–12-mm wide, teeth simple, triangular-acuminate	<i>C. grande</i>	
- Plants 50–80 cm; leaves ending in a spiny curl or a tendril; 5–6 pairs of leaflets, broadly cuneate or obovate, 10–17 (–20)-mm long, 6–10-mm wide; teeth broadly acuminate, bipartite	<i>C. korshinskyi</i>	
24. Leaflets small, 1–5-mm long, in 5–11 pairs; inflorescences 1–2 flowered		25
- Leaflets larger, 5–10 (–13)-mm long; inflorescence 1-flowered	<i>C. pungens</i>	
25. Stipules consisting of one long horizontal spine, 10–25 mm and a vertical short spine, 1–10 mm; leaflets obovate or obovate-elongate, 3–5 (–8)-mm long; inflorescences 1-flowered, rarely 2–3 flowered	<i>C. macracanthum</i>	
- Stipules shorter, vertical spinelets, up to 8 mm, or foliate		26
26. Leaflets mostly spine shaped	<i>C. stapfianum</i>	
- Leaflets foliate		27
27. Stipules horizontal lanceolate perules, 2–5-mm long; leaflets in 3–7 pairs	<i>C. acanthophyllum</i>	
- Stipules minute foliate perules, adpressed to the stem, triangular-lanceolate; leaflets very small, 1–5 mm		28
28. Plant upright, ca 40 cm; leaf rachis ending in a sturdy spine; leaflets in 5–10 pairs, rotundate-ovate, 2–5-mm long, top with 3–7 teeth	<i>C. rechingeri</i>	
- Plant low, sturdy or more slender, ascendent; leaf rachis ending in a sturdy spine or a slightly incurved spiny tendril;		
- leaflets in 2–8 pairs; ovate to subrotundate, 1–5 (–6)-mm long, top with 1–3 (–5) teeth	<i>C. tragacanthoides</i>	
- Taller, slender forms from Kopet-dagh	var. <i>turcomanicum</i>	

11D. Key to the identification of *Cajanus* species

Asian and African species

1	a	Erect shrubs.....	2
	b	Climbing or creeping plants.....	8
2	a	Widely cultivated for seed, sometimes an escape to the wild; ripe seeds without strophiole or with small vestigial strophiole.....	4. <i>C. cajan</i>
	b	Occurring wild; ripe seeds with conspicuous strophiole.....	3
3	a	Leaflets elliptic-acuminate.....	4
	b	Leaflets obovate, tip rounded or acute.....	6
4	a	Leaflets with acute tip, indumentum greyish short.....	5
	b	Leaflets thick, with rounded tip, indumentum golden brown, copious, long on leaf margin (S. India, Sri Lanka, hill tops).....	29. <i>C. trinervius</i>
5	a	Leaflets short-elliptic; pod wall thick, sutures 1 mm wide, tipped by ca 10-mm style (W Africa).....	4. <i>C. kerstingii</i>
	b	Leaflet as long-elliptic; pod wall thin, sutures inconspicuous, tipped by ca 2-mm style (E. Central India).....	5. <i>C. cajanifolius</i>
6	a	Leaves pinnately trifoliolate, leaflets rounded-obovate, whitish below; pods 4–6 seeded (Myanmar).....	22. <i>C. niveus</i>
	b	Leaves digitately trifoliolate, leaflets obovate-oblong, glaucous-green below	7
7	a	Leaflets broad, with acute to rounded tip, stipules short, 2–3 mm (India, W Ghats)..	18. <i>C. lineatus</i>
	b	Leaflets narrow, with rounded tip, stipules long, above 5 mm (India, W Ghats, E. Ghats)..	28. <i>C. sericeus</i>
8	a	Annual creeper in grass, pods flat, broad, papery.....	23. <i>C. platycarpus</i>
	b	Perennial creepers or twiners, pods narrower, more rounded and thicker.....	9
9	a	Leaves pinnately trifoliolate.....	10
	b	Leaves (sub)digitately trifoliolate.....	16
10	a	Leaflets membranaceous, thinly puberulous, pods with long caducous hairs.....	11
	b	Leaflets thick, more or less short indumentum.....	12
11	a	Calyx with few conspicuous bulbous-based hairs (Philippines).....	32. <i>C. volubilis</i>
	b	Calyx with fine hairs (India, Sri Lanka).....	13. <i>C. heynei</i>
12	a	Leaflets small, elliptic or obovate-obtuse, twiner in grasses (Asia, Africa, Australia)	27. <i>C. scarabaeoides</i>
	b	Leaflets larger, obovate-acuminate, climber in shrubs and trees.....	13
13	a	Flowers large, ca 25–30 mm (NE. India, China) corolla persistent, calyx with bulbous-based hairs.....	12. <i>C. grandiflorus</i>
	b	Flowers generally smaller, less than 15–28-mm long, calyx hairs not bulbous-based.....	14
14	a	Indumentum fine, spreading, green, bracts very hairy; corolla not persistent (India, SE. Asia).....	11. <i>C. goensis</i>
	B	Indumentum short, dense and grey or golden brown below, bracts short-puberulous; corolla persistent.....	15
15	a	Leaflets semi-coriaceous, densely grey-hairy below, end leaflets longer than broad; pods 8–10 seeded; flowering after the monsoon (Himalayan foothills above 800 m).....	21. <i>C. mollis</i>
	b	Leaflets coriaceous, brown-pubescent below, end leaflets broader than long; pods 3–5 seeded; flowering the first months of the year (India, below 800 m, SE. Asia).....	9. <i>C. crassus</i>
16	a	Leaflets obovate-rounded (S. India, Sri Lanka).....	17
	b	Leaflets obovate-acuminate (NE. India).....	18
17	a	Strong climber in trees, leaflets silvery below; pods (3–) 5–6 seeded.....	2. <i>C. albicans</i>
	b	Twiner in grasses, leaflets reticulate, densely grey-hairy below, pods (2–)3–4 seeded	26. <i>C. rugosu</i>

- 18 a Slender herbaceous twiner in grasses, woody rootstock; pods small 2–2.5 × 0.5–0.8 cm, reticulate, 3–4 seeded, glabrescent.....10. *C. elongatus*
 b More robust twiner; pods larger 2–3.5 × 0.8–1.1 cm, not reticulate, 5–6 seeded, densely pubescent with long brown hairs30. *C. villosus*

