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Manual of Genebank Operations and Procedures



International Crops Research Institute for the Semi-Arid Tropics

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ICRISAT

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Preface

Gene banks are biodiversity reservoirs and sources of alleles for sustainable genetic enhancement of crops plant. Efforts have been made to collect landraces, cultivars that were becoming obsolete, genetic stocks, and in some cases wild species important for crop improvement. *Ex situ* collections were assembled, followed by phenotypic and biochemical characterization. Well-endowed genebanks also conducted agronomic assessment after field-testing or resistance screening against pests and diseases. Seed or propagule regeneration and multiplication became a routine activity in many genebanks, despite the costs involved in maintaining a large germplasm collection. The operations of a modern genebank are not restricted to collection, characterization, regeneration and documentation. Breeding gains rely on access to useful genetic variation in the respective crop gene pools. If genes available in wild species are to be put into a usable breeding form, it is important that the long-term research agenda includes development of advanced gene pool stocks. In recent years, some genebanks have made significant investments in studies to determine the extent of genetic diversity, because this knowledge enables proper germplasm organization and development of improved parents and new cultivars. In this way, genebank curators can maximize the utilization of wild and cultivated gene pools in crop breeding. Well-documented analysis of the number and types of useful polymorphism allow genebank curators to offer specific accessions with the desired characteristics to research geneticists or applied plant breeders, who can then select material tailored to their objectives.

This manual of Genebank Operations and Procedures at ICRISAT provides information in 10 chapters regarding germplasm assembly, plant quarantine, seed processing and storage, germplasm distribution, monitoring, germplasm regeneration, characterization and preliminary evaluation, and taxonomic classification. We hope, this manual helps other genebank curators and researchers in their work for preserving plant biodiversity in *ex situ* collections. We welcome any suggestions to improve the manual in further editions.

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Manual of Genebank Operations and Procedures

Introduction

Replacement of traditional landraces by modern, less heterogeneous high yielding cultivars, as well as large scale destruction and modification of natural habitats harboring wild species are leading to genetic erosion in important food crops. Genetic variation from traditional landraces and wild species is essential for crop improvement, e.g., to combat pests and diseases and to produce better adapted cultivars for constantly changing environments. ICRISAT responded to this need by establishing a Genetic Resources Unit with the specific objective of assembly, characterization, evaluation, maintenance, conservation, documentation and distribution of germplasm of the mandate crops (sorghum, pearl millet, chickpea, pigeonpea and groundnut) and their wild relatives, and six minor millets (finger millet, foxtail millet, barnyard millet, kodo millet, little millet and proso millet).

With over 113 500 accessions from 130 countries conserved in genebank, ICRISAT acts as world repository for the genetic resources of its mandate crops. The germplasm was assembled at ICRISAT through donations from various institutes and by launching collections in areas of origin and diversity of the mandate crops, jointly with National Agricultural Research Systems (NARS). The assembled germplasm was initially held under short-term conditions, at 15–20°C and 50–60% relative humidity (RH), in isolated store rooms cooled with ordinary air-conditioners and dehumidified with commercial dehumidifiers. The collections were transferred to medium-term storage rooms at 4°C and 20–30% RH in 1980. ICRISAT, in collaboration with NARS also started collecting germplasm from priority areas, and as the world collection grew in size, the medium-term conservation facilities were expanded in 1985 to accommodate up to 150 000 germplasm accessions. The long-term conservation facilities at –20°C became operational in 1991 and germplasm transfer for conservation as base collection is now underway.

History of ICRISAT germplasm collection

In 1960s, the Indian Agricultural Programme of the Rockefeller Foundation assembled over 16 000 sorghum germplasm accessions from major sorghum growing areas, and ICRISAT acquired about 8961 accessions of this collection in 1974 through the All India Coordinated Sorghum Improvement Project (AICSIP) in Rajendranagar and another 3000 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 2000 pearl millet germplasm accessions assembled by the Rockefeller Foundation in collaboration with the Indian Council of Agricultural Research (ICAR) in New Delhi, and another 2000 accessions collected by the Institut Français de Recherche Scientifique pour le Développement en Coopération (ORSTOM) in Francophone West Africa.

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), the United States Department of Agriculture (USDA), and Karaj Agricultural University in Iran. Sets of this germplasm placed in several agricultural research institutes in India and Iran, and at the USDA were donated to ICRISAT in 1972. ICRISAT also acquired over 1200 chickpea accessions from the Arid Lands Agricultural Development Program (ALAD) with its headquarters in Beirut (Lebanon), supported by the Ford Foundation (USA), and International Development Research Center (Canada). Similarly, much of the groundnut germplasm initially assembled at ICRISAT was received from the collections maintained by the Indian national program such as the National Research Center for Groundnut (Junagadh), USDA, especially the Southern Regional Plant Introduction Station, and North Carolina State University.

ICRISAT soon assumed the responsibility to add germplasm and enlarge the world collections of the five mandate crops and special efforts were made to collect or assemble landraces and wild relatives from areas threatened by genetic erosion. Between 1974 and 1997, ICRISAT launched 212 collection missions in areas of diversity and collected 8957 sorghum, 10 802 pearl millet, 4228 chickpea, 3870 pigeonpea and 2666 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), AICSIP, and several Indian agricultural universities were responsible to enlarge the collections.

All incoming samples have been examined by the National Bureau of Plant Genetic Resources (NBPGR) for exotic diseases and pests. The Indian Government has set up a quarantine unit within ICRISAT to ensure prompt and expeditious inspection and clearance of seed shipments and receipts.

Facilities for germplasm conservation at ICRISAT

All the ICRISAT mandate crops produce orthodox seeds, which withstand desiccation to low moisture contents. Therefore, seed storage is the principle method of conservation of their genetic resources. Seeds are stored in controlled environments of 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, which do not produce adequate quantity of seeds (e.g., *Arachis* spp.), are maintained as live plants in the botanical garden and screenhouse.

The Genetic Resources Unit has the following facilities for germplasm conservation:

- 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 rooms with a capacity of 210 m³ each and four rooms with a volume of 125 m³ each, at 4°C and 20–30% RH to hold active collections.
- Three long-term storage rooms at –20°C each with a volume of 125 m³ to store base collections of germplasm.
- A seed-drying room and two drying cabinets with a combined volume of 100 m³ at 15°C and 15% RH.

- An air-cooled screenhouse with an area of 402 m² for maintaining wild species of groundnut.
- A field genebank for wild species of pearl millet, sorghum and pigeonpea.
- A seed laboratory for conducting germination tests, seed research and cytological work.
- Access to >10 ha of field space each cropping season on ICRISAT campus at Patancheru for multiplication and regeneration of germplasm and field characterization and evaluation.

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 long periods of 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.

Genebank operations (Fig. 1)

Assembly of germplasm through collection in areas of known genetic diversity or through correspondence from other plant introduction centers 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 germplasm, which is not represented in the collection. The Convention on Biological Diversity (CBD), which came into force on 29 December 1993, now provides the framework for acquisition and utilization of germplasm. Consistent with Article 15 of CBD, which recognizes the sovereign rights of nations over their biodiversity, collection and acquisition of germplasm are undertaken with prior informed consent, using material acquisition agreements 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. Further, 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 conducted in the postrainy season, and to minimize genetic drifts adequate number of plants are grown and sampled equally. 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 bonafide users. However, ICRISAT collections were later placed under the auspices of the Food and Agriculture Organization of the United Nations (FAO) following an agreement signed on 26 October, 1994 with the FAO. Germplasm accessions are now distributed only to those recipients who sign material transfer agreements that prevent the recipients from claiming intellectual property rights.

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



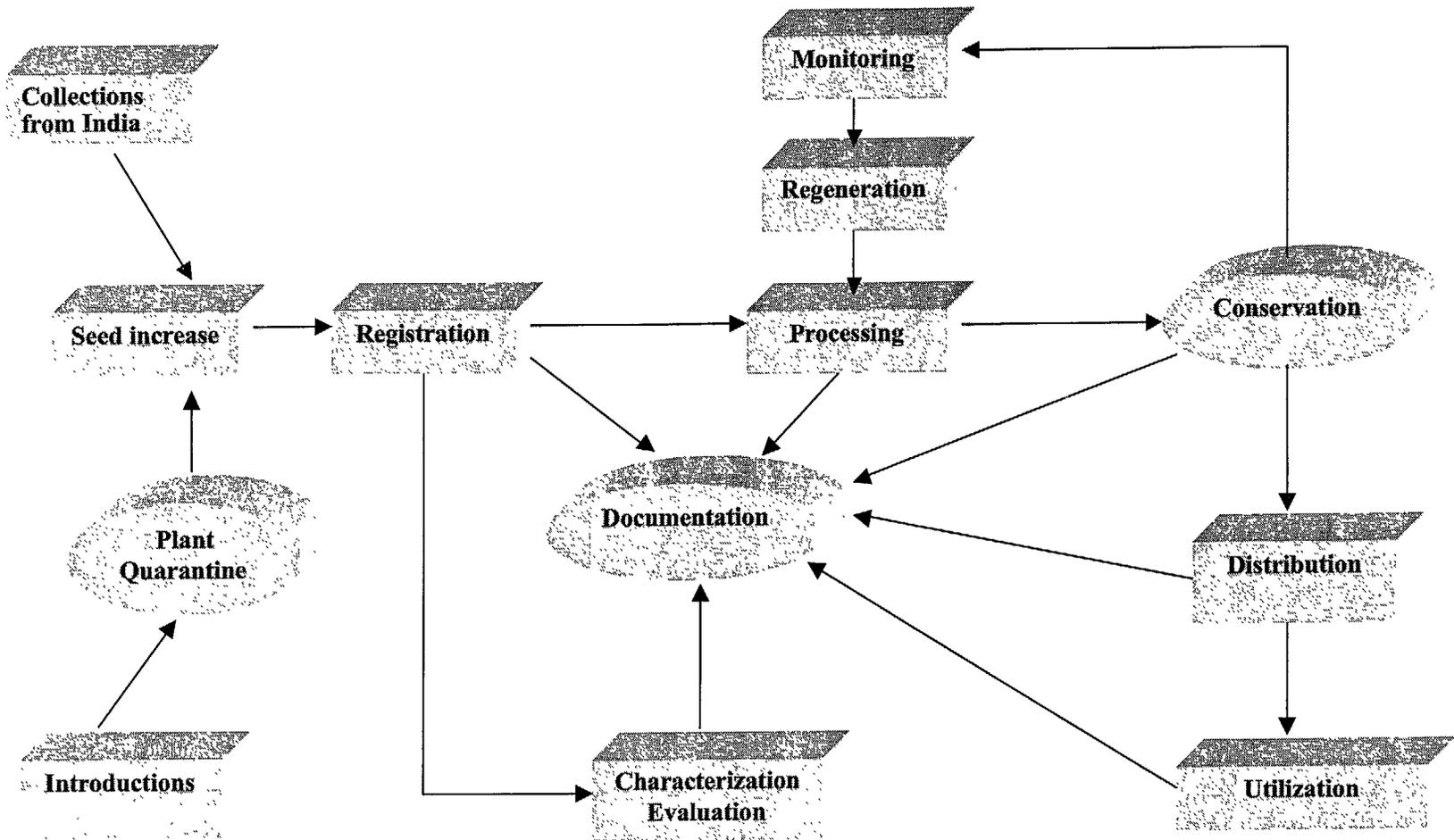


Figure 1 — Operational flow chart of ICRISAT genebank.

characters and a few environmentally influenced agronomic and quality traits. The morphological and agronomic characters are scored in field plots during the rainy and postrainy seasons. Characterization and evaluation data facilitate preliminary selection of germplasm by users, while information on country of origin, site and location of collection, and pedigree, among other passport data, permits the selection of germplasm on geographic basis.

Work with genetic resources is associated with management of large volumes of information. There are five 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 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.

The various activities involved in germplasm conservation are described in details in the following sections of this manual.

Section 1

Germplasm Assembly

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

- A. exploration and collection from farmers' fields and wild habitats, particularly in areas known as centers of diversity, and
- B. by securing materials of interest through correspondence from other plant introduction centers, individual scientists, private growers, seed companies or other germplasm suppliers.

A. 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, cold tolerance, or wild relatives, weedy types and related taxa of a crop, and
- ecosystem focussed missions to collect maximum diversity in different crops occurring and maturing almost at the same time in the region.



- *Germplasm collecting 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.*

Planning collection missions

The main focus of collection in the past has been conservation of the broad range of diversity in the landraces or wild species for use in breeding programs. The collection team consisted of an expert on the crop from the center and a local expert. The collection sites were selected based on the knowledge of the crop specialist or priorities set by the breeders. Subsequent to the coming into force of 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, i.e., national governments providing the resource. 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 NARS. The purpose of collection could be for :
 - national genebank,
 - breeding programs,
 - development 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 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-Government Organizations (NGOs), and
 - community seed banks or other seed distribution agencies.

Once the decision is made that collecting is necessary, technical and logistic planning begins. 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 are other important considerations in determining collection priorities, and
- 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.*
- *Critically study the provenance data on the herbarium sheets and 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 and, therefore, timing of the collection mission is important. Crop cycle and the 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.

Technical preparation

Collecting germplasm requires meticulous planning. The explorer needs to be in the right area at 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, and
- 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, and*
- *bilateral material acquisition agreements for transfer of the collected material incorporating provisions for further handling, storage, regeneration, utilization and distribution (sample Germplasm Acquisition Agreement [GAA] given as Annexure 1.1).*

Collecting team

Collecting teams should always be small — not more than three or four; and include a woman. The team leader should be preferably a botanist or an agronomist. The team should consist of:

- representative of NARS,
- a local expert, and
- a driver with some knowledge of the target region, who should be able to speak local language and carry out basic repairs of 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.

Equipment

The basic equipment needed for collection is as follows:

1. Harvesting

- cloth bags or nylon net bags
- seed envelopes
- strong knife or secateurs
- scissors
- absorbent paper for pressing specimen
- drying stove and stand
- packing sacks
- digger and sieves for sifting soil
(important for collection of groundnut
and its wild species)
- field or collector's notebooks
- rubber bands for closing bags
- labels (preferably tear-off tags) for
labeling specimens
- plant press with corrugated aluminum sheets
- pencil, pens and permanent markers of
different colors
- stapler and staples
- pocket knife
- 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
- film rolls
- 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
- visiting cards
- Import Permit or other required permits
- information on fuel points
- printed slips with institute's address
- collection data sheets
- herbarium and quarantine labels

4. Personal care

- lightweight 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

- card-board 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
- first-aid kit
- snake-bite kit for commonly found poisonous snakes
- anti-itch creams or antihistamines
- antiseptic cream or liquid
- insecticide sprays or repellent creams
- pain-killer pills
- antipyretics (paracetamol or aspirin)
- antacid tablets
- 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
- one set of spare parts and tools
- two spare tyres
- pump and pressure gauge
- puncture repair kit
- two jerricans for petrol
- engine driven winch and chain or nylon rope

When explorations are planned in foreign countries, transportation arrangements and acquisition of above listed articles 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 sleeve shirts with several 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, and*
- *Import Permit from home country if the material is to be transported back.*

Sampling strategy

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

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 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.
 - Samples can be taken from 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 major routes since introduction of advanced cultivars begins in regions close to major roads.
-

- Ascertain source of material 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.

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 (e.g., 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 quantity of seeds 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 nonrandom samples may be collected if the collector sees interesting forms, which are not included by random sampling.

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 in the logbook.
- Hold discussions with local officers, block or village extension workers, old farmers, school teachers in area of halt 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 jeep 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 and photograph herbarium specimens (especially wild species).
- 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 and before retiring, re-equip your bag with items needed for the next day's collecting.

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,



- *Be inquisitive to acquire information on anything interesting.*
- *Do not be overzealous 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.*

- status of sample (wild, weedy, cultivated)
- source of collection (field, market sample or farm store), and
- labeling of the collection bags both within and outside.

For convenience in the field, carry standardized collecting record books (specimen page presented as Annexure 1.2).

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

- Identify the collection site precisely. 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, including:
 - farmers' name and description of environment,
 - landrace or cultivars characteristics as described by the farmer,
 - end use of the landrace or cultivars and its specific properties,
 - normal cultural practices used with the landrace or cultivars, and
 - history of the landrace or cultivars with the farmer.

A farmer's survey form incorporating the above details developed and used by Bramel-Cox and Christinck (1998) for sorghum is in Annexure 1.3.

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 (e.g., muslin bags) when panicles or mature pods are 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.

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 the time the collection ends and it is transported back to the genebank.

In line with the revised procedures, ICRISAT's primary focus is 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. Only those cultivars of value are acquired by ICRISAT using appropriate GAA (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.

B. 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 concurrence with Article 15 of 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.

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 "MATCH" developed at ICRISAT.

Acquiring unique germplasm from other genebanks

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

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 program "MATCH" was developed to handle such events.

- Prepare the final list of unique accessions to be acquired.
- If the material is to be received in India from abroad, obtain an Import Permit from the National Plant Quarantine Service, Government of India by applying to the Director, NBPGR, New Delhi on a prescribed form (Annexure 1.4).
- Send the final list of accessions along with the Import Permit, green labels for affixing on seed packages, with the following guidelines to the consignor of seed export to India.

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 1.5).
- The green labels are to be affixed or pasted on the outside of the seed package.
- The consignors should not address the seed consignment to ICRISAT, but he/she should send it directly to Director, NBPGR, New Delhi.
- The seed material should be free from soil.
- The seeds should be free of infections or infestations and free of weed seeds, crop residues and inert material.
- Seed samples should not be treated with chemicals.

C. Germplasm assembly from internal programs

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.

Acquisition of elite breeding material

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

D. 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.

Germplasm Acquisition Agreement (GAA) for Material Intended for Designation

[Nation or Supplier] grants germplasm and related information to the *International Crops Research Institute for the Semi-Arid Tropics (ICRISAT)* under the terms and conditions of this agreement. The germplasm being provided is identified in the attached list, which forms part of this agreement.

[Nation or Supplier] warrants that it is legally free to provide the germplasm to *ICRISAT*, and that all necessary permissions have been obtained.

ICRISAT will hold the germplasm in trust under the terms of an agreement between *ICRISAT* and *FAO* (attached), place it in its genebank, periodically regenerate it, duplicate it for security reasons, and provide long-term conservation.