Australian species

- 1 a Shrubs, erect or with straggling branches.....2
 b Prostrately creeping plants, branches twining at the ends.....13
 2 a Cultivated, in Australia rather a new crop, or as an escape to the wild; ripe seeds without strophiole or with small vestigial strophiole.....4. *C. cajan*
 b Occurring wild, ripe seeds with conspicuous strophiole.....3
 3 a Leaflets narrow-lanceolate, 3 (or 1) per leaf.....15. *C. lanceolatus*
 b Leaflets rhomboid, ovate, obovate or rounded, 3 per leaf.....4
 4 a Leaves digitately trifoliolate.....7 *C. confertiflorus*
 b Leaves pinnately trifoliolate.....5
 5 a Leaflets thin-coriaceous to membranaceous, pubescence very short, apex acute.....6
 b Leaflets thick-coriaceous, pubescent, apex more obtuse.....8
 6 a Shrub with straggling branches, leaves viscid31. *C. viscidus*
 b Shrub, erect, leaves glandular but not sticky.....7
 7 a Leaflets elongate to rounded-ovate, apex acute, almost non-aromatic, pods (1–)2–4 seeded.....1 *C. acutifolius*
 b Leaflets broadly ovate, apex, acute aromatic; pods (6–) 8–10 seeded.....3. *C. aromaticus*
 8 a Stems very thick also towards the apex, whitish-pubescent; leaves very thick.....9
 b Stems thin also towards the apex, pubescence grey or brown; leaves reticulate, not so thick10
 9 a Indumentum white very dense, covering stems and leaves; inflorescences much longer (up to 14 cm) than the leaves (up to 7 cm)8. *C. crassicaulis*
 b Leaves woolly, green with yellow-brown veins, young stems and peduncles visible through the white hairs; inflorescence as long as the leaves (up to 8–9 cm)..16. *C. lanuginosus*
 10 a Calyx teeth lanceolate or acuminate.....11
 b Calyx teeth broad-acuminate.....17. *C. latisepalus*
 11 a Leaflets often large, rhomboid to rounded, up to 12.5 cm long, tip acute to rounded, pubescence relatively thin, hairs long, on new leaves and branches dense and conspicuously golden brown, more rarely grey; calyx teeth linear-lanceolate, curved in open flower.....25. *C. reticulatus*
 b Leaflets smaller, to 5 (~7) cm long, elliptic to obovate, tip obtuse, pubescence silvery grey to brown; calyx teeth short-acuminate.....12
 12 a Leaflets quite thick, upper side reticulate, veins concolorous, top leaflets with 5–6(–8) pairs of major secondary veins, pubescence short, greyish below, not filling reticulations; pods narrow, short, pubescent, sutures narrow..... 24. *C. pubescens*
 b Leaflets thick, upper side flat, veins whitish, top leaflet with 7–9 pairs of major secondary veins, pubescence very short, close, velvety, filling reticulations; pods broad, grey-velvety, pubescence very short, sutures broad.....6. *C. cinereus*
 13 a Leaflets rounded, apex obtuse or emarginate or acuminate; pods flat, broad.....20. *C. marmoratus*
 b Leaflets obovate or lanceolate14
 14 a Leaflets lanceolate; pods broad, flat, variegated with purple19. *C. mareebensis*
 b Leaflets obovate; pods small, more rounded, uniformly colored27. *C. scarabaeoides*

11E. Key to the identification of *Arachis* species

(Source: T. Stalker, unpublished)

1. Leaf with 3 leaflets. Erect plants; hypocotyls in the form of tube-flowers and pods grouped at the base.
 1. Peg horizontal at the surface, much spread..... **Section Trierectoides**
 2. Leaflets linear-lanceolate, rigid, up to 22.5-cm long \times 0.6-cm wide..... *A. guaranitica*
 2. Leaflets obtuse, elliptical-lanceolate, 2.5-cm long \times 1-cm wide, obovate and smaller towards the base of the branch..... *A. tuberosa*
1. Leaf with 4 leaflets — hypocotyl with cylindrical form.
 3. Plant without rhizomes.
 4. Pods with 2 or 3 segments. Cotyledons with veins very deep in the upper side. Branch decumbent. Flowers and pods spread all along the branch. Standard with red lines in both sides. Perennial plants..... **Section Triseminatae**
 - Root axonomorphic, without thickenings. Main stem erect. Stipules joined at the base making a short tube and subulatus. Small flowers, hypanthium 45-mm long. Standard orange with a prominent purple spot at the base. Pod with 3 segments: peg and isthmus long, horizontal; segments with one seed, pericarp flat, with a dense cap of hair..... *A. triseminata*
 4. Pods with 1 or 2 segments. Cotyledons with the upper face flat.
 5. Standard with red lines in the down face or in both sides. Procumbent branches.
 6. Perennial plants, root thickened. Standard with red lines only on the lower side. All flowers normal, with corolla expanded..... **Section Extmervosae**
 7. Leaflets 3–4 \times longer than wide, upper face of the leaflet glabrous, lower face with bristles at the marginal veins..... *A. setinervosa*
 7. Leaflets less than 3 \times longer than width, bristles if present perpendicular to the margin, not Qt the veins.
 8. Upper leaflet face glabrous.
 9. Leaflets 2 \times longer than wide. Segment of the pod 10–15-cm long \times 6–7-mm wide..... *A. macedoi*
 9. Leaflets less than 1.6 times longer than wide.
 10. Erect plants. Leaflets with the margin thickened, 40-mm long \times 25-mm wide. Segment of the pod 14–16-mm long. \times 5–8-mm wide..... *A. marginata*
 10. Prostrate branch. Leaflets with the margin not marked, less than 20 mm long. Segment of the pod less than 9-mm long \times 6-mm wide.
 11. On the prostrate branch the leaflets are oblong or elliptical to obavate (1.4–1.8:1).
 12. Pod's pegs with adventitious roots. Leaflets elliptical to obovate (1.4–1.5:1)..... *A. prostrata*
 12. Pegs without adventitious roots. Leaflets oblong, to obovate (1.6–1.8:1)..... *A. lutescens*
 11. Leaflets suborbiculate (1.0:1). Pegs with adventitious roots..... *A. retusa*
 8. Upper face of leaflets hairy, principally on the younger leaves.
 13. Primary branches with 5-cm long. Lateral branches prostrate with leaflets up to 13-mm long \times 10-mm wide. Segment of the pod less than 11 \times 7 mm..... *A. burchellii*
 13. Primary branches with more than 40-cm long.
 14. Segment of the pod with 8-mm long \times 5-mm wide. At the laterals branches leaflets 12–23-mm long \times 5–10-mm wide..... *A. pietrairellii*
 14. Segment of the pod 16–23-mm long \times 8–10-mm wide. Leaflets 16–44-mm long \times 5–16-mm wide..... *A. villosulicarpa*