ICRISAT will be free to make the germplasm and related information, its progeny, and genes in it available to any third party for agricultural conservation, research and breeding purposes, but may do so only under a material transfer agreement, with terms intended to restrict the recipient from obtaining intellectual property rights on the material itself and to require similar commitments from any further recipients.

Signed

Signed

Farmer's Survey Form

Farmer's name _____

Site characteristics

Topography (local name) _____

Site (local name) _____

Soil (local name) _____

Farmer's description of landraces

Landrace name _____

Type within landrace characteristics _____

Farmer's:

Collector's:

Morphological

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	
Building	Low	Medium	High	

Disease problems

Problem	Susceptible	Resistance

Insect problems

Problem	Susceptible	Resistance

Weed, storage pest, or bird problems

Problem	Susceptible	Resistance

PTO

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

	Bitter	Sweet
Taste		
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



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

APPLICATION FOR PERMIT TO IMPORT SEEDS/PLANTING MATERIALS
(FOR RESEARCH PURPOSE)
(Please type/write in block letters)

The Director
National Bureau of Plant Genetic Resources
Pusa Campus
New Delhi 110 012

I hereby apply for a permit authorising the import of seeds/planting materials for research purpose as per details given below:

1. Name and address of the applicant :
2. Exact description of seed/planting material to be imported :
 - a) common name and botanical name :
 - b) germplasm / variety / hybrid / composite / synthetic :
 - c) form of material required (seed / rooted plants / scions / tubers / cuttings / bulbs etc) :
 - d) parentage, if known :
 - e) place of collection / origin of the material to be imported (country / state) :
 - f) name and address of the organisation/ institution producing the material :
3. Quantity to be imported (separately for each accession/variety/hybrid) :
4. Suggested source of availability of material including published reference, if known :

PTO

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5. Whether the aforesaid germplasm/variety/ hybrid was imported by you earlier? If so, details thereof (year, quantity, source etc.)
Was the material shared with other scientists National Gene Bank at the NBPGR? :
6. Expected date and mode of arrival in India (Airmail/air freight/accompanied baggage) :
7. Place where imported seeds/planting material will be grown and scientists under whose supervision the seeds/planting material will be grown :

DECLARATION

I hereby declare that the germplasm under import has no commercial value / exclusive ownership and may be shared freely for research purposes.

Place:

Signature of the Applicant

Date:

Address:

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Section 2

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Plant Quarantine

Plant quarantine helps in safe introduction of new seed samples from other countries. The work is done with assistance from NBPGR, the plant quarantine authority of the Government of India.

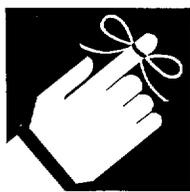
Plant quarantine regulations for seed import

Requirements for seed import

Seeds cannot be imported into India without an Import Permit issued by the NBPGR. The Genebank Curator must obtain the required permit and send it to the donating institution before ordering the seeds. The incoming seeds must be accompanied by a PSC obtained from the National Plant Quarantine Service of the exporting country.

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.

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



The Genebank should not acquire imported seed, unless it is cleared by National Plant Quarantine Services.

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

Pearl millet: Certification that seeds are free of downy mildew.

Chickpea: Certification that seed samples were collected from mother plants free of *Aschochyta rabiei* and viral diseases such as stunt and mosaic.

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.***

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.

Sorghum, pearl millet, pigeonpea and groundnut are required to be grown in Post-Entry Quarantine Isolation Area (PEQIA) to avoid possible introduction of seedborne diseases and pests.

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Sowings are done under the close supervision of the Plant Quarantine Officer. Optimum number of plants (minimum sample size) are grown to harvest sufficient quantity of seeds for storage and to maintain genetic integrity of the sample. A few seeds are saved for replanting in case of crop failure. If the number of plants is less than the number required for maintenance of genetic integrity, all seeds are used and the bottleneck recorded in genebank documentation. Optimal planting and crop management practices are used to ensure production of high quality seeds (see Section 7). 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,
- elimination of alien pollen by bagging inflorescence where needed,
- ensuring appropriate female-male combinations,
- harvesting at optimal maturity, and
- equal contribution of seed from each parent.

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

In the event of crop failure, Plant Quarantine is 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.

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

- Registration is done by assigning each sample a **unique accession number**, which distinguishes it from all other accessions in the genebank.
 - Record the accompanying data on country of origin, collection site, local names, and other basic information.
 - Request for any missing passport data when the material is logged-in or else 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.
- Assign an accession number only if the sample received is unique and sufficient seeds are available.
- 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.
- If the material is already represented in the genebank, grow the putative duplicate along side the existing accession to compare the morpho-agronomic characters. If convinced that they are duplicates, they can be bulked and maintained by the existing accession number.

A simple method for registration of the sample is given below:

1. Arrange the material received by genebank according to alphabetical order of names of variety or numeric order of number depending on the identification provided on the packets/bags.
2. Check all the packets against the list provided with the samples.
3. If no list was provided or seeds do not correspond with the list, prepare a new list. Check again to confirm that all packets have been included.
4. Check the passport database file or collectors' number and other information to ensure that the sample does not exist in the genebank.
5. Open each packet of seeds and inspect their condition by checking for any insect damage, fungal growth, damaged, broken, empty or shriveled seeds which probably would not germinate.
6. If the seeds are in poor condition, discard the sample and make note in the files of the action taken and reasons for it. Write to the donor for fresh samples.

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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.

Minimum standards for registration

Register and assign accession number to the incoming material only when the following criteria are met:

Passport information

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

- source country,
- location of collecting site (if relevant),
- local name or cultivar name,
- pedigree information for breeding lines and improved varieties, and
- source of collection (farmers' field, farm store, market, etc., if the sample is collected during a germplasm expedition)

Seed health

- Seeds should be absolutely free from pathogens and insects.

Seed quality and quantity

- Percent germination should not be <85% for cultivated and <75% for the wild species accessions (see Section 4D for germination testing).
- Seed quantity should be sufficient to conduct at least four regenerations.



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 meets the above standards and does not exist in the genebank, assign accession number following the procedure described below:

Procedure for registration

1. Check the passport data file to determine the last accession number used for the crop.
2. Assign the next ascending accession number to the first sample on the list and consecutive numbers to each sample (see Table 3.1 for crop identity codes).
3. Write the accession number clearly on the packet using a permanent marker and on the list of the new samples.
4. Enter the details in the passport data files 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 data file.
 - 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.
5. Once the sample is registered, clean, dry and pack the seeds in appropriate containers for storage as outlined in Section 4.
6. Pack a small sample (5–10 seeds or pods in legumes, 5 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.

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 in highly cross-pollinating crops such as pearl millet and pigeonpea.

Useful tips on numbering

The numbering system should be simple and practical to use.

- Use a strictly numeric system that is sequential in operation, e.g., 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 (e.g., IS 1, IS 2, IS 3... for sorghum, IP 1, IP 2, IP 3... for pearl millet, as practiced in ICRISAT genebank) (see Table 3.1).
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 3.1 — Botanical names and crop identity codes used for genebank accessions at ICRISAT.

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.) Millisp.	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 crus-galli</i> (L.) Beauv.	Iec
Proso millet	<i>Panicum miliaceum</i> L.	Ipm
Little millet	<i>Panicum sumatrense</i> Roch. ex Roem.& Schult.	Ipmr

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.

Passport data (based on International Plant Genetic Resources Institute [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.

Collecting 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.

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

Species: Species name in lowercase letters plus authority.

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

Accession 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.

Location of the collecting site: Location information below the country level that describes where the accession was collected starting with the most detailed information. Such information may include distance in kilometers and direction from the nearest town, or village.

Latitude of collecting site: Degrees and minutes followed by N (North) or S (South) (e.g., 1030S). Missing data (minutes) should be indicated with hyphen (e.g., 10-S).

Longitude of collecting site: Degrees and minutes followed by E (East) or W (West) (e.g., 07625W). Missing data (minutes) should be indicated with hyphen (e.g., 076-W).

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

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

Biological Status of sample:

- 1 Wild
- 2 Weedy
- 3 Traditional cultivar/Landrace
- 4 Breeder's line
- 5 Advanced cultivar
- 0 Unknown
- 99 Others

Collecting source:

- 1 Wild habitat
- 2 Farm
- 3 Market
- 4 Institute/research organization
- 0 Unknown
- 99 Others

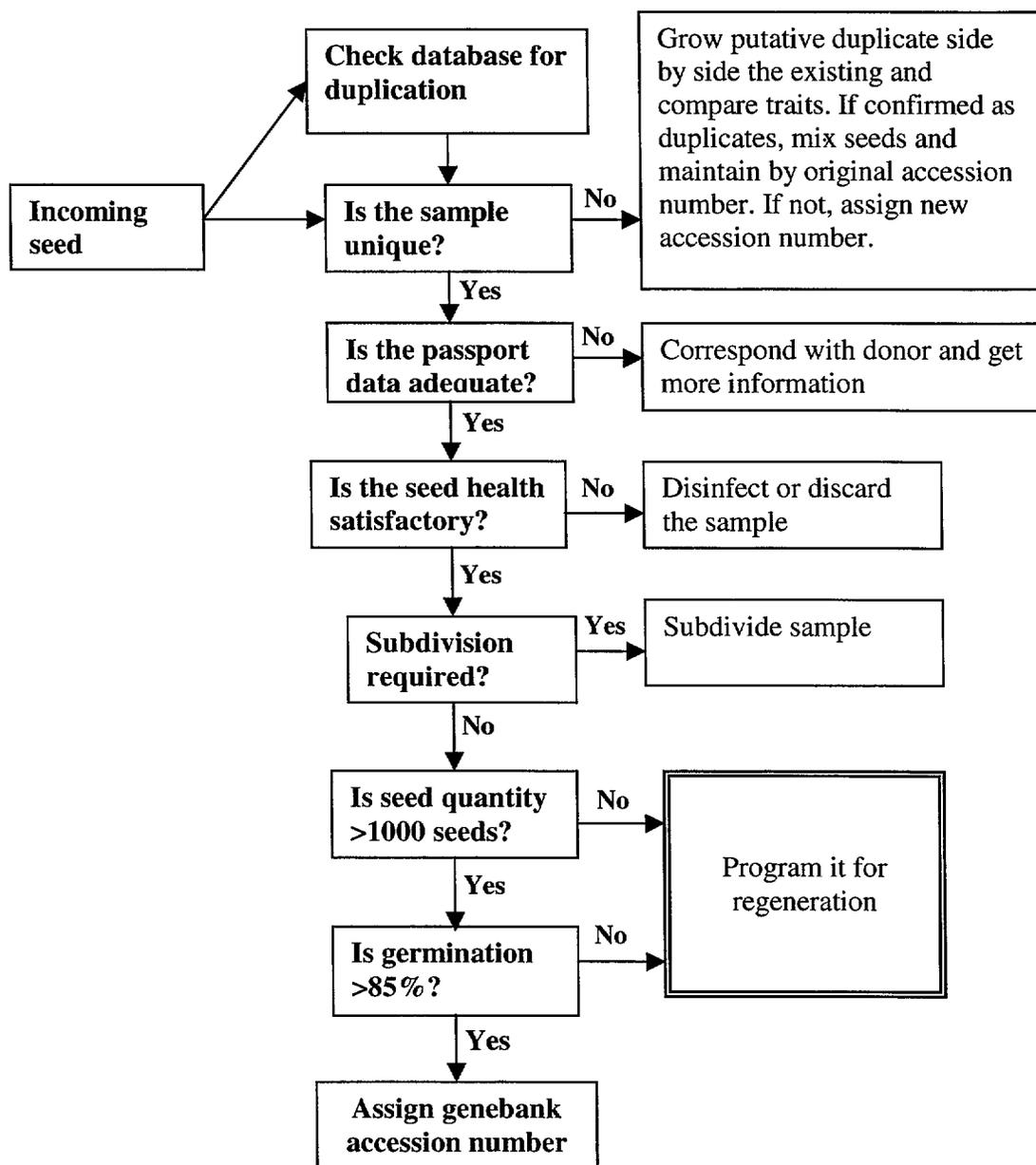
Donor institute code: Code for the donor institute. The code consists of a 3-letter ISO 3166 country code of the country where the institute is located.

Donor number: Number assigned to an accession by the donor. Letter should be used before the number to identify the genebank or national system.

Other number(s) associated with the accession: Any other identification number known to exist in other collections for this accession. Letters should be used before the number to identify the genebank or national system.

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 (e.g., Collecting source = roadside). Separate remarks referring to different fields are separated by semicolon.

Flow chart for registering new germplasm



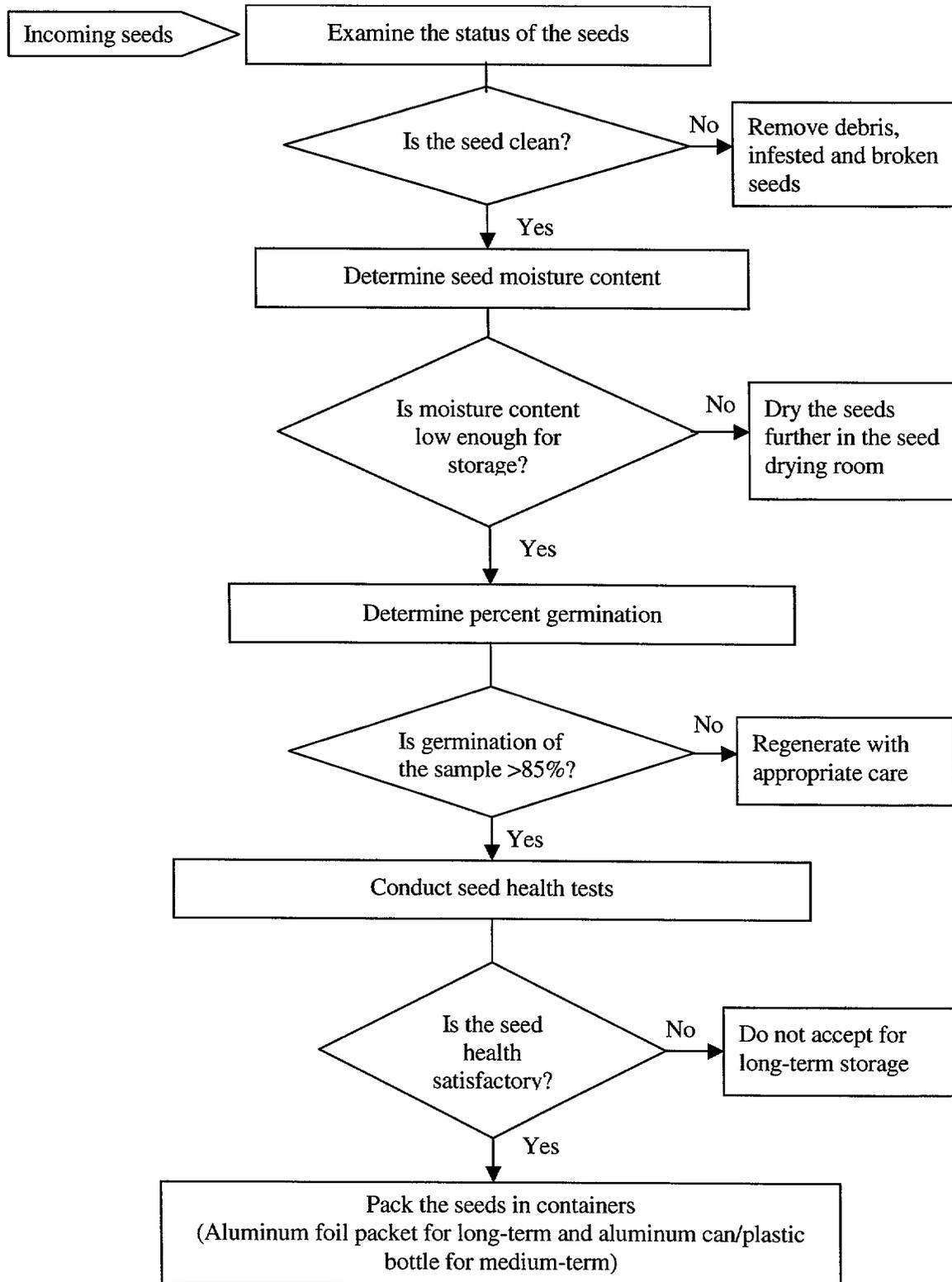
Section 4

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 nondestructive methods (e.g., Burrows Digital Moisture Computer) 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 it is predicted that the seed moisture content has reached 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. Seed lots that meet the minimum prescribed standards for seed health are only 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 which are regularly used or distributed (see Section 4E).
- The seeds are placed in the genebank and data on inventory is recorded, along with seed viability and related information.

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A. Seed cleaning

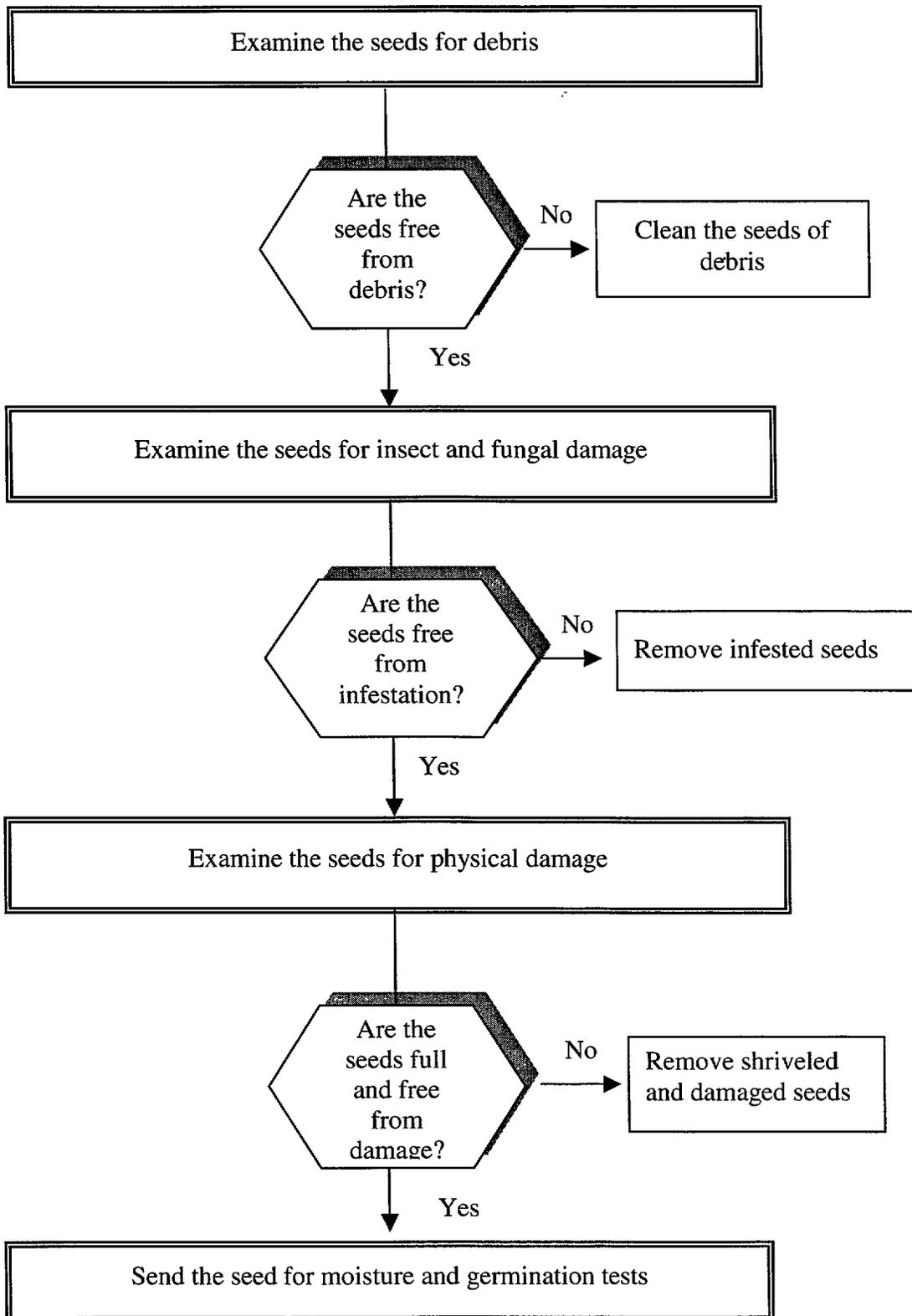
The cost of maintaining an accession is high and space is limited. Debris and damaged seeds can spread infection. Therefore, place only good quality viable seeds in storage.

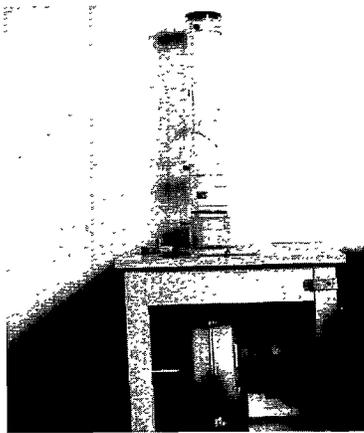
Seed cleaning involves removal of debris, low quality, infested or infected seeds and seeds of different species (weeds).

- Clean the seeds immediately after registration 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.
- Groundnut requires shelling before storage.
 - Shell the seeds manually.

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

1. Empty the contents of each packet into a labeled tray and assess the need for cleaning.
2. If cleaning is required, check that the seeds are dry enough to be cleaned without damage.
3. If the samples are moist, place them in the drying room to reduce the moisture content to <15%.
4. Remove debris from seeds by graded sieves (Fig. 4A.2), ensuring that small sized seeds in the sample are not separated.
5. Separate lightweight material and empty glumes by gentle winnowing or using the seed blower.
6. 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).
7. Discard any visually damaged, shriveled, infected or infested seeds.
8. Destroy any waste material to prevent the spread of the disease or insects to other material.

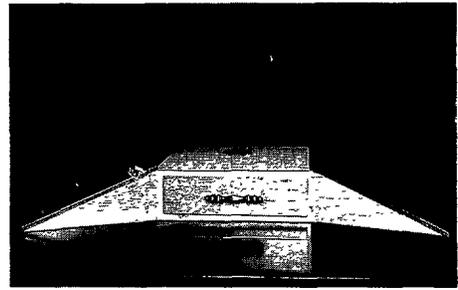




1. Seed blower



2. Graded sieves

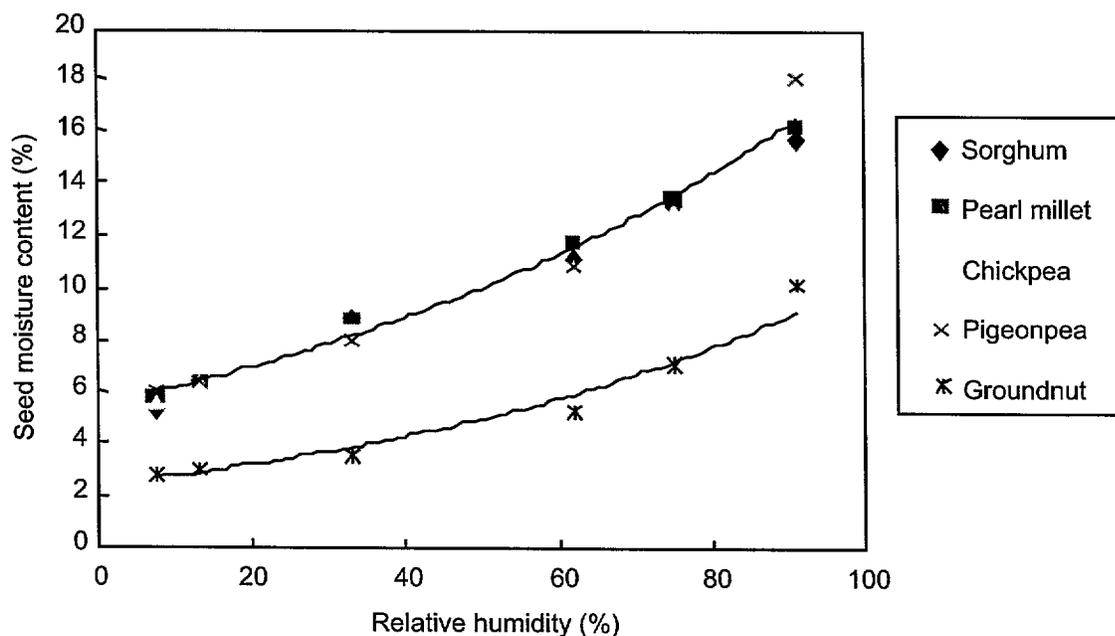


3. Purity workboard

Figure 4A — Seed cleaning equipment used in genebanks.

B. Seed moisture testing

The moisture content is the amount of water in the seed and is usually expressed as a percentage. Under all storage conditions, the moisture content of seeds comes to an equilibrium with the RH of the surrounding atmosphere. For a given species there is a definable equilibrium relationship between RH and seed moisture content. The equilibrium relationship between seed moisture content and RH at 25°C for ICRISAT mandate crops is presented below:



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 (nonoily seeds).

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

Moisture content determination

Equipment

The following equipment is necessary for determining the moisture content (see Fig. 4B.1):

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

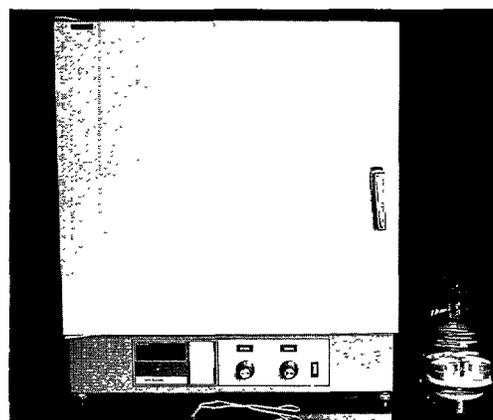
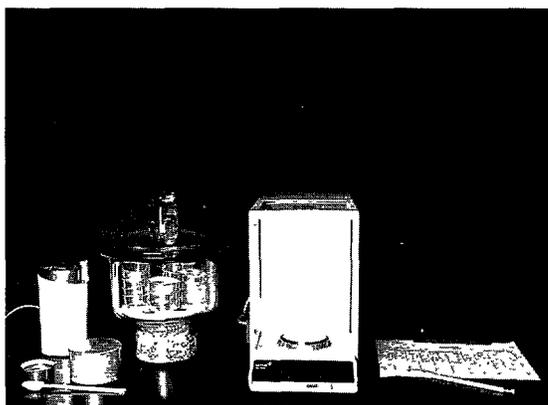


Figure 4B.1 — Equipment to determine seed moisture content

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, and
 - should not cause undue heating.
4. Analytical balance:
 - capable of weighing 0.01 mg.

5. Desiccator:
 - fitted internally with a thick metal or ceramic plate to promote rapid cooling of the containers, and
 - containing a desiccant like 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 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, and
- 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 lab bench, and
- weigh again to determine the loss of weight.

Determine the moisture content as described below:

1. Dry the containers at 130°C for 1 h and allow them to cool in the desiccator for 1 h.
2. Label and weigh each dish with the lid.
3. Place about 0.5–1.0 g of the sample (predried and ground if necessary) in the container, replace the lid and weigh again.
4. Place dishes with lids removed in the oven maintained at 130°–133°C for samples of nonoily seeds and at 103°±2°C for groundnut.
5. Dry:
 - sorghum and pearl millet samples for 2 h,
 - chickpea and pigeonpea for 1 h, and
 - groundnut for 16 h.

samples are kept in the oven and the oven door is closed.

6. Replace the lid on each dish from which it was removed at the end of the drying period.
7. Move the containers to a desiccator and allow them to cool for about 45 min.
8. Record the weight of the containers.
9. Calculate the moisture content on wet weight basis and express it as percentage to one decimal place.
10. 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:

$$\text{Percent (\%)} \text{ moisture content} = \frac{M_2 - M_3}{M_2 - M_1} \times 100$$

where, M_1 is the weight of the container with lid, M_2 is the weight of container and sample before drying and M_3 is the weight of container and sample after drying.

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

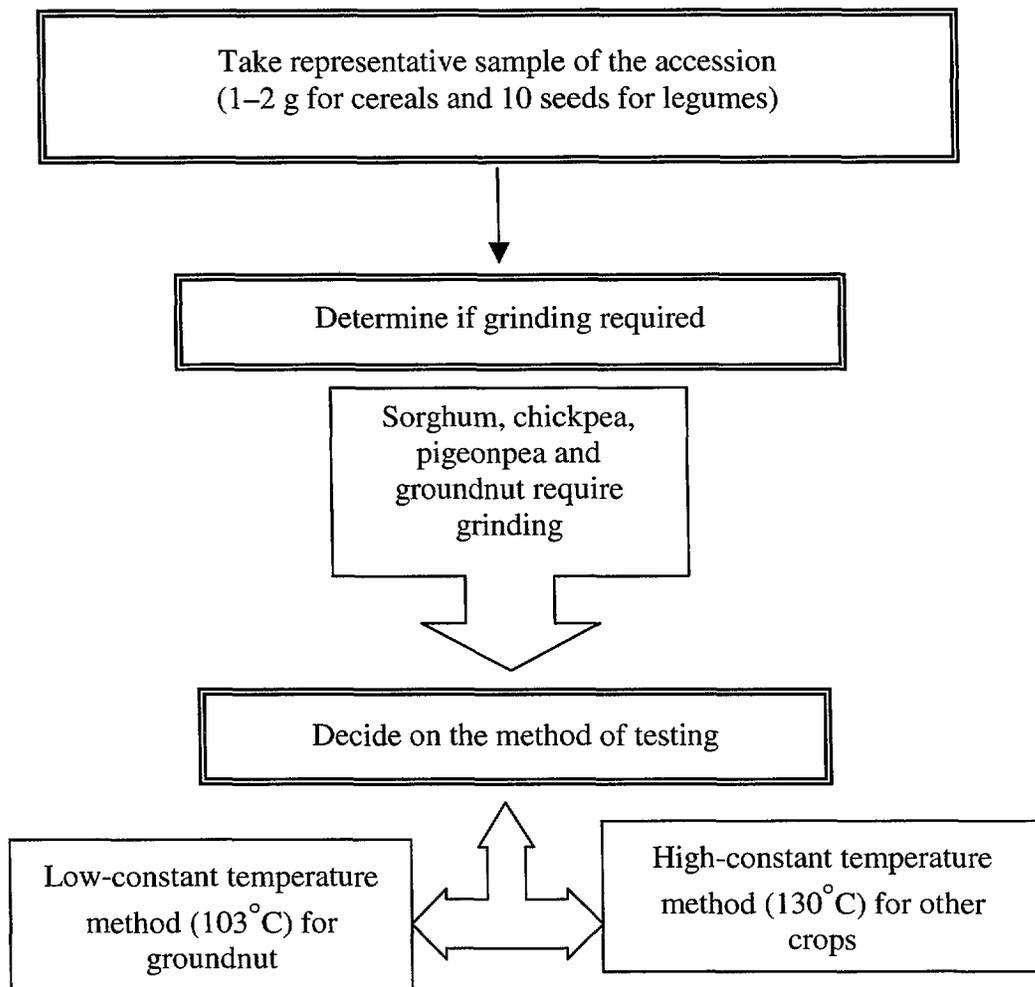
$$\text{Final moisture content (\%)} = S_1 + S_2 - \frac{(S_1 \times S_2)}{100}$$

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

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



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



C. 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

1. Estimate the moisture content of seeds using methods described in Section 4B and assess the need for drying depending on where the seed will 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.

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

The bags used for drying should allow moisture escape easily. Muslin cloth bags are best suited for this purpose.
3. Do not keep too many seeds in the same bag.
4. Close the bags properly to ensure there is no spill over and mixing of seeds.
5. 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.

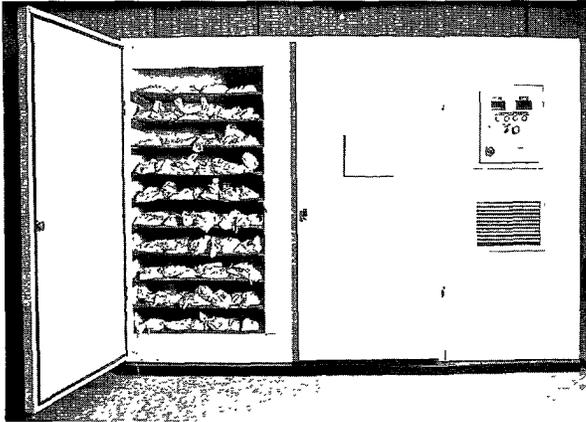
Dehumidified drying

The FAO/IPGRI Genebank Standards recommend the use of $15\pm 5\%$ RH 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 market (Fig. 4C.1)¹. Larger genebanks, however, need modular walk-in seed-drying rooms as shown in Fig. 4C.2.

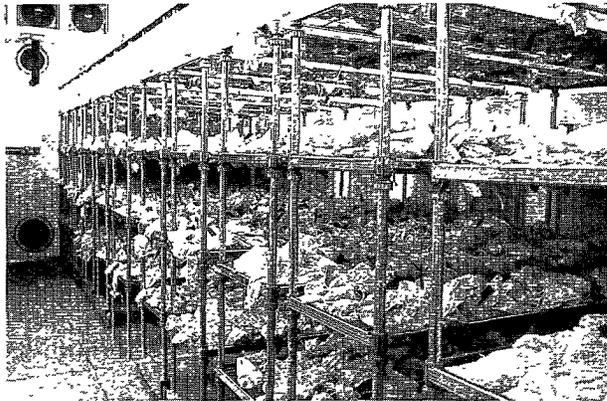
1. Place the seeds packed in muslin cloth bags on the open racks of the drying room or seed-drying cabinet.
2. Leave the seeds in the drying room or cabinet until the moisture content is likely to be in the range required for storage.

¹ Munters Limited, 2 Glebe Road, Huntingdon, Cambs. PE18 7DU, UK.





1. Seed-drying cabinet



2. Inside view of seed-drying room



3. Silica gel drying

Figure 4C — Seed-drying facilities and apparatus used at ICRISAT genebank.



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

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

Prediction of drying period by weight loss

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

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

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

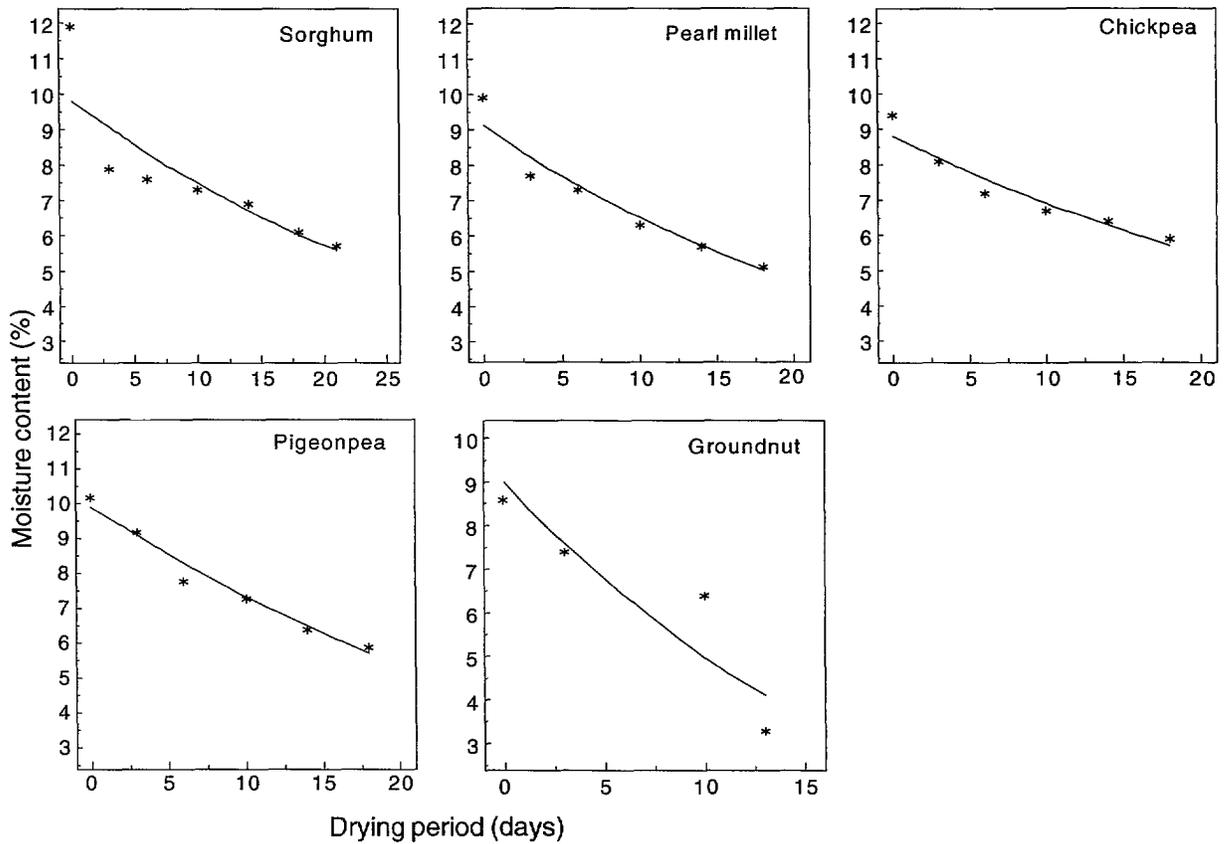
If there is no previous experience of drying seeds of particular species, it may be necessary to do some experimental work to predict the appropriate drying period.