- 6 Annual plants, roots without thickenings, Standard with red lines on lower side or both sides, lowers dimorphic, normally opens very little with corolla smaller than the calyx..... **Section Heteranthae**
- 15 Stem, stipules and petiole covered exclusively by rigid bristles, 2–3-mm long. Petioles canal very narrow, separated from rachis by prominent hairs on the side of canal. Leaflets with the upper face glabrous, Standard with red lines in both sides..... *A. giacomettii*
- 15 Stem, stipules and petioles covered by hair of different lengths, hairs are 2-mm long, smooth, undulated, not rigid, Petiole-rachis's canal large, separated or not separated.
- 16 Standard orange with red lines only in the back face; yellow wings, Petioles canal separate the rachis's canal by transverse line with hairs. Bristles at the lower face are scattered. Pericarp flat. Leaflet almost always with hairs in both faces, rarely with the upper face glabrous..... *A. sylvestris*
- 16 Standard with red lines in both faces. Upper face glabrous.
- 17 Petiole's canal separated from the rachis's canal by a transverse line with hairs. Frequently with ordinate bristles line parallel to the margin on the lower face. Pericarp flat. Yellow wings with the apices and margin interior orange..... *A. pusilla*
- 17 Petiole's canal almost always separated from the rachis' canal. Lower face without bristles. Pod's segment reticulated; when drooping, epicarp vicious yellow wings..... *A. dardani*
- 5 Standard with red lines in the upper face.
- 18 Erect plants or decumbent. Flowers density grouped in the base of the plants. Normally only these flowers produce pods. But the flowers at branch's base that are inside the soil also produce pods. Roots and branch are thick (except in *A. stenophylla* and *A. paraguayensis*)..... **Section Erectoides**
- 19 Roots with laterals thickened. Branch straight, not undulated.
- 20 Leaflet suborbicular, ovate or ovate-lanceolate, length/width ratio smaller than 4:1 (can go up to 4.2:1 in *A. douradiana, maximum*).
- 21 Leaflets apical obovate to suborbicular and this basal leaflets elliptic, small, 7–12-mm longer × 4–9-mm width, upper face very short hairs. Base face woolly, with bristles. Stipules woolly, without bristles..... *A. mattii*
- 22 Short petiole, 2–4-mm long. Stipules and petioles woolly. Ellipsoid seeds, 11-mm long × 3.5-mm wide..... *A. brevipetiolata*
- 22 Petiole longer than 5 mm. Seeds more thick, 5–7-mm width.
- 23 Leaflets with the upper face glabrous and with the lower face with addressed hairs.
- 24 Leaflets with the margin very marked in both sides, very hairy. Leaflets usually obovate and frequently with bristles in the lower side. Stipules and petiole without bristles. Petiole 8–15 (up to 20)-mm long *A. oteroi*
- 24 Leaflets with the margin slightly marked only at the lower side, commonly elliptical.
- 25 Stipules and petioles with bristles. Petioles 15–28-mm long..... *A. hatschbachii*
- 25 Stipules and petioles without bristles. Petiole 30–38-mm long..... *A. cryptopotamica*

- 23 Leaflets with short hairs over all the surface in the upper face, and some times only at the base on the main vein.
- 26 Big plants, very high branching, with n+3 branch. Leaflets commonly without bristles in the lower face.....*A. major*
- 26 Smaller plants, little branching, erect secondary branches, very little arch at the base, the branching ends in short n+2 branch, no longer than 10 cm
- 27 Leaflets with height/width ratio 1.5-2.5:1 (rarely 3), frequently with bristles in lower face, ciliated at margins, bristles rare. Petiole 10-30-mm long.....*A. benthamii*
- 27 Leaflets with height/width ratio 2.3-4.2:1, without bristles. Petioles commonly 6 mm or longer, rarely 9-mm long.....*A. douradiana*
- 20 Leaflets large-elliptical, ovate-lanceolate, length/width ratio 3-8:1 (less ratio at the base of branch, but at end of branch the leaflets have a ratio bigger than 5:1), generally with bristles in the lower face.
- 28 Petioles and stipules with bristles stipules with margins joined making a short tube. Length/width ratio of the leaflets 4-8:1. Petioles 10-25-mm long.....*A. gracilis*
- 28 Petioles and stipules without bristles.
- 29 Tube at the base of the stipule up to 9-mm long. Leaves spaced, internode up to 50-mm long, petiole 15-35-mm long. Length/width ratio 37:1.....*A. hermannii*
- 29 Tube at the base of the stipules up to 4-mm long. Leaves grouped towards the top of the branch, internodes 10-15-mm long. Petiole frequently 10-15-mm long (rarely up to 30 mm).....*A. archeri*
- 19 Root taxonomorphic, with slender branching, without thickenings. Stem undulated or something twisted. Margin of the leaflets marked.
- 30 Leaflets linear-lanceolate, less than 10-mm wide, length/width ratio 7-12:1.....*A. stenophylla*
- 30 Leaflets with more than 10-mm wide, length/width ratio less than 5:1.
- 31 Length/width ratio of the upper leaflets 2.6-3.4:1 and of the down leaflets 3.3-4.3:1. Margin and veins very marked in the lower face.....*A. paraguariensis* ssp. *paraguariensis*
- 31 Leaflets wider, with veins and margins not as visible, length/width ratio of the two distal leaflets 2-3:1 and of the lower leaflet 2.5-3.5:1.....*A. paraguariensis* ssp. *capibarensis*
- 18 Branch procumbent. Base of the plant without flowers; inflorescence and pods spread by the branch. In *A. appressipila* (sect. Procumbentes) the branch is not decumbent, and does not have flowers grouped in the base of the plant.
- 32 Stem with roots at the nodes.....**Section Caulorhizae**
- 33 Leaflets more than 2.5 × more height than width. Plants without bristles, rarely exceptions where little bristles are present on petiole.....*A. repens*
- 33 Leaflets less than 2 × more height than width. Bristles present on stem, petiole and rachis, frequently too on leaflet down face.....*A. pintoii*
- 32 Stem without roots at the nodes, sometimes roots seen at both basal internodes, in the soil.
- 34 Horizontal peg, very spreading and superficial.....**Section Procumbentes**
- 35 Lateral branch's leaflets with length/width ratio less than 2:1
- 36 Short leaves, lateral branch's leaflets up to 19 mm × 10 mm, glabrous. Stem without adventitious roots. Standard organge.....*A. lignosa*