Prediction of drying period from mean drying curves

1. Determine the moisture content of the seed lots using methods described in Section 4B.
2. Keep the seed lots in labeled muslin cloth bags and place them in drying environment.
3. Remove a small sample and repeat moisture determination of the seed lot every day.
4. Plot the moisture content of the seeds on a graph with percent 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 as shown in the graphs:



The above 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.

Silica gel drying

Small samples can be dried using silica gel (see Fig. 4C.3).

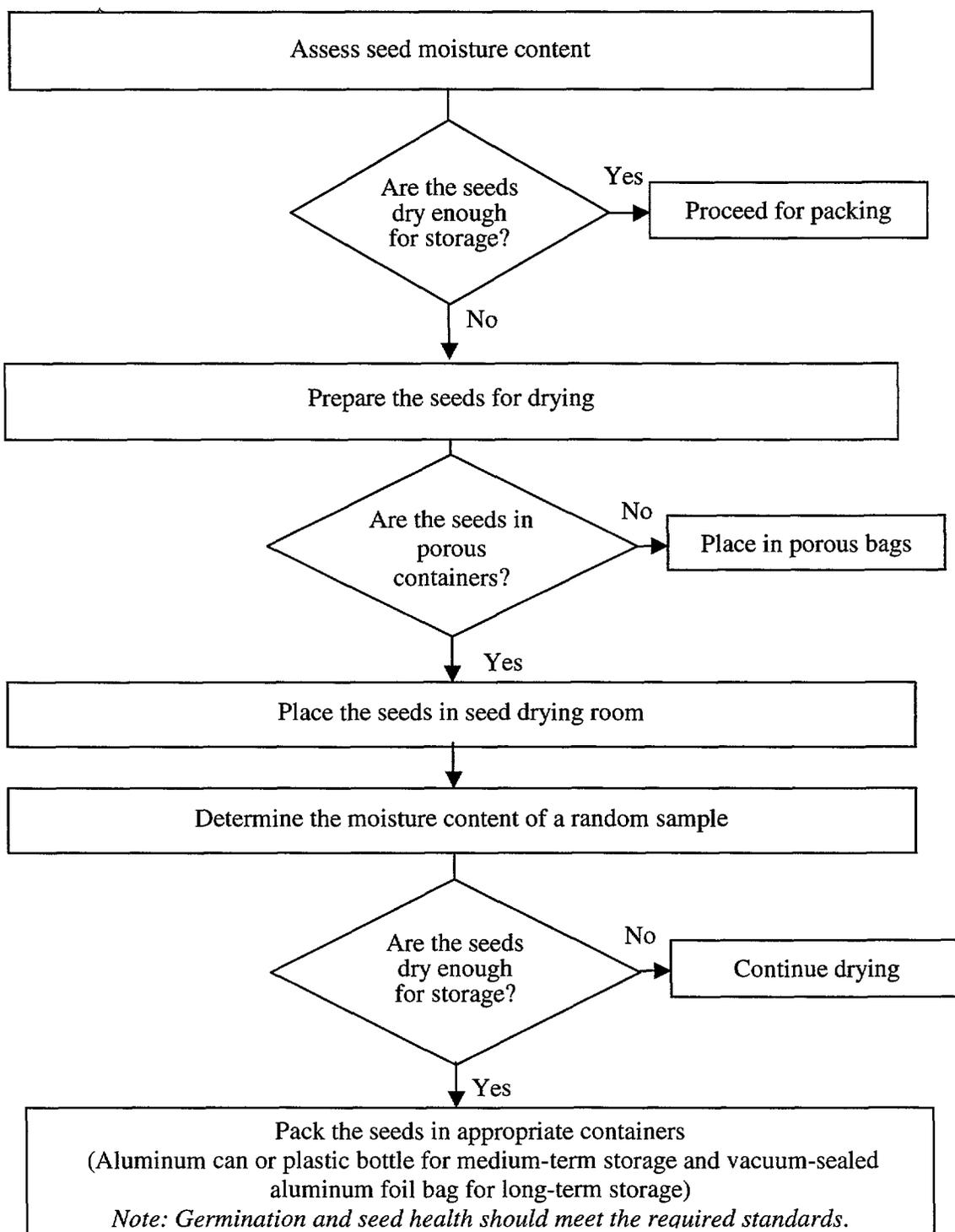
- Place dried silica gel (deep blue in color) in desiccators or glass jars with an airtight seal. The weight of the silica gel used should be equal to the seeds for efficient drying.
- Place the seeds in muslin cloth bags and keep them in close proximity to the silica gel.
- Keep the desiccator at 15°–20°C.
- 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.



D. 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 is the germination test.

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 below (Table 4D.1).

Table 4D.1 — Recommended conditions for germinating seeds of ICRISAT mandate crops.

Crop	Substrate*	Temperature	Special requirements**
Sorghum	BP	20/30°C (16/8 h); 20°C	0.2% KNO ₃ for wild species
Pearl millet	TP	20/30°C (16/8 h); 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 (16/8 h)	
Foxtail millet	TP	20/30°C (16/8 h)	
Little millet	TP	Not available	
Proso millet	TP	20/30°C (16/8 h)	Light 12 h d ⁻¹
Barnyard millet	TP	20/30°C (16/8 h)	Prechill, light
Kodo millet	TP	20/30°C (16/8 h)	

* TP = Top of paper, BP = Between paper

** Freshly harvested seeds and wild species of most crops show dormancy, i.e., 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

1. 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 quantity.
2. Take a random sample of seeds from the container.
3. 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 is used as substrate for germination in both the methods.

Paper substrate quality

- 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 substrate should be tested for their quality on receipt.

Simple test for paper quality

A. Presence of toxic substances

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

B. Paper strength

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

C. Moisture absorption

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

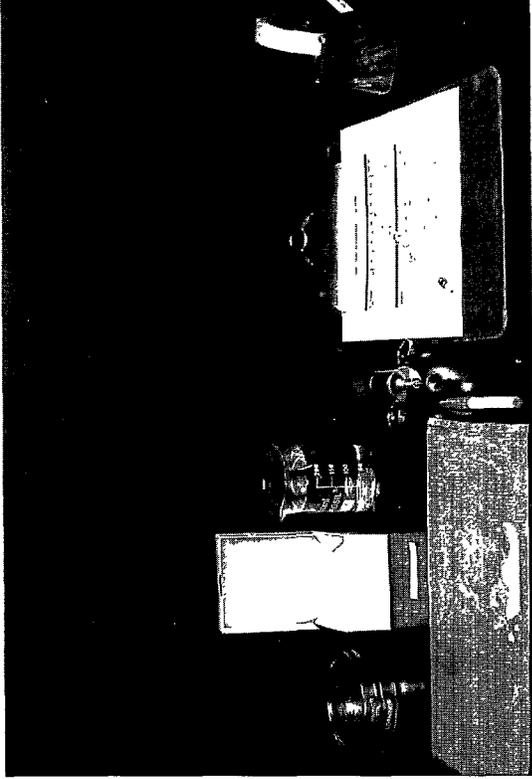
A. Top of paper method

Seeds are germinated on top of moist paper (Whatman Grade 181) in a petri dish (Fig. 4D.1.1–4).

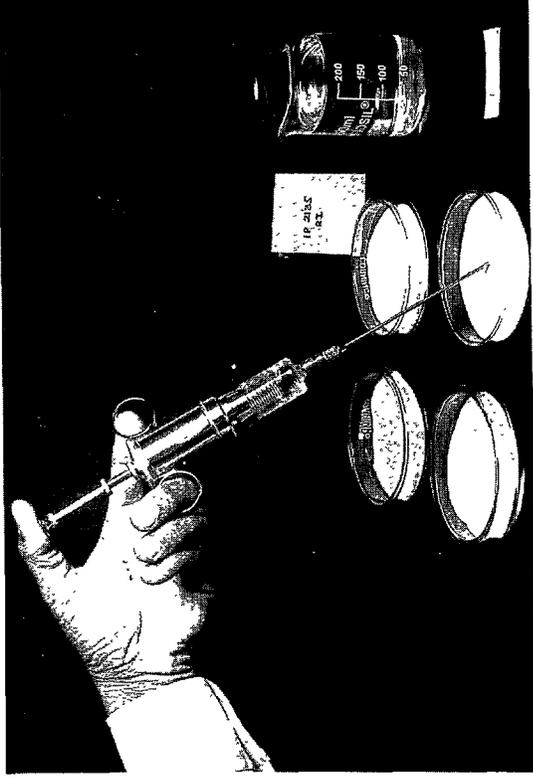
1. Place the paper in 9-cm petri dishes (Fig. 4D.1.1).
2. Moisten it with about 4 mL distilled water (Fig. 4D.1.2).
3. Put a label in the petri dish with accession number, number of replicate and date of the test.
4. Spread the seeds uniformly on the surface of the paper (Fig. 4D.1.3).
5. Cover the petri dishes and keep them in a plastic bag to prevent drying (Fig. 4D.1.4)
6. Place the petri dishes in an incubator maintained at the recommended optimum temperature.



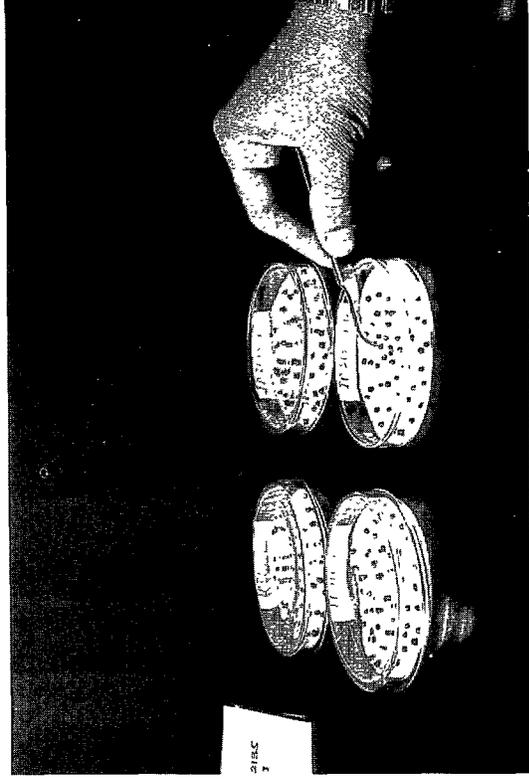
1



2



3



4

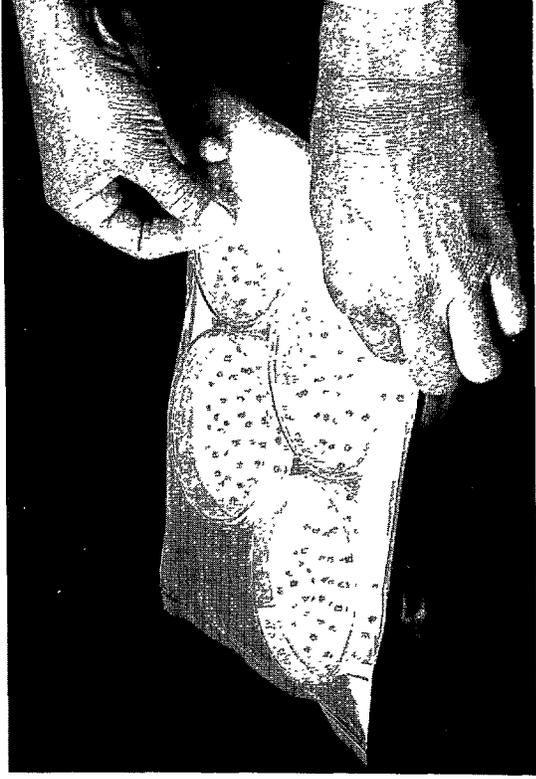


Figure 4D.1.1-4 — Testing germination of seeds on the top of filter paper.

B. Between paper method

Seeds are germinated between two layers of moist paper (Fig. 4D.2).

1. Cut the paper to a convenient size to hold one replicate of the seeds (Fig. 4D.2.1).
2. Label the paper on the outside at one end with the accession number, replicate number and the date of testing (Fig. 4D.2.2).
3. Moisten the paper with water.
4. Arrange the seeds in rows at regular intervals 4 cm from the top edge, leaving 3–4 cm gap on sides (Fig. 4D.2.3).

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

5. Cover the seeds with another sheet of dry paper (Fig. 4D.2.4).
6. Roll the paper loosely from the label end (Fig. 4D.2.5).
7. Put a paper clip to hold the rolled papers from falling apart (Fig. 4D.2.6).
8. Keep the rolls in a plastic tray (Fig. 4D.2.7).
9. Add sufficient quantity of distilled water (covering the bottom 3-cm of rolls) to the tray.
10. Place the tray in an incubator maintained at recommended temperature (see Table 4D.1).

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

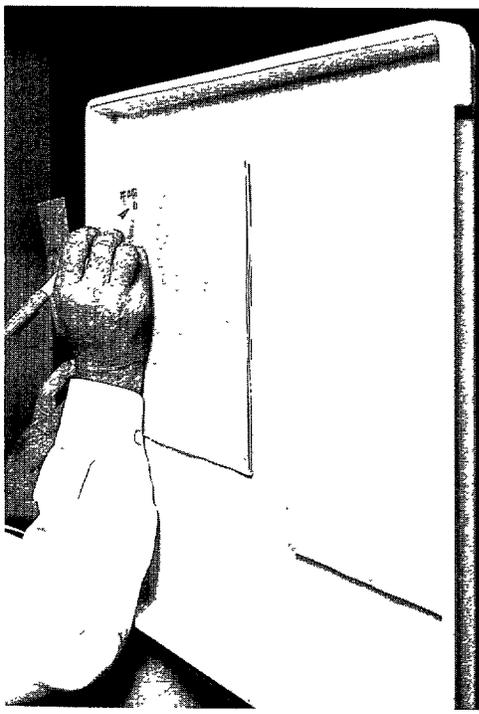
1. Use proper spacing of seeds — increase the distance between seeds and use greater number of replicates.
2. Provide optimum environment for germination — temperature regime should be suitable and the test environment must be well aerated.
3. Ensure cleanliness of germination test media and containers — make sure that these are not sources of inoculum.
4. Avoid imbibition injury (by prior humidification of the seeds) that could lead to leakage of cell contents and provide source of nutrients to fungi.
5. Promptly remove decaying seeds to prevent the spread of fungi to neighboring seeds.
6. Remove seed covering structures before tests when these are found to be sources of infection.
7. Remove sprouted seeds (seeds, which germinated before harvest and subsequently dried) that can be a severe source of infection.
8. Treat seed with thiram (tetramethylthioperoxydicarbonic diamide).

Evaluation of germination tests

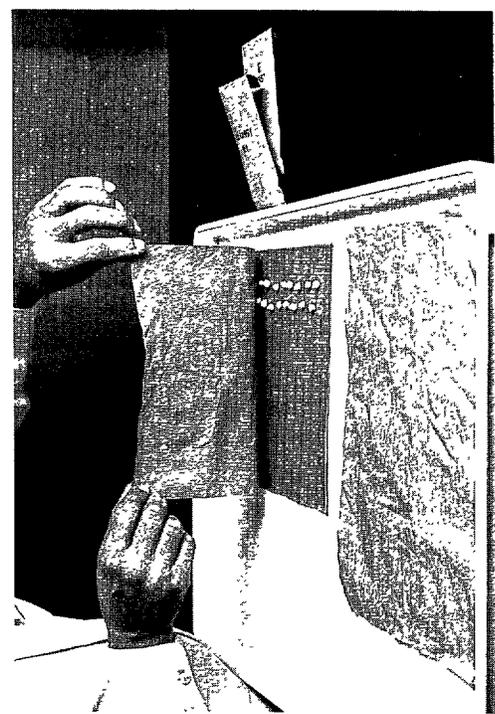
1. Evaluate the seedlings 7 days after sowing.
2. Scarify the hard and ungerminated seeds of chickpea and pigeonpea and evaluate at 14 days after sowing.

2000

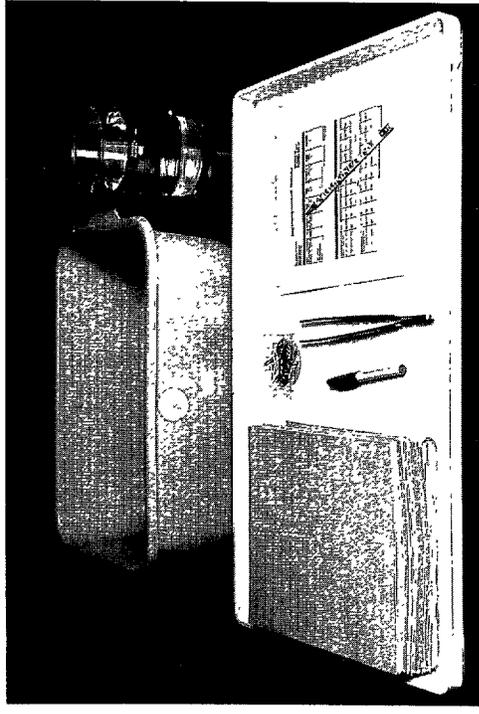
2



4



1



3

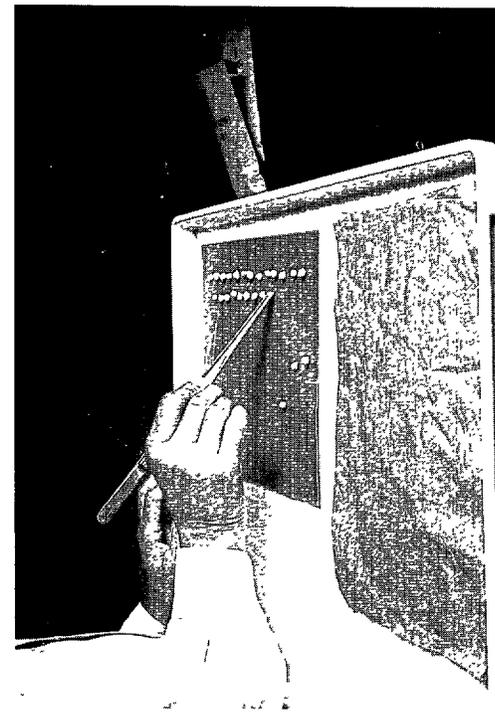


Figure 4D.2.1-7 — Testing germination of seeds between moist paper towels.

Contd...

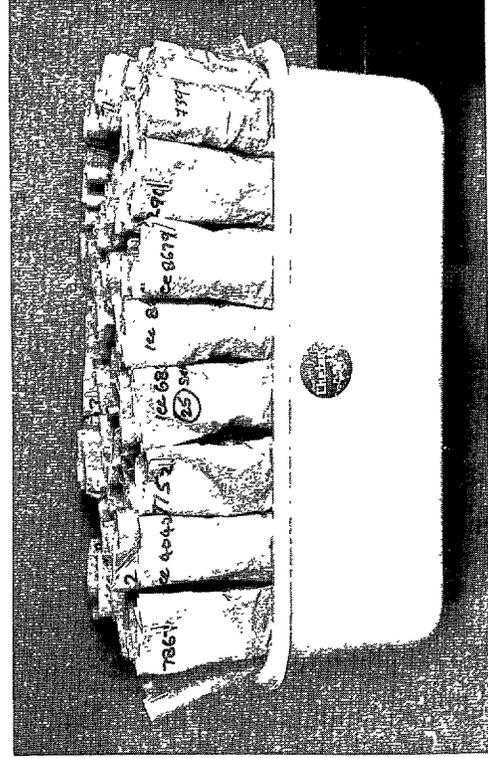
6



5



7



05

3. 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 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.3 and 4D.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.
4. Record observations in the data sheet shown as Annexure 4D.1.
5. Update the inventory database with information from germination test.
6. Repeat the germination test if the difference between the two replicates exceeds the maximum tolerance limits at 2.5% probability (Appendix 1).

ICRISAT Genebank

Crop/species:
Accession number:
Date of storage:
Date of testing:

Substrate: BP/TP
Temperature:
Incubation time: 7 days

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 (%)

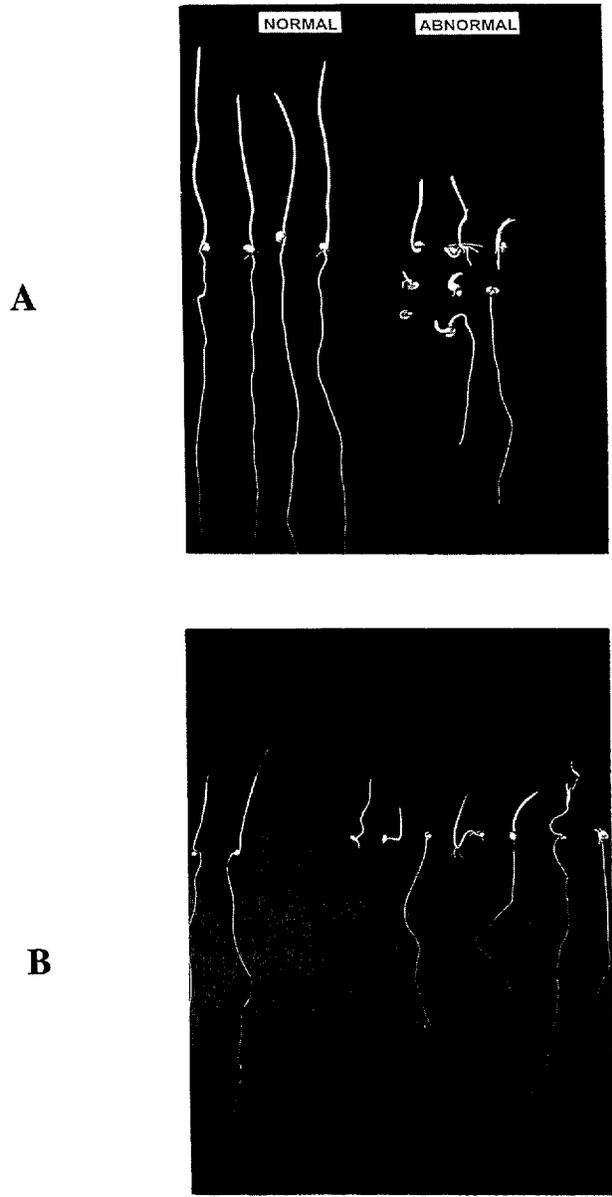
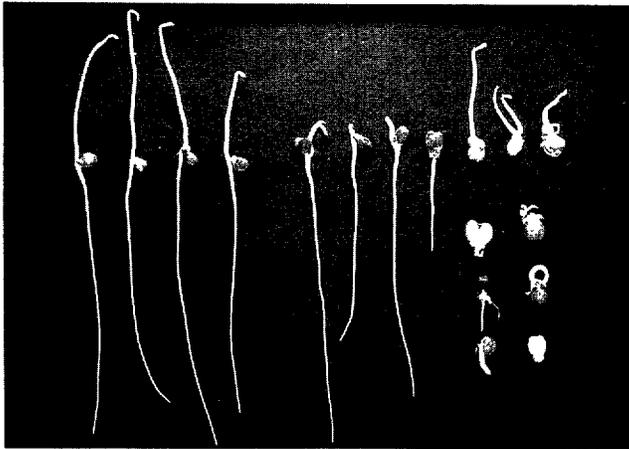
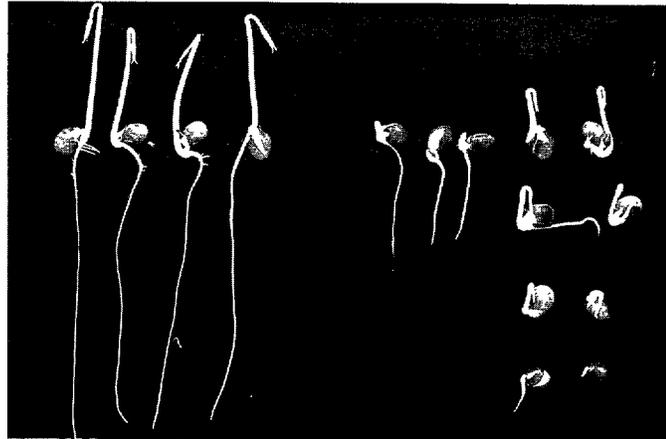


Figure 4D.3 — Normal and abnormal seedlings of sorghum (A) and pearl millet (B).



A

Chickpea



B

Pigeonpea

C

Groundnut

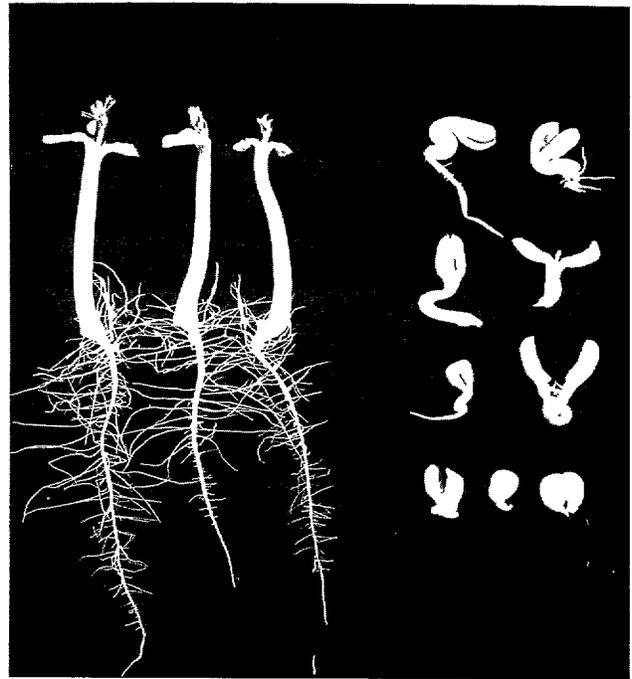


Figure 4D. 4 — Normal (left) and abnormal (right) seedlings in chickpea (A), pigeonpea (B), and groundnut (C).

Tropical tetrazolium test for viability

The tetrazolium test can be used as a backup procedure for 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

1. Remove the seed covering structures (glumes, etc.).
2. 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 at the end of a germination test are evaluated.

Staining

1. Bisect the seeds longitudinally through the embryo with a razor blade.
2. Discard one-half of the seed and place the other half in the staining solution at recommended concentration (Table 4D.2) in a glass vial.
3. Place the vials in an incubator maintained in dark at recommended temperatures and duration (Table 4D.2).

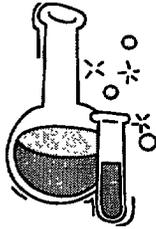
Table 4D.2 — Concentration, temperatures and period of staining with tetrazolium solution

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>	-	-
<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

4. After staining, wash the seeds several times in distilled water to remove excess stain.
5. 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.
6. 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.

7. 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.

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 require 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 h from the beginning of the germination test (Annexure 4D.2).

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.2). 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.2). 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.

Seed vigor studies

Crop:
Accession number:

Date of testing:
Temperature: 20/25°C

Date of storage:

Radicle emergence data

Rep No. Of seeds tested		I 50	II 50	III 50	IV 50	Total (n)	t x n	Remarks
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. Root length, mm	1	2	3	4	5	6	7	8	9	10
	11	12	13	14	15	16	17	18	19	20
	21	22	23	24	25	Mean				

Rep II

Seedling no. Root length, mm	1	2	3	4	5	6	7	8	9	10
	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)

11/11/2019
11/11/2019
11/11/2019

E. Seed health testing

Seedborne 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 seedborne pathogens. The methods employed to detect the pathogens are referred as seed health testing methods.

The commonly used seed health testing methods are:

Visual examination

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

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.

- 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 forcep.
- 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.

Agar plate method

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

- Prepare the medium by mixing Potato Dextrose Agar (PDA) powder with appropriate quantity of water.
- Sterilize the mixture in an autoclave for 15–20 min 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 pretreating 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 seedborne pathogens on the basis of colony and spore characteristics.

Some times 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.

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 >25%, the seeds are unsuitable for conservation as base collection:

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

F. 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, and
- prevent contamination by insects and diseases.

Pack the seeds immediately after drying.

Types of container

Different types of containers are available for packaging. The choice depends on storage conditions and species. The packing material should be impermeable to water and suitable for long-term use. If the RH 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 RH is not controlled.

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 mm) 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:

1. Fill the container with regenerated silica gel and seal it.
2. Accurately determine the weight of the container with an analytical balance.
3. Hold the container over water in a desiccator for about a week.
4. Remove the container from desiccator and allow the surface to dry.

5. 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.
6. 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.
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.1A), or
 - large size plastic bottles with inner lid and screw cap for groundnut (Fig. 4F.1A).
 - base collections conserved for long-term, are stored in resealable laminated aluminum foil packets² (Fig. 4F.1B).

Packing procedures

Base collections

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

- accession number,
- identity, and
- 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	140 × 160	75
Chickpea, pigeonpea and groundnut	190 × 160	200
Small millets	110 × 80	25

1. Take out a few samples at a time from the drying room to minimize reabsorption of moisture by the seeds.
2. Weigh the amount of seed being prepared for storage.

2. Barrier Foils Products Co., C.C.E. Business Park, Windmill Lane, Denton, Manchester M34 3QS, U.K.

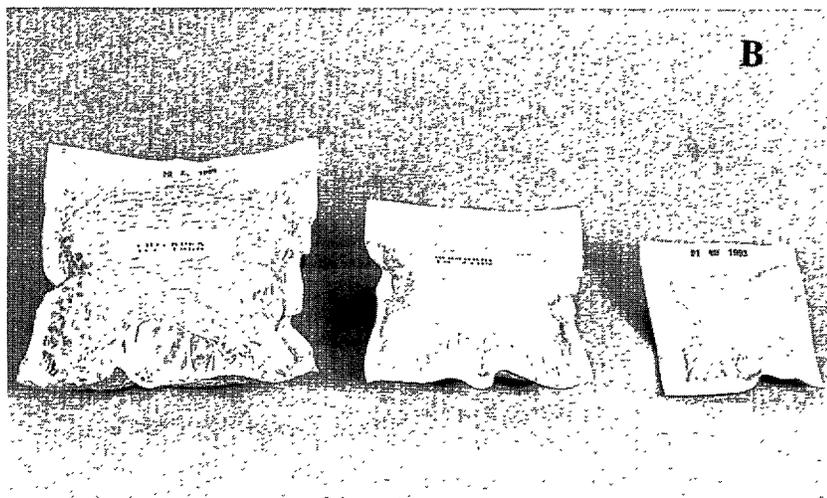
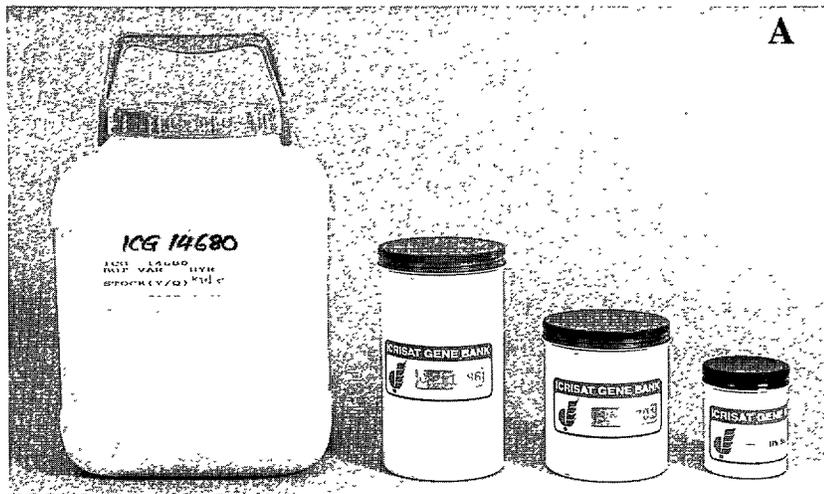


Figure 4F.1 — Containers used for medium-term (A) and long-term (B) storage at ICRISAT.

3. Fill the container with seeds leaving at least 2.5 cm headspace for sealing.
4. Inside the container place a nonadhesive label with:
 - accession number,
 - identity, and
 - season of harvest — to help in identification of the accession if the label outside is lost.
5. Seal the container at 0.6 mbar vacuum using Audion Vac sealer (Fig. 4F.2).
6. Check for any deficiencies in packets and in sealing.
7. Print the date of sealing on the packet (if available).
8. Move the packets into long-term store.

Active collections

1. Use the container of the following type, size and cap color (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 stored in the same room.

2. Paste adhesive metallic labels pre-engraved with accession number or use permanent marking pens for labeling the containers.
3. Weigh the amount of seed being prepared for storage.
4. Place a label with accession number, identity and season inside the container.
5. Fill the container with seed and close the cap tightly.
6. 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.*

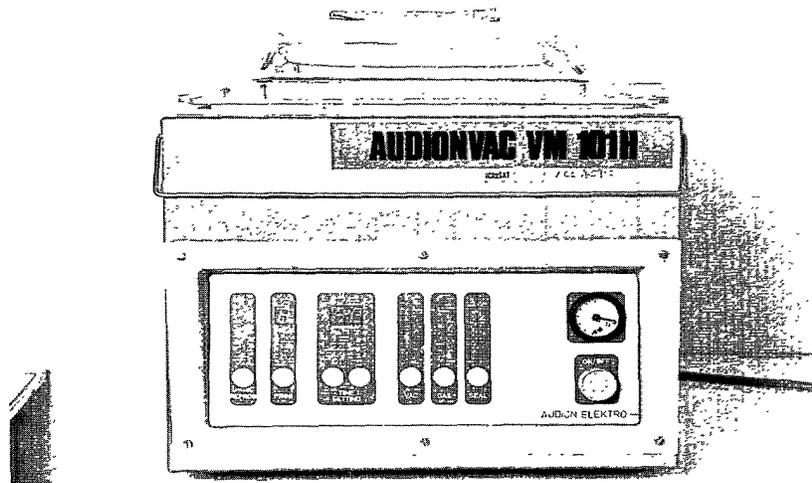
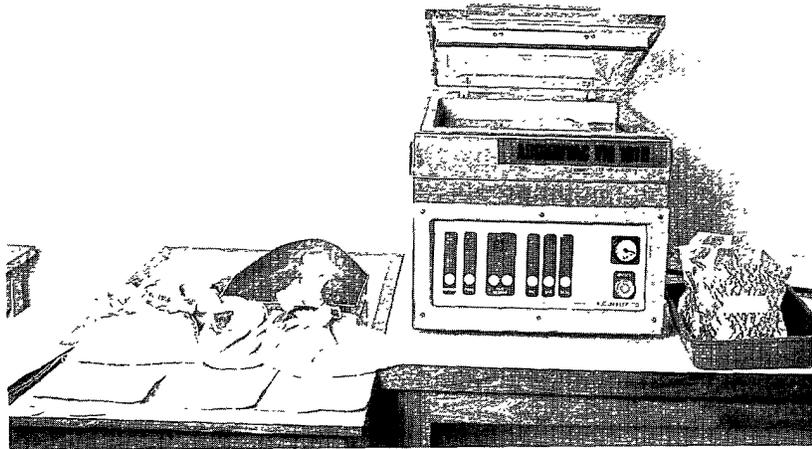


Figure 4F.2 — Vacuum sealing for packing seeds for long-term conservation at ICRISAT.

Section 5

Seed Storage

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

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.