- 36 Bigger leaves.
- 37 Leaflet glabrous, up to the 35 mm × 19 mm *A. kretschmeri*
- 37 Lower face with small adpressed hairs.
- 38 Leaflets up to 30 mm × 17 mm. Yellow corolla. Stem without adventitious roots *A. rigonii*
- 38 Leaflets up to 24 mm × 18 mm. Standard orange. Stem with adventitious roots *A. chiquitana*
- 35 Lateral branch's leaflets with length/width ratio more than 2.5:1.
- 39 Leaflet length/width ratio of 2.5–3.5:1.
- 40 Lateral branches procumbent, with leaflets up to the 43 mm × 13 mm, glabrous. Stipules violet in the base
- 40 Lateral branches decumbent, with leaflets up to 50 mm × 16 mm, with glabrous upper face and the back face with adpressed hairs. Stipules green *A. appressipila*
- 39 Leaflet length/width ratio of 4 to more than 7:1.
- 41 Stipule with very short bristles. Peg thickened, hollow *A. vallsii*
- 41 Stipules with long bristles or without bristles. Peg delicate, compact *A. subcortacea*
- 34 Peg almost vertical **Section Arachis**
- 42 Pod with two segment, separated by one isthmus. Peg fragile.
- 43 Annuals or biennial plants.
- 44 Villous peg with bristles.
- 45 Pod's segment reticulated *A. glandulifera*
- 44 Peg glabrous or with little hairs, without bristles.
- 45 Pod's segment plain *A. cruziana*
- 46 Pod's segment with marked reticulations.
- 47 Lower face of leaflet subglabrous. with hairs adpressed very short and with little hairs long at the medium vein and at the margin
- 48 Stipules with bristles.
- 49 Pod's segment up to the 21 mm × 9 mm, 2n = 40 *A. monticola*
- 49 Pod's segment up to the 17 mm × 9 mm, 2n = 20 *A. magna*
- 48 Stipules without bristles. Segment up to the 17 mm × 10 mm. 2n = 20 *A. ipaensis*
- 47 Lower face only with hairs with 2 mm long at the medium vein and at the margin.
- 50 Stipules without bristles. Pod's segment up to the 17 mm × 10 mm *A. valida*
- 50 Stipules with bristles. Pod's segment up to 12 mm × 7 mm *A. williamsii*
- 46 Pod's segment plain or smooth reticulated.
- 51 Standard yellow, with color pink-violet at the back face. Stipules with bristles. Lower face with hairs 2 mm long *A. batizocoi*
- 51 Standard orange or yellow, without violet color.
- 52 Lower face with hairs 2-mm long, scattered.
- 53 Stipules without bristles. Upper face glabrous *A. duranensis*
- 53 Stipules with bristles. Frequently too with long hairs at the upper face of the younger leaf *A. hoehnei*
- 52 Lower face glabrous
- 54 Stipules without bristles.
- 55 Pod's segment with 14–22-mm long × 5–7-mm width *A. stenosperma*
- 55 Segment with less than 13-mm long × 6-mm wide
- 56 Calyx and hypanthium glabrous to subglabrous. Leaves glabrous *A. praecox*
- 56 Calyx and hypanthium with hairs.

- 57 Calyx with little silky hairs, without bristles. Leaflets glabrous *A. palustris*
57 Calyx villosus and with bristles. Lower face glabrous or with little long hairs at the median vein.
58 Stem only with little hairs at the younger parts *A. benensis*
58 Stem vicioux *A. trinitensis*
54 Stipules, petiole and rachis with numerous bristles *A. decora*
- 43 Perennial plants.
- 59 Lower face with hair 2-mm long, upper face glabrous. Stipules without bristles. Pod's segment plain *A. herzogii*
59 Lower face glabrous to villous, with hair not longer than 1 mm.
60 Pod's segment very reticulated.
61 Pod's segment up to 9 mm × 4 mm. Upper face glabrous, lower face with adpressed hairs *A. microsperma*
61 Segment 10–15 mm × 7–8 mm. Leaflets with both faces vicioux *A. villosa*
60 Segment plain or with reticulations only marked.
62 Leaflets glabrous, with some short bristles at the margin *A. helodes*
62 Leaflets with hairs, at least at the lower face.
63 Leaflet's margin with two classes of the hairs: short adpressed, and long (up to 2 mm) and frequently with 'almost' bristles *A. correntina*
63 Leaflet's margin with one or two classes of hairs.
64 Pod's segment short, 7–11-mm long × 4–8-mm wide.
65 Leaflet's margin with hairs, present on both faces *A. simpsonii*
65 Leaflet's margin only marked at the lower face.
66 At the lateral branches, leaflets always rounded, obtuse, big (1.4–1.7:1), with hairs margin and with almost bristles *A. cardenasii*
66 Leaflets short (1.6–2.9:1); margin with adpressed hairs and almost bristles *A. kempff-mercadoi*
64 Segment big, 10–14-mm long × 5–7-mm wide.
67 Leaflets from lanceolate to oblong-lanceolate (2.5–4.8:1), upper face with very little hairs *A. diogoi*
67 Leaflets oblong-lanceolate to obovate (1.4–2.3:1), upper face glabrous *A. kuhlmannii*
68 Main stem without flowers and n+1 branches in plants that alternate with regularity two vegetative branches and two reproductive (alternate branching) *A. hypogaea*
69 Leaflets with the lower face glabrous or with almost hairs at the main vein subsp. *hypogaea* var. *hypogaea*
69 Leaflets with hair at the lower face with 1–2-mm long, scattered over all the surface subsp. *hypogaea* var. *hirsuta*
68 Main stem with flowers and lateral branches at the reproductive and vegetative branch without order (sequential branching) subsp. *fastigiata*
70 Pods with more than two seeds. Extended frutification.
71 Leaflets with the down face glabrous or with hairs only under the median vein.
72 Pods with smooth or lightly marked reticulations, without surpassing longitudinal ribs
72 Reproductive branch almost always short and delicate ... subsp. *fastigiata* var. *fastigiata*
72 Pods always with prominent reticulation and longitudinal ribs.
Long reproductive branches (5–6 cm), strong; main stem like the lateral branches subsp. *fastigiata* var. *peruviana*
- 42 Pod without segment, with 1–5 seeds. Peg tenacious