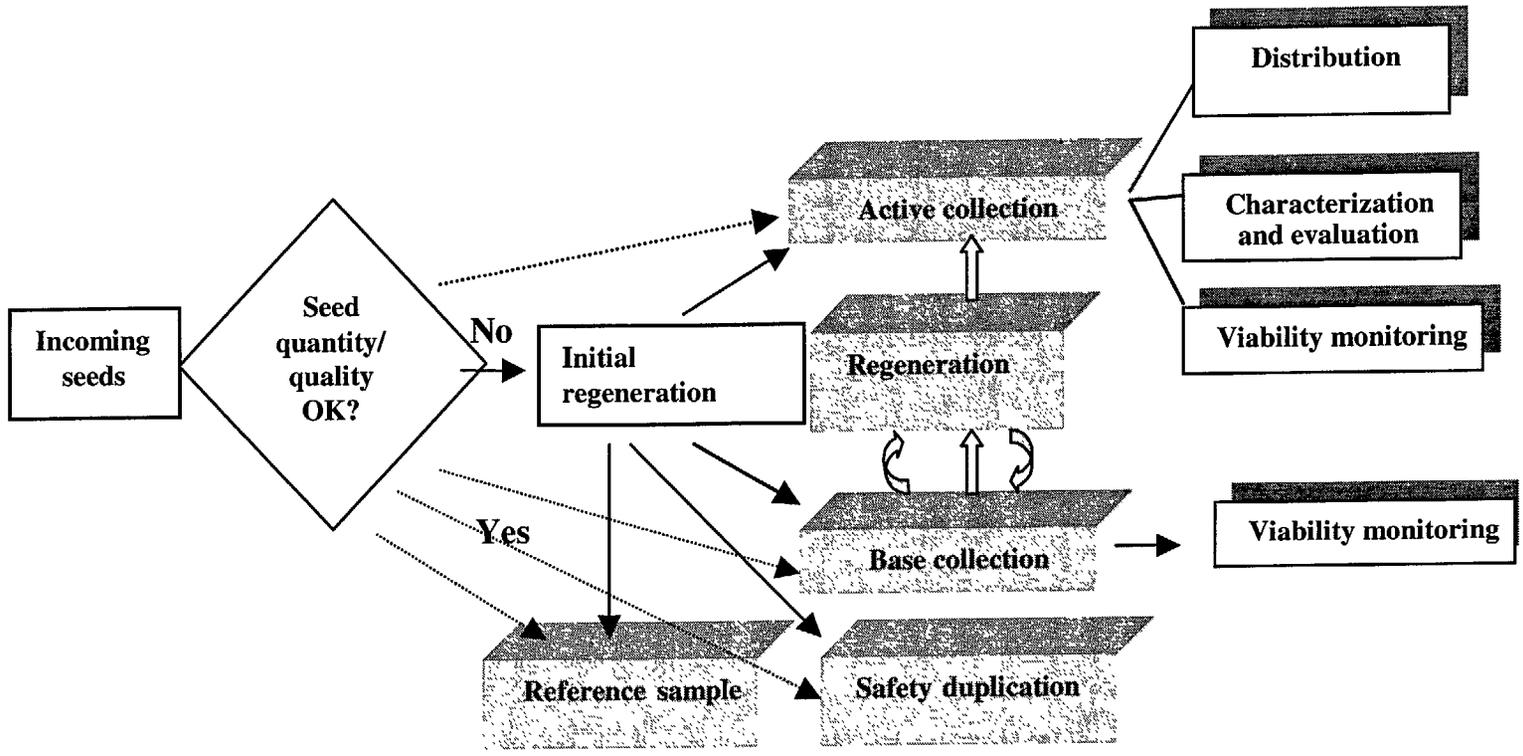
If the genebank has distribution of germplasm as a function, adopt a two-step storage system and maintain *active collections* of the sample under medium-term conditions.



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% 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 high viability begins to decline. Then some seeds are taken out from long-term store and multiplied. The multiplied seeds are stored in medium-term for further distribution.*
- *These cycles are repeated until the seeds in long-term storage 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 fresh stock in medium-term and further stock 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.



Explanatory diagram of a two-step storage system

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:
 - -18°C or cooler with 3–7% seed moisture content, depending on species.
- Acceptable storage conditions are:
 - Subzero temperature with 3–7% moisture content.

Accession size

- At least 1000 viable seeds, but preferably 1500–2000 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 accessions should consist of at least 4000 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.

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

Temperature ($^{\circ}\text{C}$)	Moisture content (%)	
	Groundnut	Sorghum, millets, chickpea and 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 are maintained at 4°C and 20–30% RH.

Accession size

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

- sorghum, pearl millet, chickpea and pigeonpea: 400 g,
- groundnuts: 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,

$$400 \text{ g contain: } 100 \times 400/2.5 = 10\,000 \text{ seeds}$$

Germination

Seeds placed in 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 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 shows the location of sample as Tray no. 12 in Room 3, Rack B and Bay IV.

Assigning location code

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

Safety duplication

Safety duplicate 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. This provides insurance against loss of material. Under the trust Agreement with FAO, ICRISAT has responsibility for 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 sole 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 PSC from the originating country. Similarly, the Plant Quarantine Authority in destination country needs to permit the importation of seeds by the recipient, bypassing the routine quarantine examination.

- *Active* — when the duplicate collection is incorporated into the recipient's collection thus being subject to regeneration, multiplication and distribution by the recipient.
- *Base* — maintained under suitable conditions for long-term seed storage and is incorporated into the recipient's collection.

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 $>85\%$, and
- vacuum sealed in laminated aluminum foil packets.

The minimum sample size can be smaller, i.e.,

- approx. 25 g for sorghum and millets and 100 g for legumes.

To save time, the samples for safety duplication can be simultaneously prepared when processing the seeds for long-term conservation.

Storage policy of ICRISAT genebank

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

The following are conserved as base collection:

- germplasm currently designated to FAO,
- all landrace accessions collected or acquired in future, complete with passport information

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

The medium-term storage conserve working collections of:

- frequently distributed material,
- core collections,
- genetic stocks,
- nondesignated stable breeding lines,
- wild species, and
- emergency national holdings.

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 accession 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 accessions was regenerated

Container: Type of container used for storage, e.g., 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-resistant envelopes.

Procedures for seed distribution within India

1. 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 (i.e., 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.

2. 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.

3. If seeds are available for distribution, register the request by assigning a request number.
4. Prepare the list of accessions available for distribution and obtain a Material Transfer Agreement (MTA) signed for the selected accessions by sending:
 - Germplasm Order Form for registered genebank accessions (Annexure 6.1),
 - Breeding Material Order Form for ICRISAT developed varieties registered as genebank accessions but not designated to FAO (Annexure 6.2).
5. Generate labels for the selected accessions using Genebank Information System.
6. Paste the labels on seed envelopes used for distributing seeds to requestor (Fig. 6.1). Use:
 - coin envelopes for cereals, and
 - metal fold paper envelopes or aluminum foil packets for other crops.
7. Check the inventory file and note the location of the container in the genebank.
8. Pick the containers from genebank and move them out into a dehumidified room *the previous evening* to allow them to warm up to room temperatures before opening. (If the number of samples to be distributed is small, then draw the seeds from containers in the genebank itself).
9. Ensure absolute accuracy in identification of accessions while drawing the seed from the genebank.

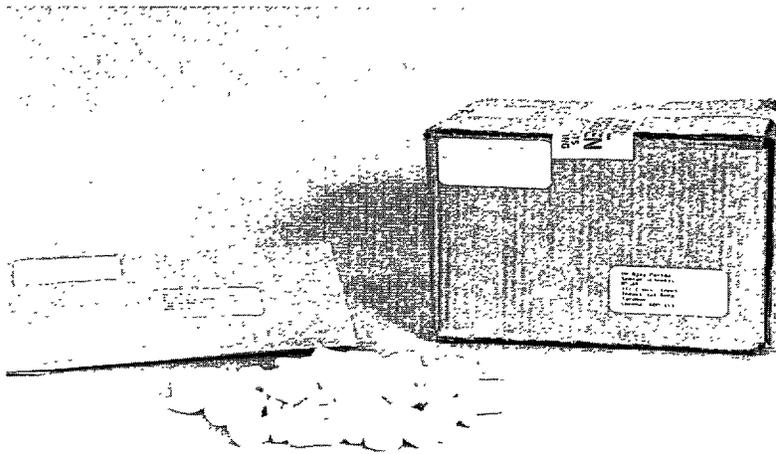
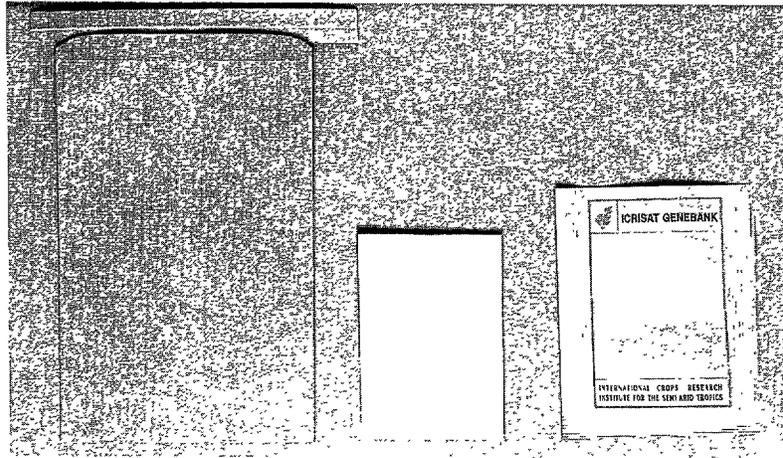


Figure 6.1 — Containers used for seed distribution at ICRISAT.

10. Open the container and quickly draw the required amount of seeds into the labeled envelopes (see Table 6.1)

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

Table 6.1 — 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	10 seeds

11. 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.

12. Proofread the list of accessions drawn from the genebank with the labels on the envelopes.
13. 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.
14. Prepare a covering letter.
15. Pack the seed envelopes, covering letter and the seed list in a jiffy bag (if the number of samples is few) or a cardboard box and label it with the complete address of the consignee.
Use filling material to avoid damage to seeds during transit.
16. Send the seed parcels by airmail or airfreight to avoid delay and possible loss in seed quality during transit.
17. Record the shipment details into the distribution data file.
18. Update inventory data deducting the number of seeds supplied in each accession.

Procedures for seed distribution outside India

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

1. 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.

2. Draw the seeds from genebank as described above and prepare seed lists.
3. Fill the Request for Export of Seed/Plant/Plant Products form (Annexure 6.3).
4. Check with the plant quarantine office if additional declarations are required confirming freedom of seeds from specific diseases and pests and obtain them from concerned persons.
5. Send the seed samples with the export request and IP to the Plant Quarantine Unit for exit quarantine certification and issue of PSC.
6. When the samples are ready, prepare a covering letter and the final list of accessions (deleting accessions detained at quarantine) along with passport data and send to the Plant Quarantine Unit to dispatch along with seeds.
7. Record the shipment details in the distribution data file.
8. Update seed inventory by deducting the number of seeds supplied.

Procedures for seed 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 6.4).

Processing internal seed request

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

Seeds from 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 handle them according to the provisions of ICRISAT/FAO Agreement.



- *Do not send designated germplasm without an MTA.*
- *Accessions not bred at ICRISAT, accessions not designated to FAO and accessions acquired after 1993 should be supplied according to the provisions in GAA made with the donor institute or country.*
- *If no GAA 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 (e.g., *Arachis* and *Pennisetum* spp.)

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

Feedback on germplasm utilization

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 6.5.

Documenting distribution data

The Genebank Curator needs to keep 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
- an accession details file containing information about the 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, e.g., 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 (e.g., commercial company, national institute, NGO, 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.

Descriptors for 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.

Germplasm repatriation

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

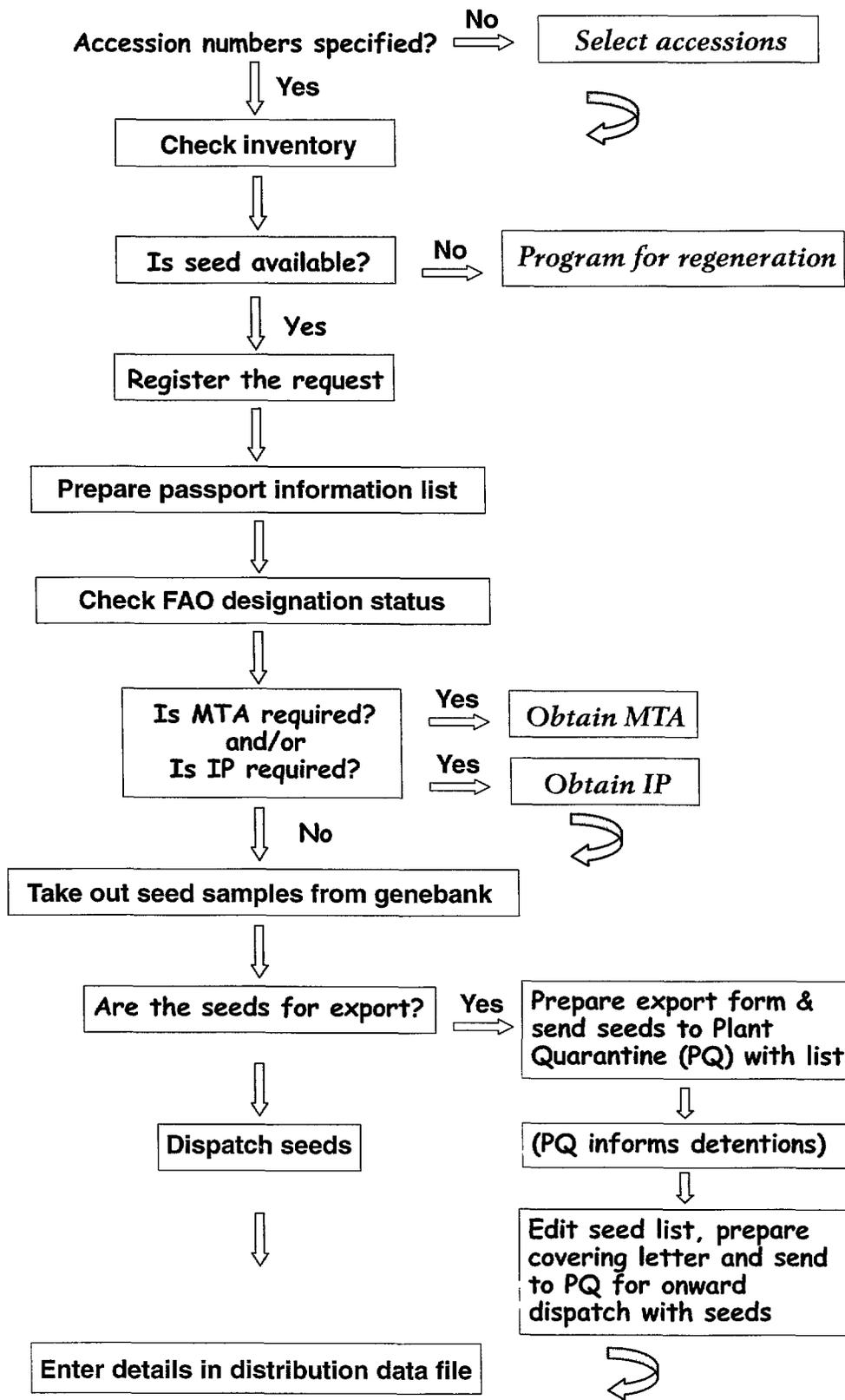
- establish or add to the genebank of their own, or
 - rebuild their collections lost due to inadequate facilities, natural calamities, civil disorder, etc.
1. Ask the requestor for the list of accessions required to be repatriated.
 2. Supply seed samples if the distribution would not reduce the stocks below accepted levels for conservation.

The quantity distributed should be sufficient to conduct at least two regenerations (see Section 8).

3. 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.

Germplasm Seed Distribution – Flow Chart



ICRISAT
Standard Order Form
Consecutive Number: SOF/Year/Number

I/we order the following material:

In so far as this material is "designated germplasm" under the Agreement between ICRISAT and the Food and Agriculture Organization of the United Nations (FAO) placing Collections of Plant Germplasm under the Auspices of FAO dated 26 October 1994.†

I/we agree:

not to claim ownership over the material received, nor to seek intellectual property rights over that germplasm or related information;
to ensure that any subsequent person or institution to whom I/we make samples of the germplasm available, is bound by the same provision.

Place/date

Name of person or institution requesting the germplasm

Address

Shipping address (if different from the above)

Authorized signature _____

† Whether or not the material is "designated germplasm" will be indicated on the seed list attached to the Shipment Notice and on the seed packets.

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 whom 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 of 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 Unit**

Request For Export Of Seed/Plant Products/Other Material¹
(To be type written only)

Crop	:	RPMIS Project no:
Year of harvest and season²	:	
No. of samples	:	Weight:
Origin/source of material	:	
Inspection of crop at flowering/pre-harvest state³	:	
Description	:	
Objective	:	
Consignor	:	
Program	:	Budget code:
Consignee and address	:	

Cable:

Telex:

Dispatch instructions: AIR MAIL/AIR FREIGHT/COURIER SERVICE/ACCOMPANIED BAGGAGE
(Strike out whichever is not applicable)

Special instructions (if any) :

Import Permit No. :
(Attach original, along with additional declaration certificate if any)

Date: **Scientist⁴:** **Program Director/Group Leader/Program Leader**

Seeds treated with chemicals are not acceptable

1 Plant products = Flour, dahl, *Rhizobium* cultures; Other materials = Soil, pests (insects, fungi, nematodes etc.)

2 Rainy or postrainy

3 Location where parent plants were grown

4 Names of pathologists/virologist/entomologist/nematologist or alternatively an experienced research associate.

100

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: _____

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Feedback on germplasm received from ICRISAT Genebank

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 Number	Requester's identification number	Special characters observed

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

Name of respondent:

Address:

Date:

Signature:

Section 7

Monitoring

The viability of seeds stored in the genebank decreases gradually during storage.

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

- Monitor the viability of seeds at regular intervals.
- The monitoring interval depends on the species, 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 nonoil 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.

Monitoring viability

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

1. Identify and make list of the accessions, which require testing on a monthly basis, using genebank documentation system.
2. Find the location of the containers in storage from inventory database.
3. Remove the containers from storage and leave them overnight at room temperature to warm up.
4. Open the container and draw a sample of seeds needed for the test and close the containers.

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

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 when the quantity or viability of seeds falls below minimum at regular intervals to program the accessions for regeneration.*

Section 8

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 RH 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.

Reasons for regeneration

Germplasm is regenerated for the following purposes:

1. *Initial seed increase*

In case of new collections or materials received as donations, the quantity of seeds received by 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.

2. *Long-term conservation*

Seed accessions that are not yet in base collection need long-term conservation.