- 71 Leaflets with hair at the lower face with 1–2-mm long, scattered. Reproductive branches long, principally at the lateral branch. Main stem almost always with inflorescence or short reproductive branch subsp. *fastigiata* var. *aequatoriana*
- 70 Pods almost always with two seeds. Pods clustered at the base of the plant. Frequently with compound spike subsp. *fastigiata* var. *vulgaris*
3. Plants with rhizomes **Section Rhizomatosae**
- 73 Leaflets coriaceous, with the margin marked in both faces. Standard with red lines on both faces. $2n = 20$ **Section Rhizomatosae** series **Prorhizomatosae**
 Leaflets coriaceous, with the margin salient in both sides. Standard orange with lines red in the both sides *A. burkartii*
- 73 Leaflets with margin lightly marked only at lower face. Standard with red lines at the upper face. $2n = 40$ **Section Rhizomatosae** series **Rhizomatosae**
 74 Plant all creeping, with the leaves placed back to back at the soil. Upper face shiny, almost always with small scattered bristles.
 Pericarp reticulated *A. pseudo villosa*
 74 Plant somewhat tall, with the leaves separated from the soil. Upper face plain, without bristles. Pericarp plain *A. glabrata*
 75 Leaflets more or less oblong *A. glabrata* var. *glabrata*
 75 Leaflet lanceolate *A. glabrata* var. *hagenbeckii*

Further Reading

Bhattacharjee R, Khairwal IS, Bramel PJ and Reddy KN. 2007. Establishment of a pearl millet [*Pennisetum glaucum* (L.) R. Br.] core collection based on geographical distribution and quantitative traits. *Euphytica* 155:35-45.

Bramel-Cox PJ and Christinck A. 1998. Participatory methods to enhance the quality of germplasm collections. Pages 1-8 *in* Participatory Plant Improvement: proceedings of the Workshop on Farmer Participatory Methods in Research and Development for the Semi-Arid Tropics, 27–28 October 1998, ICRISAT, India. Chennai, India: MS Swaminathan Research Foundation.

Brown AHD. 1989. The case for core collection. Pages 136-155 *in* The use of plant genetic resources. (Brown AHD, Frankel OH, Marshal DR and Williams, eds.). Cambridge: Cambridge University Press.

Chakrabarty SK, Anitha K, Girish AG, Sarath Babu B, Prasada Rao RDVJ, Varaprasad KS, Khetarpal RK and Thakur RP. 2005. Germplasm exchange and quarantine of ICRISAT mandate crops. Information Bulletin no. 69. Rajendranagar 500 030, Andhra Pradesh, India: National Bureau of Plant Genetic Resources; and Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics. 80 pp. ISBN 92-9066-481-9.

Clayton WD and Renvoize SA. 1982. Pages 672-690 *in* Flora of Tropical East Africa, Gramineae (Part 3). (Polhill RM, ed.). Rotterdam: AA Balkema..

Ellis RH, Hong TD and Roberts EH. 1985. Handbook of seed technology for genebanks. Volume I. Principles and methodology. Handbook for genebanks. No. 2. Rome, Italy: International Board for Plant Genetic Resources. 210 pp.

Engels JMM and Ramanatha Rao V. 1998. Regeneration of seed crops and their wild relatives. Proceedings of a consultation meeting, 4–7 December 1995, ICRISAT, Hyderabad, India. Rome, Italy: International Plant Genetic Resources Institute.

Food and Agriculture Organization of the United Nations/International Plant Genetic Resources Institute. 1994. Genebank Standards. 13 pp.

Frankel OH. 1984. Genetic perspective of germplasm conservation. Pages 161-170 *in* Genetic manipulations: Impact on man and society. (Arber WK, Llimensee K, Peacock WJ, Starlinger P, eds.). Cambridge: Cambridge University Press.

Grenier C, Bramel PJ and Hamon P. 2001a. Core collection of sorghum:1. Stratification based on eco-geographical data. *Crop Science* 41:234-240.

Grenier C, Hamon P and Bramel-Cox PJ. 2001b. Core collection of sorghum: II. Comparison of three random sampling strategies. *Crop Sci.* 41:241-246.

Guarino L, Ramanatha Rao V and Reid R. (eds.). 1995. Collecting plant genetic diversity. Technical Guidelines, Wallingford, UK: CAB International. 747 pp.

Hamilton NRS and **Chorlton KH**. 1997. Regeneration of accessions in seed collections: a decision guide. (J Engels, ed.). Handbook for Genebanks No. 5, Rome, Italy: International Plant Genetic Resources Institute.

Hanson J. 1985. Procedures for handling seeds in genebanks. Practical Manual for Genebanks: No. 1. Rome, Italy: International Board for Plant Genetic Resources. 115 pp.

Harlan JR and **de Wet JMJ**. 1972. A simplified classification of cultivated sorghum. *Crop Science* 12:172-176.

International Bureau for Plant Genetic Resources (IBPGR) and International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). 1992a. Descriptors for groundnut. International Board for Plant Genetic Resources, Rome, Italy and International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India. 123 pp.

International Bureau for Plant Genetic Resources (IBPGR) and International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). 1993a. Descriptors for sorghum [*Sorghum bicolor* (L.) Moench]. International Board for Plant Genetic Resources, Rome, Italy and International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India. 38 pp.

International Bureau for Plant Genetic Resources (IBPGR) and International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). 1993b. Descriptors for pearl millet [*Pennisetum glaucum* (L.) R. Br.]. International Board for Plant Genetic Resources, Rome, Italy and International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India. 43 pp.

International Bureau for Plant Genetic Resources (IBPGR) and International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). 1993c. Descriptors for pigeonpea [*Cajanus cajan* (L.) Millsp.]. International Board for Plant Genetic Resources, Rome, Italy and International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India. 31pp.

International Bureau for Plant Genetic Resources (IBPGR), International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and International Center for Agricultural Research in the Dry Areas (ICARDA). 1993. Descriptors for chickpea (*Cicer arietinum* L.). International Board for Plant Genetic Resources, Rome, Italy; International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India and International Center for Agricultural Research in the Dry Areas, Aleppo, Syria. 31 pp.

Johns MA, Skroch PW, Nienhuis J, Hinrichson P, Bascur G and Munoz-Schick C. 1997. Gene pool classification of common bean landraces from Chile based on RAPD and morphological data. *Crop Sci.* 37:605-613.

Kameshwara Rao N and Paula J Bramel. (eds.). 2000. Manual of Genebank Operations and Procedures. Technical Manual no. 6. Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.

Lazarides M, Hacker JB and Andrew MH. 1991. Taxonomy, cytology and ecology of Indigenous Australian sorghums (*Sorghum bicolor* Moench: Andropogoneae: Poaceae). Australian Systematic Botany 4:591-635.

Liu K and Muse SV. 2005. Power marker: Integrated analysis environment for genetic marker data. *Bioinformatics* 21:2128-2129.

Mehra KL, Arora RK and Wadhi SR. (eds.). 1981. Plant Exploration and Collection (South Asian Training Course Lectures). NBPGR Science Monograph No. 3. New Delhi, India: National Bureau of Plant Genetic Resources. 132 pp.

Pande S, Kishore GK, Upadhyaya HD and Narayan Rao J. 2006. Identification of sources of multiple disease resistance in mini-core collection of chickpea. *Plant Disease* 90:1214-1218.

Perrier X, Flori A and Bonnot F. 2003. Data analysis methods. Pages 43-76 in Genetic diversity of cultivated tropical plants (Hamon P, Seguin M, Perrier X and Glaszmann JC, eds.). Montpellier: Enfield, Science Publishers.

Prasada Rao KE and Ramanatha Rao V. 1995. The use of characterization data in developing a core collection of sorghum. Pages 109-116 in Core collection of plant genetic resources (Hodgikin T, Brown AHD, van Hintum Th JL and Morales BAV, eds.). Chichester, UK: A Wiley-Sayee Publication.

Reddy LJ, Upadhyaya HD, Gowda CLL and Sube Singh. 2005. Development of core collection in pigeonpea [*Cajanus cajan* (L.) Millsp.] using geographic and qualitative morphological descriptors. *Genetic Resources and Crop evolution* 52:1049-1056.

Upadhyaya HD, Bhattacharjee R, Hoisington DA, Chandra S, Varshney RK, Reddy KN and Saxena KB. 2006a. Molecular characterization of pigeonpea [*Cajanus cajan* (L.) Millspaugh] composite collection. Poster presented in the Generation Challenge Program Annual Research Meeting, 12-16 September 2006, Sao Paulo, Brazil.

Upadhyaya HD, Bramel PJ, Ortiz R and Sube Singh. 2002. Developing a mini core of peanut for utilization of genetic resources. *Crop Science* 42:2150-2156.

Upadhyaya HD, Bramel PJ and Sube Singh. 2001a. Development of a chickpea core subset using geographic distribution and quantitative traits. *Crop Science* 41:206-210.

Upadhyaya HD, Dwivedi SL, Gowda CLL and Sube Singh. 2007a. Identification of diverse germplasm lines for agronomic traits in a chickpea (*Cicer arietinum* L.) core collection for use in crop improvement. *Field Crops Res.* 100:320-326.

Upadhyaya HD, Gowda CLL, Buhariwalla HK and Crouch JH. 2006b. Efficient use of crop germplasm resources: identifying useful germplasm for crop improvement through core and mini-core collections and molecular marker approaches. *Plant Genetic Resources* 4(1):25-35.

Upadhyaya HD, Gowda CLL, Pundir RPS, Reddy VG and Sube Singh. 2006c. Development of core subset of finger millet germplasm using geographical origin and data on 14 quantitative traits. *Genet Res. and Crop Evolution* 53:679-685.

Upadhyaya HD, Gowda CLL, Reddy KN and Sube Singh. 2009a. Augmenting the pearl millet [*Pennisetum glaucum* (L.) R. Br.] core collection for enhancing germplasm utilization in crop improvement. *Crop Science* 49:573-580.

Upadhyaya HD and Ortiz R. 2001b. A mini core subset for capturing diversity and promoting utilization of chickpea genetic resources in crop improvement. *Theor. Appl. Genet.* 102:1292-1298.

Upadhyaya HD, Ortiz R, Bramel PJ and Singh S. 2001c. Development of a groundnut core collection from Asia region. Pages 43-44 *in* Proceedings of the Diamond Jubilee Symposium (Abstracts). Hundred years of Post-Mendelian Genetics and Plant Breeding - Retrospect and Prospects, 6-9 November 2001. (Kharakwal MC, Mehra RB, eds.). New Delhi, India: Indian Society of Genetics and Plant Breeding.

Upadhyaya HD, Ortiz R, Bramel PJ and Singh S. 2003. Development of a groundnut core collection using taxonomical, geographical and morphological descriptors. *Genet. Resour. Crop Evol.* 50:139-148.

Upadhyaya HD, Pundir RPS, Dwivedi SL, Gowda CLL, Reddy VG and Singh S. 2009b. Developing a mini core collection of sorghum for diversified utilization of germplasm. *Crop Sci.* 49:1769-1780.