3. *Replenish seed stocks in active and base collections*

Increase seed of accessions that have:

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



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

4. 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 requiring 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:

1. Suitability of environment to minimize natural selection.
2. Special requirements, if any, to break dormancy and stimulate germination (e.g., scarification).
3. Correct spacing for optimum seed set.
4. Breeding system of the plant and need for controlled pollination.

Procedures for regeneration

- If possible, regenerate germplasm in ecological region of its origin. Alternatively, seek a location that does not select 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

- Regeneration of 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 regeneration plots

Soil

- 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 (e.g., fertilizers, lime, irrigation, or solarization).

Solarization

Solarization is heating soil by covering it with polyethylene sheets during hot summer to control soilborne diseases. It is particularly useful to control fusarium wilt in chickpea and pigeonpea, which is a major limitation to crop growth during regeneration. Solarization is conducted for at least 6 weeks during the hottest part of the year.

1. Thoroughly cultivate the land and level it to minimize protrusions.
2. Give 50 mm irrigation before laying of the polythene sheets.
3. Use clear transparent polythene sheet, 25–100 mm thick.
4. Insert two edges of the polythene sheet in the furrows, and bury the edges in the soil tightly.
5. Place weights to prevent flapping and tearing of polythene sheets in the wind.
6. 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.
7. Do not allow irrigation water to flow in from other areas after solarization and during crop growth.

Weeds

- Identify the problem weeds, pests and pathogens, by inspection and prior experience.
- Consider reducing such problems during preparation of regeneration plots by application of appropriate treatment.

Cleanliness

- Keep the plots absolutely clean from alien seed and plants by
 - herbicide spray,
 - sterilizing soil,
 - ploughing to encourage germination of weeds followed by herbicide spraying, and
 - deep ploughing to kill emerging weeds.
 - Consider the risk of contamination with alien pollen and take appropriate measures to reduce it during plot preparation and by intercultivation and hand weeding.
 - Ensure that the method of plot preparation is appropriate for the chosen method of establishing plants, e.g., ridges and flat beds.
 - Prepare the regeneration plot considering:
 - number of accessions to be regenerated,
 - number of plants per accession,
 - spacing between rows and between plants, and
 - mechanical access for weeding.
 - Method of preparation depends on:
-

- soil structure,
- species to be sown or transplanted and its cultural requirement, and
- need for plant supports, e.g., for climbers such as *C. albicans* or *C. volubilis*.

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 and allow them to warm up,
 - 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 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 screenhouse under close supervision.

Seed pretreatments

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

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

Sowings 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 large losses of alleles:

- use 100 or more plants in cross-pollinating species,
- provide suitable conditions for growth to trigger abundant flowering,
- eliminate competition by weeding alien plants, and

- ensure maximum survival.

Regular inspection of plants is mandatory to achieve these objectives.

Sowing date

- Sow at an optimum time so that maturity and harvesting coincide with the most favorable weather conditions.
- If there is much variation between accessions in flowering time, sort on maturity (e.g., early and late) based on previous documentation and adjust the planting dates so that all accessions mature under uniform favorable environment.

Planting on maturity basis also makes it convenient for crop management and harvesting.

- 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.
- Thinning should not be normally undertaken. If required, thin plants at random.
- 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 waterlogging.

Meiosis and anthesis are sensitive stages during 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.
- When materials are grown in rows, plants growing off-row may be eliminated.

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 bags, and
- erecting temporary pollen-proof or pollinator-proof nets around 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 postharvest 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, e.g., 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 to avoid maternal effects.
- 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 purpose-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 (e.g., 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

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 contents improves longevity of some species, while it can damage other species. Seeds dried to low moisture contents 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 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.

A. Sorghum regeneration

Seed multiplication is undertaken in postrainy season. All tropical photosensitive 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).

Land preparation

Vertisols (black soil) are used for seed regeneration. The field should have good drainage. It should be free from weeds at the time of sowing.

- Select fields in which sorghum was not grown in the previous year.
- 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 layout

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

Fertilization

Apply diammonium phosphate @ 150 kg ha⁻¹ as basal dose, and 100 kg ha⁻¹ urea as top dressing 30 days after sowing.

Sowing method

Use one row of 9 m for regenerating each accession. Use the four-cone planter for seeding. It requires about 8 g seeds row⁻¹.

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 at random when the seedlings are 2-week-old. Maintain a plant-to-plant distance of 10 cm, which provides a stand of at least 90 plants row⁻¹.

Weed management

Apply Glycel (1–2%) as a preemergence herbicide. Use shallow intercultivation during early stages of crop growth. Hand weeding, if required, is undertaken at later stages.

Pest and disease control

Follow normal cultural practices to control diseases and pests.

Irrigation

Irrigate the field after sowing and to save the crop later. 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 waterlogging damages the crop.

Pollination control

Genetic integrity of sorghum accessions is maintained by selfing.

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

Verification of accession identity

1. Eliminate off-types and plants growing off-row.
2. 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
3. Rogue the plants that are genuine mixtures.

Harvesting

Seed maturity can be identified by black layer formation on the 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.

1. 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 secature.
2. 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.

3. Dry the panicles in shade for a week until the seed moisture content is reduced to 12%.
4. Thresh individual panicles by beating gently, and clean the seeds of debris by winnowing.
5. Take equal quantity of seeds from each panicle (plant) and bulk them together to reconstitute the accession.
6. Prevent spillover and contamination of accessions during threshing and subsequent handling.
7. 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

1. Grow the wild species in botanical garden to avoid possible outcrossing of germplasm with related species, and avoid introduction of new weeds.
2. Prepare broadbeds of 1.5-m width and 6-m length.
3. Germinate the wild species in paper cups and transplant them at a distance of 20 cm.
4. Follow all crop husbandry practices of cultivated sorghums.
5. Cover the panicles in parchment paper bags before stigma emergence to prevent outcrossing.
6. Harvest the panicles individually as they mature, i.e., before shattering.
7. Collect the seeds from each plant into a labeled paper envelope.
8. Dry the seeds under shade and clean them by gentle blowing or winnowing.
9. Take equal quantity of seed from each plant and reconstitute the accession for further drying and subsequent storage.

B. Pearl millet regeneration

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

Soil preparation

Alfisols (red soils) are best suited for seed multiplication. Choose a field, which was not under millet cultivation during 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.

Field layout

Mark the field of 4-m tiers, leaving 1-m walking space (alleyway) in between tiers. Assign row numbers in serpentine pattern.

Fertilization

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

Sowing date

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

Sowing method

Grow each accession in three rows, each of 4-m length. Sowing is done using a four-cone planter. About 5 g of seeds is used for each row.

Labeling

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

Weed management

Apply Glycel (1–2%) as a preemergence herbicide. Use shallow intercultivation during early stages of crop growth. Hand weeding, if required, is undertaken at later stages.

Plant husbandry

Thin 2 weeks after sowing to maintain a distance of 10 cm between plants within the row to provide about 120 plants accession⁻¹. Care should be taken to thin at random.

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.

Pest and disease control

Follow normal cultural practices to control diseases and pests.

Pollination Control

Pearl millet inbred lines and genetic stocks are maintained by selfing. Landraces are maintained by sibbing.

Landraces

1. Cover 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.
2. As anthers begin to dehisce, remove the bags from panicles, collect the pollen into a common paper bag, gently tapping the panicles. Cover the panicles with bags after collecting the pollen.
3. Remove the bags from panicles with stigmas emerged, dust the collected pollen on to the stigmas and cover the panicles 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.
Ensure that all plants within the accession are either sibbed or selfed.
 - Remove the bags 2 weeks after flowering (at dough stage) and clasp them around the panicles to identify sibbed panicles while harvesting.

Genetic stocks

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

Verification of accession identity

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

Harvesting

The optimum time to harvest seeds with maximum quality is 5–6 weeks after anthesis.

1. Cut the bagged or selfed panicles just below the base — one from each plant, from **at least 120 plants** accession⁻¹.
2. Collect the panicles within the row (accession) into a gunny bag labeled both within and outside using tear-off tags.
3. Dry the panicles under shade for about a week to reduce the moisture content to about 12%.
4. Thresh the panicles individually by gently beating with sticks.
5. Clean the seed by winnowing.
6. Take equal quantity of seeds from each plant to reconstitute the accession.
7. Prevent seed mixtures during threshing and seed handling.

Seed health

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

Wild species

1. Maintain the wild species as living plants in botanical garden to avoid possible outcrossing of germplasm with related species and the introduction of new weedy species.
2. Prepare broadbeds of 1.5-m width and 6-m length.
3. Germinate the wild species in paper cups and transplant them at a distance of 20 cm.
4. Cover the panicles in parchment paper bags before stigma emergence to prevent outcrossing and to prevent seed loss due to shattering.
5. Harvest the panicles individually, 5–6 weeks after anthesis.

6. Separate the seeds by crushing the florets between hands.
7. Clean the seeds and take equal quantity of seed from each plant to reconstitute the accession.
8. Prune the perennial and rhizomatous species up to 30 cm from the ground level during the rainy season to avoid mixing with adjacent accessions.

C. Chickpea regeneration

Soil preparation

Chickpea multiplication is conducted in Vertisols (black soils). The field should have good drainage and 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.

Field layout

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

Sowing date

Sow the seeds in the middle of October. Use two rows of 4 m, providing *at least 80 plants* for regenerating an accession.

Fertilization

Apply a basal dose of diammonium phosphate @ 100 kg ha⁻¹.

Sowing method

Sowing is done by hand. Dibble 2–3 seeds at a distance of 10 cm on the ridge.

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.

Weed management

Apply Glycel (1–2%) as a preemergence herbicide. Intercultivation is done twice during early stages of crop growth. If required, hand weeding is undertaken at later stages.

Verification of accession identity

1. Eliminate off-types and plants growing off-row.
2. Verify accession identity by comparing the following traits in characterization data:
 - growth habit,



- flower color,
 - seed color, and
 - seed shape.
3. 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.

1. Hold the stem at the base and pull out the plants from soil.
2. Tie the uprooted plants from a row into small bundles and label them with accession number and field plot number.
3. Thresh the pods from individual plants on a tarpaulin by gently beating with sticks and collect the seeds into paper packets.
4. Ensure that spillover and seed mixing do not occur during threshing.
5. If limited number of pods are available, separate out seeds manually.
6. Clean the seeds of debris.
7. Take equal quantity of seeds from each plant and place them in muslin cloth bags labeled within and outside with tear-off tags.
8. Move the bags into temporary storage area for further drying.

Seed health

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

Wild species

Raise seedlings in small pots and then transfer them to large pots or to the field. Pasteurize the soil mixture to protect plants from soilborne diseases such as wilt and collar rot.

Raising seedlings

1. Fill small pots (earthen or plastic pots, 10 × 10 cm with a hole at bottom) with pasteurized mixture of 3:1 soil and farm yard manure.
2. Scarify the seeds by making a small cut to the seed coat to improve water absorption and germination.

3. Dress the seeds with Benlate®.
4. Put two seeds in each pot at about 2-cm depth.
5. 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.

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



D. Pigeonpea regeneration

Seed multiplication is carried out in Vertisols (black soils). Late sowing results in reduced plant height, and thus allows whole plants to be conveniently covered with muslin cloth bags to control outcrossing. It is also possible to control pollination by covering the whole plot using dismantable frames covered with nets. When small number of cultivars are to be multiplied for large-scale seed production, geographic isolation of about 100 m is desirable. Soil tests should be carried out prior to sowing to ensure satisfactory fertility.

Soil preparation

Prepare the field by deep ploughing followed by 2–3 harrowings. Level the field and make ridges spaced 75 cm apart.

Field layout

Mark the field into tiers of 9 m with 1-m path between tiers. Use two rows of 9 m, providing a *minimum of 180 plants* for regenerating each accession.

Fertilization

Apply a basal dose of diammonium phosphate @ 100–115 kg ha⁻¹.

Sowing date

Sow seeds in August at Patancheru.

Sowing method

Sowing is done by hand. Dibble 4–5 seeds in holes at a distance of 10 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.

Weed management

Apply 1–2% Glycel as a preemergence herbicide. Intercultivation is done twice during early stages of crop growth. Hand weeding is undertaken, if required at later stages.

Thinning

Reduce the stand to one or two plants hole⁻¹ by thinning after 15 days.

Pollination control

Pigeonpea is cross-pollinating (0–40%, depending on genotype and insect pollinator populations). Seed increase must preclude cross pollination.

- Cover at least 180 plants in muslin cloth bags (Fig. 8D.1) or in pollination cages before flowering.
- Spray Thiodan® (@ 2 mL L⁻¹) before covering the plants.

Verification of accession identity

1. Eliminate off-types and plants growing off-row.
2. 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.
3. Rogue the plants, which 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 labeled jute sack (63 × 33 cm).

Dry the pods under shade for 2–3 days to reduce the seed moisture content to about 12%.

1. Thresh the pods on a tarpaulin by gentle beating and collect the seeds into paper packets.
2. Ensure that spillover and seed mixing do not occur during threshing.
3. If limited number of pods are available, separate seeds manually.
4. Clean the seeds of debris.
5. Take equal quantity of seeds from each plant and put them in muslin cloth bag labeled within and outside with tear-off tags.
6. Move the bags into temporary storage area for further drying.

Seed health

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



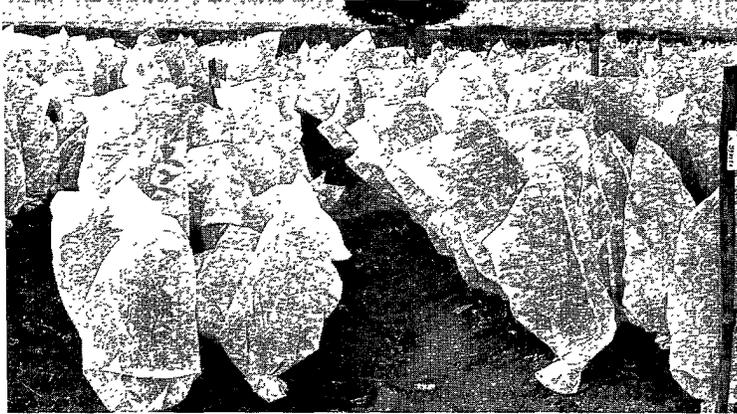


Figure 8D.1 — Pigeonpea plants bagged to maintain genetic integrity during regeneration.

Wild species

Raise seedlings in small pots and then transfer them to large pots or to the field. Pasteurize the soil mixture to protect plants from soilborne diseases such as wilt and collar rot.

Raising seedlings

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

Transplanting

1. Transplant in the evening.
2. 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.
3. Large pots (size 30 × 30 cm) filled with a pasteurized mixture of 3:1 soil and farm yard manure 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.
4. Turn the plastic cup upside down holding the plant with your fingers.
5. Tap gently until the seedling with all the soil comes out into the palm.
6. Transplant the seedling in the desired pot or field and water it with a rose can.
7. Keep the new pots in shade for 2 days, providing optimum moisture.
8. 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.
9. Use a sample size of 8–10 plants for each accession.
10. Provide bamboo stakes to support the climbers such as *C. albicans*, *C. crassus*, *C. goensis*, *C. heynei* and *C. mollis*.
11. Collect the ripe pods from individual plants into paper envelopes before they shatter.
12. Bulk equal quantities of seeds from each plant to reconstitute the accession.



E. 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 during previous 2 years. Groundnut is day-neutral, and seeds can be regenerated both during rainy and postrainy seasons (preferable). During rainy season, they are sown in June and harvested in October, where as during postrainy season, they are sown in November and harvested in April at Patancheru.

Soil preparation

Prepare the field by deep ploughing, followed by 2–3 harrowings. Level the field and make ridges spaced 75 cm apart.

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.

Fertilizer

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.

Irrigation

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

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 Glycel (1–2%) as 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

1. Eliminate off-types and plants growing off-row.
2. Verify accession identity as the plants grow by comparing characterization data on:
 - branching pattern,
 - leaflet shape,

- flower color,
 - pod constriction and
 - primary seed color.
3. Rogue the plants, which are genuine mixtures.

Harvesting

Check harvest-maturity by digging samples from below ground. Prominent symptoms of maturity are yellowing of leaves, spotting and shedding of old leaves. The pods become hard and tough with a dark tannin discoloration inside the shell. The kernels become unwrinkled and 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:

- manually lifting the vines from the soil with pods intact, and
- separating the pods from the vines.

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 soil by digging with a spade.

Stripping pods from vines

1. Leave the harvested plants to dry in the field with pods turned uppermost in windrows for 2–3 days. Alternatively, tie the plants into small bundles, label, and dry them under shade.
2. Strip the dry pods from the plant by hand and collect them into paper bags.
3. Clean the pods of the soil and dry them further to about 8–9% moisture content by slow drying.
4. Bulk equal number of pods from each plant to make up the accession.
5. 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, portion of the testa should peel off.*

Seed health

1. Coordinate periodic field inspection by pathologists and virologists during the growing season.
2. Send a representative sample of the harvested seed for health testing.
3. Process the material for storage if the level of infection is within allowable limits.
4. 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 bottom) or concrete rings (65 cm circumference × 85 cm height) to grow wild *Arachis* species.

1. Cover the hole at the bottom of the pots with pieces of rubble.
2. Fill the pot or concrete rings with 3 red soil : 2 sand : 1 farm yard manure mixture, pasteurized (3 cycles of 1 h each) at 82.2°C (180°F) and 34.5×10^3 Pa (5 psi).
3. Apply a basal dose of urea and diammonium phosphate (25 g pot⁻¹) at a depth of 7.5 cm .
4. Dress the seeds in a 2:3 mixture of Bavistin® and Thiram® and sow them at a depth of about 3.75 cm.
5. Apply 2–3 drops of 0.2% Etherel (2-chloroethylphosphonic acid, 39%) solution (3 mL L⁻¹) on seeds before covering them with soil.
6. Top the soil in concrete rings with 5.0–7.5 cm sand.
7. Water the pots after seeding and then twice a week.
8. Apply gypsum @ 10 gm pot⁻¹ 50 days after sowing.

Yellowing of foliage and formation of dark lining inside the shell indicate maturity.

9. Stop watering the pots 2 days prior to harvesting.
10. Sift the soil through a sieve and strip the pods.
11. Dry the pods in shade.
12. Transfer them to a temporary holding room for further drying and processing.

Rhizomatous species

1. Use rhizomes of 20-cm length, cut from mother plant.
2. Soak the rhizomes in Bavistin® suspension (@ 3 g L⁻¹ water) for 5 min.
3. Plant the rhizomes in a potting mixture consisting of 3 parts of red soil, 2 parts of sand, and 1 part of farm yard manure.