Upadhyaya HD, Pundir RPS, Gowda CLL, Reddy KN and Sube Singh. 2005. Geographical pattern of diversity for qualitative and quantitative traits in the pigeonpea germplasm collection. *Plant Genetic Resources: Characterization and Utilization* 3:332-352.

Upadhyaya HD, Pundir RPS, Gowda CLL, Reddy VG and Sube Singh. 2008. Establishing a core collection of foxtail millet to enhance the utilization of germplasm of an underutilized crop. *Plant Genetic Resources: Characterization and Utilization* 7(2):177-184.

Upadhyaya HD, Reddy LJ, Gowda CLL, Reddy KN and Sube Singh. 2006d. Development of mini core subset for enhanced and diversified utilization of pigeonpea germplasm resources. *Crop Science* 46:2127-2132.

Upadhyaya HD, Reddy KN, Gowda CLL and Silim SN. 2007b. Patterns of diversity in pigeonpea [*Cajanus cajan* (L.) Millsp.] germplasm collected from different elevations in Kenya. *Genet. Resour. and Crop Evol.* 54:1787-1795.

van der Maesen LJG. 1987. Origin, history and taxonomy of chickpea. Pages 11-34 *in* The Chickpea (Saxena MC and Singh KB, eds.). Wallingford, UK: CAB International.

van der Maesen LJG. 1990. Pigeonpea: origin, history, evolution and taxonomy. Pages 15-46 *in* The Pigeonpea (Nene YL, Hall SD and Sheila VK, eds.). Wallingford, UK: CAB International.

Ward J. 1963. Hierarchical grouping to optimize an objective function. *J. Am. Stat. Assoc.* 38:236-244.

Appendix 1

Maximum tolerable difference between the number of seeds, which is rarely exceeded by chance alone, (probabilities of 5.0% 2.5% 1.0% or 0.1%) for two 100-seed replicates of a germination test.

Average germination (%) of replicates		Probability (%)			
		5%	2.5%	1%	0.1%
99	2	3	4	4	5
98	3	4	5	5	7
97	4	5	6	6	8
96	5	5	6	7	9
95	6	6	7	8	10
94	7	7	8	9	11
93	8	7	8	9	12
92	9	7	9	10	13
91	10	8	9	10	13
90	11	8	9	11	14
89	12	9	10	11	15
88	13	9	10	12	15
87	14	9	11	12	16
86	15	9	11	13	16
85	16	10	11	13	17
84	17	10	11	13	17
83	18	10	12	14	17
82	19	10	12	14	18
81	20	11	12	14	18
80	21	11	13	14	19
79	22	11	13	15	19
78	23	11	13	15	19
77	24	11	13	15	19
76	25	12	13	15	20
75	26	12	14	16	20
74	27	12	14	16	20
73	28	12	14	16	21
72	29	12	14	16	21
71	30	12	14	16	21
70	31	12	14	17	21
69	32	13	14	17	21
68	33	13	15	17	22
67	34	13	15	17	22
66	35	13	15	17	22
65	36	13	15	17	22
64	37	13	15	17	22
63	38	13	15	17	22
62	39	13	15	17	22
61	40	13	15	18	23
60	41	13	15	18	23
59	42	13	15	18	23
58	43	13	15	18	23
57	44	13	15	18	23
56	45	14	16	18	23
55	46	14	16	18	23
54	47	14	16	18	23
53	48	14	16	18	23
52	49	14	16	18	23
51	50	14	16	18	23

Maximum tolerable difference between the number of seeds, which is rarely exceeded by chance alone, (probabilities of 5.0% 2.5% 1.0% or 0.1%) for two 50-seed replicates of a germination test.

Average germination (%) of replicates		Probability (%)			
		5%	2.5%	1%	0.1%
99	2	2	2	3	4
98	3	3	3	4	5
97	4	3	4	4	6
96	5	4	4	5	7
95	6	4	5	6	7
94	7	5	5	6	8
93	8	5	6	6	8
92	9	5	6	7	9
91	10	5	6	7	9
90	11	6	7	8	10
89	12	6	7	8	10
88	13	6	7	8	11
87	14	6	7	9	11
86	15	7	8	9	11
85	16	7	8	9	12
84	17	7	8	9	12
83	18	7	8	10	12
82	19	7	8	10	13
81	20	7	9	10	13
80	21	8	9	10	13
79	22	8	9	10	13
78	23	8	9	10	13
77	24	8	9	11	14
76	25	8	9	11	14
75	26	8	9	11	14
74	27	8	10	11	14
73	28	8	10	11	14
72	29	9	10	11	15
71	30	9	10	11	15
70	31	9	10	12	15
69	32	9	10	12	15
68	33	9	10	12	15
67	34	9	10	12	15
66	35	9	10	12	15
65	36	9	10	12	15
64	37	9	11	12	16
63	38	9	11	12	16
62	39	9	11	12	16
61	40	9	11	12	16
60	41	9	11	12	16
59	42	9	11	12	16
58	43	9	11	12	16
57	44	9	11	13	16
56	45	9	11	13	16
55	46	9	11	13	16
54	47	10	11	13	16
53	48	10	11	13	16
52	49	10	11	13	16
51	50	10	11	13	16

Maximum tolerable difference between the number of seeds, which is rarely exceeded by chance alone, (probabilities of 5.0% 2.5% 1% or 0.1%) for two 25-seed replicates of a germination test.

Average germination (%) of replicates		Probability (%)			
		5%	2.5%	1%	0.1%
99	2	1	2	2	3
98	3	2	2	3	3
97	4	2	3	3	4
96	5	3	3	3	5
95	6	3	3	4	5
94	7	3	4	4	5
93	8	3	4	5	6
92	9	4	4	5	6
91	10	4	4	5	7
90	11	4	5	5	7
89	12	4	5	6	7
88	13	4	5	6	7
87	14	4	5	6	8
86	15	5	5	6	8
85	16	5	5	6	8
84	17	5	6	6	8
83	18	5	6	7	9
82	19	5	6	7	9
81	20	5	6	7	9
80	21	5	6	7	9
79	22	5	6	7	9
78	23	6	6	7	9
77	24	6	6	7	10
76	25	6	7	8	10
75	26	6	7	8	10
74	27	6	7	8	10
73	28	6	7	8	10
72	29	6	7	8	10
71	30	6	7	8	10
70	31	6	7	8	10
69	32	6	7	8	11
68	33	6	7	8	11
67	34	6	7	8	11
66	35	6	7	8	11
65	36	6	7	8	11
64	37	6	7	8	11
63	38	6	7	9	11
62	39	6	7	9	11
61	40	6	7	9	11
60	41	7	8	9	11
59	42	7	8	9	11
58	43	7	8	9	11
57	44	7	8	9	11
56	45	7	8	9	11
55	46	7	8	9	11
54	47	7	8	9	11
53	48	7	8	9	11
52	49	7	8	9	11
51	50	7	8	9	11

Appendix 2

Plant quarantine Import Permit (IP) requirements of different countries in respect of ICRISAT mandate crops (as in 2005). Four countries: El Salvador, Guatemala, Mexico and Sri Lanka also require Certificate of origin of germplasm.

List of the countries for Import permit requirement.

Date:

S.No.	Name of the country	Import Permit	S.No.	Name of the country	Import Permit
	Africa:			Asia:	
1	Algeria	Not required	1	Afghanistan	Not required
2	Angola	Not required	2	Bahrain	Not required
3	Benin	Not required	3	Bangladesh	Required
4	Botswana	Required	4	Bhutan	Not required
5	Burundi	Not required	5	Cambodia	Not required
6	Burkina Faso	Not required	6	China	Required
7	Cameroon	Not required	7	East Timor	Required
8	Canary Is.	Not required	8	Hong Kong	Not required
9	Cape Verde Is.	Not required	9	Indonesia	Not required
10	Central African Rep.	Not required	10	Iran	Required
11	Chad	Not required	11	Iraq	Not required
12	Comoro Is.	Not required	12	Israel	Required
13	Congo	Not required	13	Japan	Required
14	Côte d'Ivoire	Required	14	Jordan	Required
15	Djibouti	Not required	15	Korea DPR	Not required
16	Egypt	Not required	16	Korean Rep.	Not required
17	Eritrea	Required	17	Kuwait	Not required
18	Ethiopia	Required	18	Laos PDR	Not required
19	Gabon	Not required	19	Lebanon	Not required
20	Gambia	Not required	20	Malaysia	Required
21	Ghana	Not required	21	Maldives	Not required
22	Guinea	Not required	22	Micronesia	Not required
23	Guinea Bissau	Not required	23	Myanmar	Not required
24	Kenya	Required	24	Nepal	Not required
25	Lesotho	Not required	25	Oman	Not required
26	Liberia	Not required	26	Pakistan	Required
27	Libya	Not required	27	Philippines	Required
28	Malagasy Rep.	Not required	28	PR China	Not required
29	Malawi	Required	29	Qatar	Not required
30	Mali	Not required	30	Rep. of Palau	Not required
31	Mauritania	Not required	31	Saudi Arabia	Not required
32	Mauritius	Required	32	Singapore	Not required
33	Morocco	Not required	33	Sri Lanka	Required
34	Mozambique	Not required	34	Syria	Not required
35	Namibia	Required	35	Taiwan	Not required
36	Niger	Required	36	Thailand	Not required
37	Nigeria	Required	37	UAE	Not required
38	Rep. Fr. Mayotte	Not required	38	Uzbekistan	Required
39	Rwanda	Not required	39	Vietnam	Required
40	Senegal	Not required	40	Yemen	Not required
41	Sierra Leone	Not required			
42	Somalia	Not required			
43	South Africa	Required			
44	Sudan	Not required			
45	Swaziland	Not required			
46	Tanzania	Required			
47	Togo	Not required			
48	Tunisia	Not required			
49	Uganda	Required			
50	Zaire	Not required			
51	Zambia	Required			
52	Zimbabwe	Required			

S.No.	Name of the country	Import Permit	S.No.	Name of the country	Import Permit
Europe:			The Americas:		
1	Austria	Not required	1	Argentina	Required
2	Belgium	Not required	2	Belize	Not required
3	Bulgaria	Not required	3	Bolivia	Not required
4	Cyprus	Not required	4	Brazil	Required
5	Czech Rep.	Not required	5	Canada	Required
6	Denmark	Not required	6	Chile	Not required
7	France	Not required	7	Colombia	Required
8	Germany	Required	8	Costa Rica	Required
9	Greece	Not required	9	El Salvador	Required
10	Hungary	Not required	10	Ecuador	Not required
11	Italy	Required	11	Guatemala	Required
12	Moldavia	Not required	12	Guyana	Not required
13	Poland	Not required	13	Havai (USA)	Required
14	Portugal	Not required	14	Honduras	Required
15	Romania	Not required	15	Mexico	Required
16	Russia	Not required	16	Nicaragua	Required
17	Slovakia	Not required	17	Panama	Not required
18	Spain	Not required	18	Paraguay	Not required
19	Sweden	Not required	19	Peru	Required
20	Switzerland	Not required	20	Puerto Rico	Not required
21	The Netherlands	Not required	21	Surinam	Not required
22	Turkey	Not required	22	Uruguay	Not required
23	UK	Required	23	USA	Required
24	Ukraine	Not required	24	Venezuela	Not required
25	Yugoslavia	Not required	25	Virgin Is.	Not required
Caribbean Is.			Oceania:		
1	Antigua	Not required	1	Australia	Required
2	Bahamas	Not required	2	Caroline Is.	Not required
3	Barbados	Required	3	Fiji Is.	Required
4	Cuba	Not required	4	Niue Islands	Required
5	Dominican Rep.	Not required	5	New Zealand	Not required
6	Granada	Not required	6	Papua New Guinea	Required
7	Haiti	Not required	7	Solomon Is.	Required
8	Jamaica	Required	8	Tonga Is.	Not required
9	St. Helena	Not required	9	Vanuatu	Not required
10	St. Kitts & Nevis	Not required	10	Western Samoa	Required
11	St. Lucia	Not required			
12	Trinidad & Tobago	Required			