4. Plant the rhizomes 5-cm deep preferably in plastic or earthen pots, or on a raised nursery bed.
5. Maintain the rhizomes in a greenhouse at $25\pm 2^{\circ}\text{C}$ 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 by avoiding continuous watering until they are established.*
- *Rhizomes require 1 month for establishment after which they can be transferred to the field.*
- *Transplantation should be done in the evening.*

F. 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.

1. Apply diammonium phosphate @ 100 kg ha⁻¹ as a basal dose prior to sowing.
2. Prepare the field as fine tilth as the species will not tolerate a seedbed, which is not properly compacted.
3. Grow the crop along 4-m rows.
4. Hand-weeding is done when seedlings are about 5-cm high.
5. Thin the plants so that they are 10 cm apart.
6. Cultivars vary in their ability to resist shattering, therefore, harvest before there is any great loss of seed.
7. Harvest the panicles by hand.
8. Dry the seed heads to about 12% moisture under shade.
9. Thresh the panicles by hand.
10. Clean the seeds by winnowing.
11. Bulk equal amount of seeds from each plant to make up the accession.

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 occurred 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: Method 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 9

100

Characterization and Preliminary Evaluation

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

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

Follow the same sowing and cultural practices for the field grow-out, as described under regeneration (Section 8). Grow the accessions in single 4-m rows. Maintain row to row distance at 60 cm (pearl millet and chickpea) or 75 cm (other crops) and plant-to-plant distance at 10 cm (25 cm in pigeonpea). When the collections are large, grow and evaluate in an augmented block design. Use standard check cultivars every 10 or 20 rows.

Use the descriptors developed by ICRISAT and IBPGR for characterization and preliminary evaluation (IBPGR and ICRISAT 1992a,b and 1993a,b; IBPGR et al.1993).

A. 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 10 randomly selected plants (Fig. 9A.1).

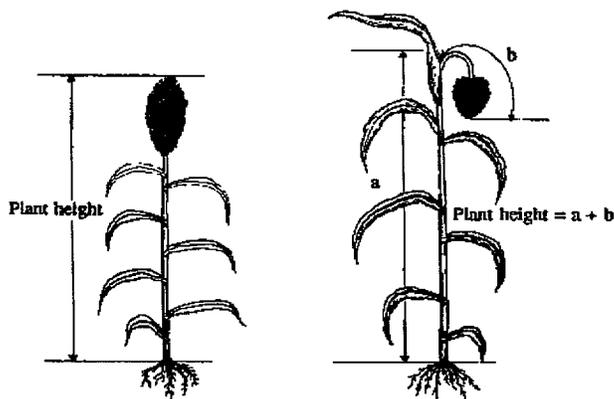


Figure 9A.1 — Plant height in sorghum.

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 stage

Days to flowering: Number of days from the date of emergence 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. 9A.2)

- 1 Slightly exerted
- 2 Exerted
- 3 Well-exerted
- 4 Peduncle recurved

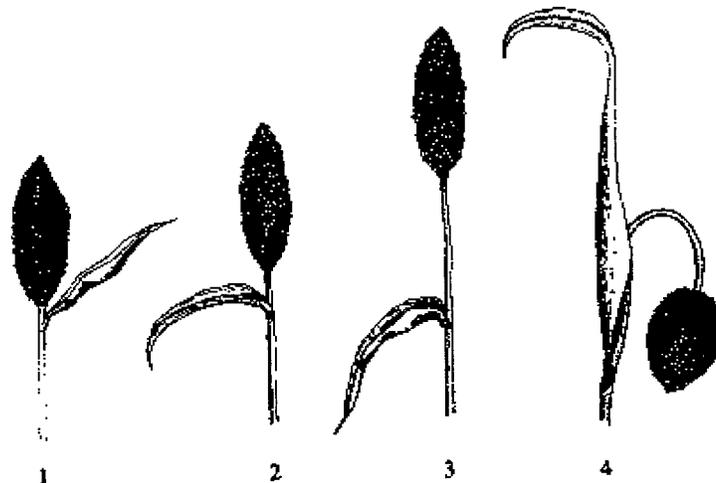


Figure 9A.2 — Panicle exertion in sorghum.

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

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

Panicle compactness and shape: Compactness and shape of the panicle (Fig. 9A.3)

- 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

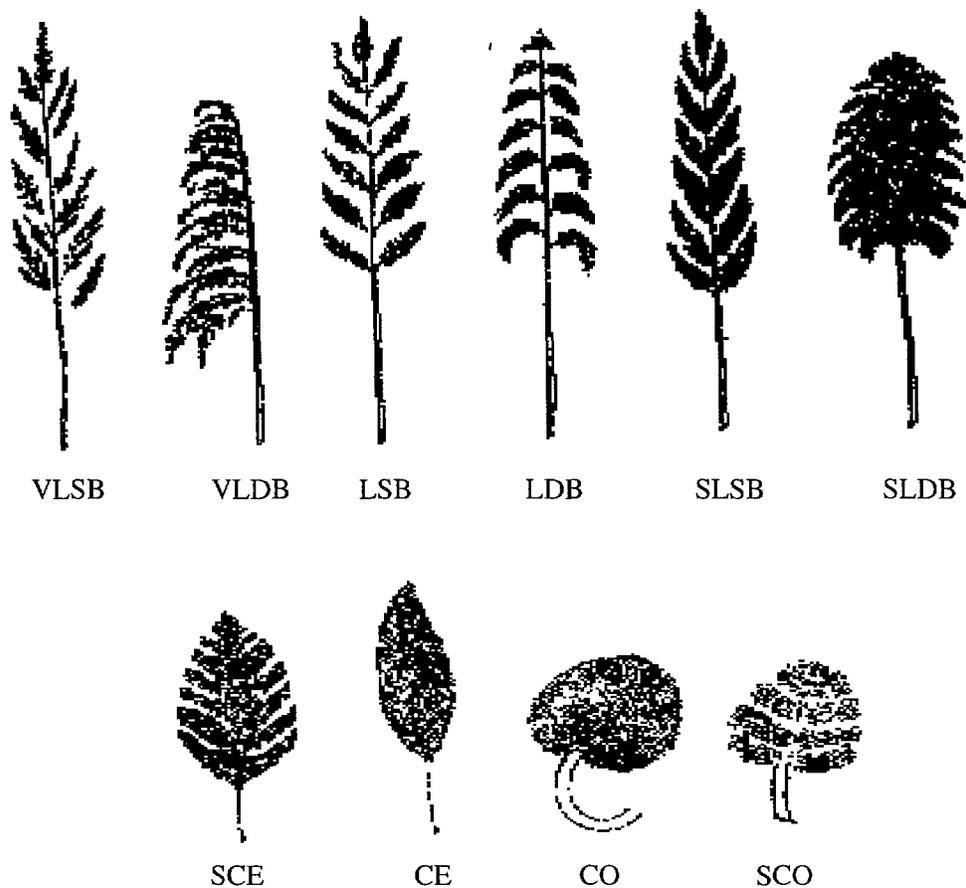


Figure 9A.3 — Inflorescence compactness and shape in sorghum.

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 grain covered by glumes at maturity (Fig. 9A.4)

- 1 25% grain covered
- 2 50% grain covered
- 3 75% grain covered
- 4 Grain fully covered
- 5 Glumes longer than grain

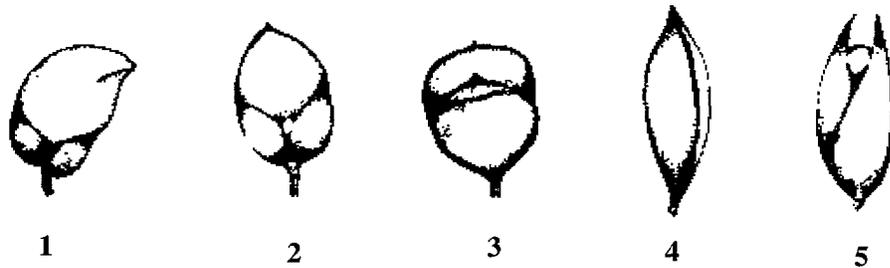


Figure 9A.4 — Extent of grain covered by glume.

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: Shine of seed

L	Lustrous
NL	Nonlustrous

Seed subcoat: 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. 9A.5)

- 1 Completely corneous
- 2 Almost corneous
- 3 Partly corneous
- 4 Almost starchy
- 5 Completely starchy

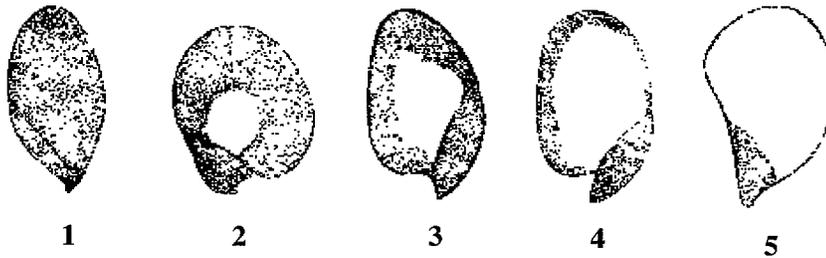


Figure 9A.5 — Endosperm texture in sorghum.

Threshability: Ease with which seeds can be separated from the panicle

- | | |
|----|---------------------|
| FT | Freely threshable |
| PT | Partly threshable |
| DT | Difficult to thresh |

B. Descriptors for characterization of pearl millet

Vegetative phase

Plant height (cm): Height of plant measured from ground level to the tip of the panicle.

Productive tiller number : Number of tillers bearing panicles counted at dough stage.

Nodal tillers: Visual score on a 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

Photoperiod sensitivity: Visual score on a 1–9 scale for sensitivity to photoperiod

3	Insensitive
5	Partly sensitive
7	Highly sensitive

Fodder yield potential: Green fodder yield potential considering tillering, leafiness and bulk at flowering

3	Poor
5	Intermediate
7	Good

Reproductive phase

Days to flowering: Number of days from sowing to when 50% of plants flower in the plot. Stigma emergence on the main panicle is considered as flowering.

Exertion (cm): Distance between ligule of the flag leaf and the base of the panicle on main plant.

Panicle length (cm): Length of panicle on main axis measured at dough stage.

Panicle thickness (cm): Thickness of panicle on main plant, measured at dough stage.



Panicle shape: Shape of panicle at dough stage (Fig. 9B.1)

- | | | | |
|---|-------------|---|--------------|
| 1 | Cylindrical | 6 | Dumb-bell |
| 2 | Conical | 7 | Lanceolate |
| 3 | Spindle | 8 | Oblanceolate |
| 4 | Club | 9 | Globose |
| 5 | Candle | | |

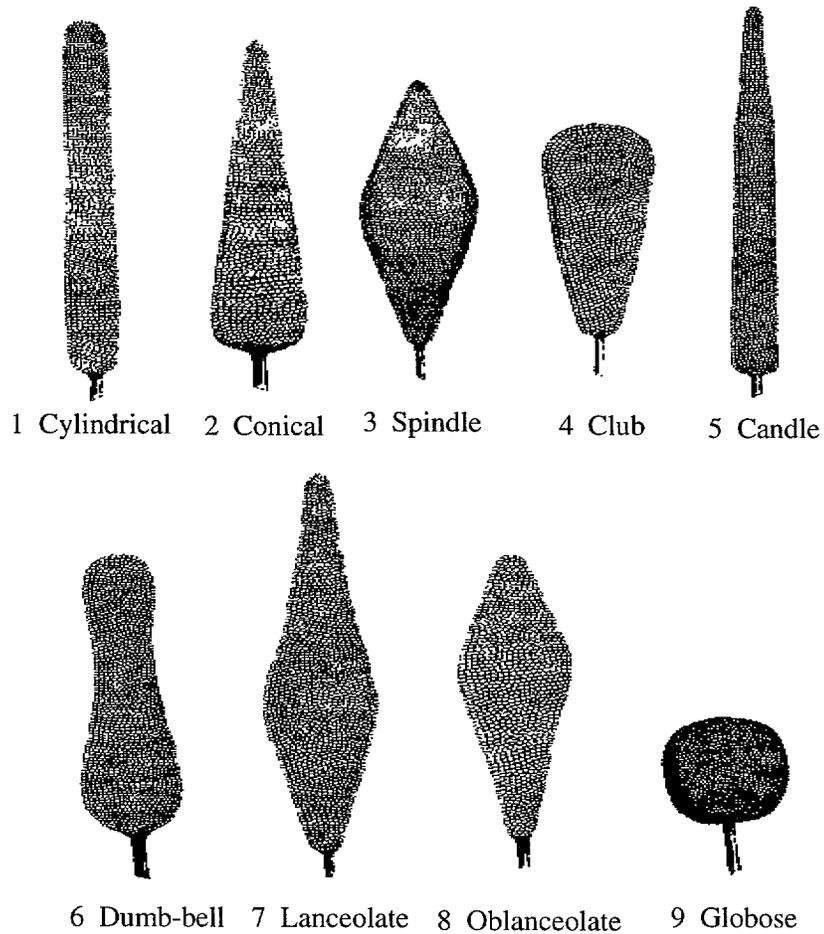


Figure 9B.1 — Panicle shape in pearl millet.

Spikelet density: Density of spikelets, visually scored on a 1–9 scale at maturity

- | | |
|---|--------------|
| 3 | Loose |
| 5 | Intermediate |
| 7 | Compact |

Synchrony of panicle maturity: Uniformity for maturity, visually scored on a 1–9 scale at dough stage

- 3 Nonsynchronous
- 5 Intermediate
- 7 Synchronous

Bristle length: Length of bristles, visually scored on a 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 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 1000 seeds drawn randomly from plot yield, at 12% moisture content.

Seed shape: Shape of seed after drying (Fig. 9B.2)

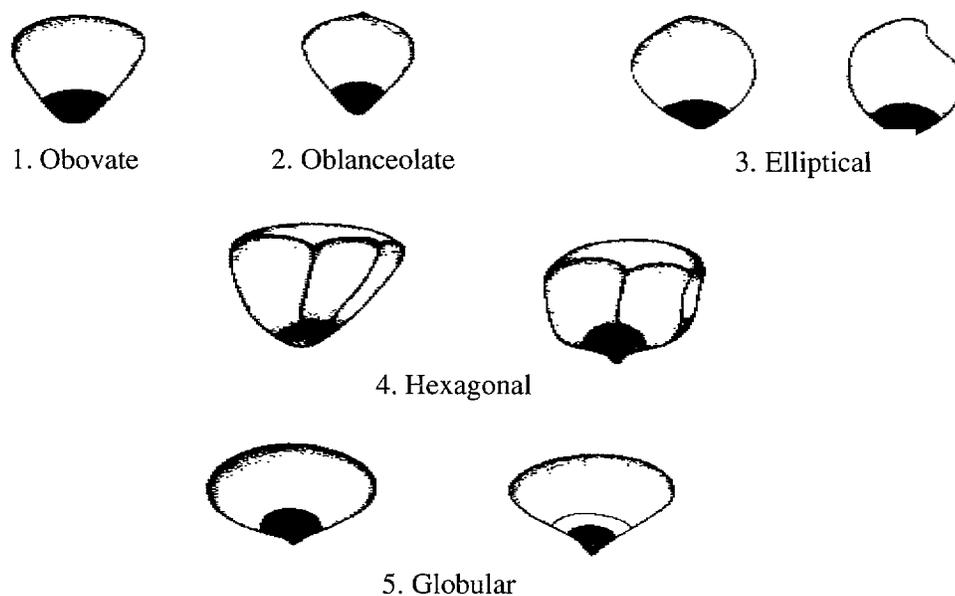


Figure. 9B.2 — Seed shapes in pearl millet.

- 1 Obovate
- 2 Oblanceolate
- 3 Elliptical
- 4 Hexagonal
- 5 Globular

Seed yield potential: Seed yield potential of the accession, visually scored on a 1–9 scale considering number, size and density

- 3 Low
- 5 Intermediate
- 7 High

Endosperm texture: Texture of endosperm visually scored on a 1–9 scale:

- 3 Low
- 5 Partly corneous
- 7 Mostly starchy



C. Descriptors for characterization of chickpea

Vegetative phase

Growth habit: Angle of primary branches, recorded at mid-pod filling stage (Fig. 9C.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

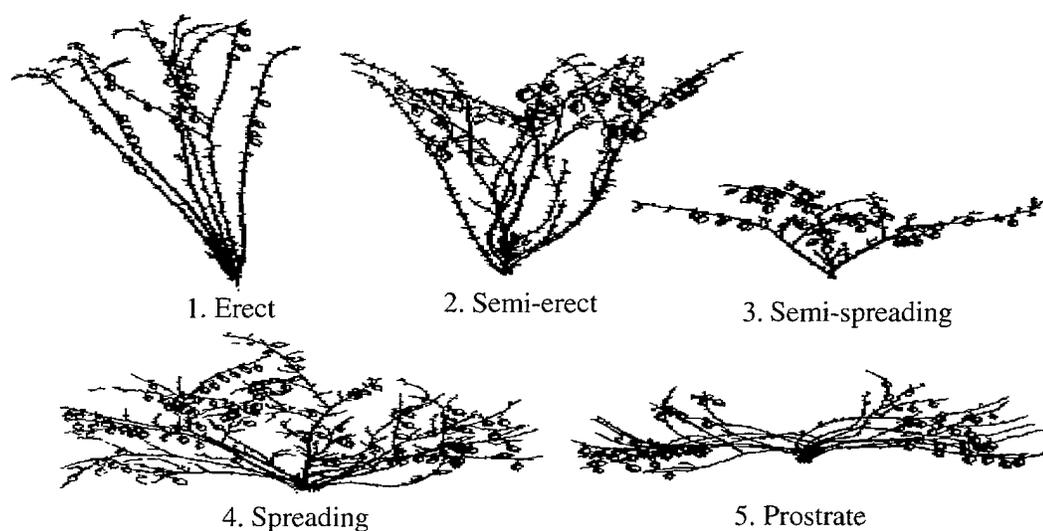


Figure. 9C.1 — Growth habit in chickpea.

Plant height (cm): Mean canopy height of five representative plants, measured from soil surface after flowering is complete.

Plant width (cm): Mean canopy spread of five representative plants, measured after flowering is complete.

Plant pigmentation: Presence of anthocyanin pigment in plant parts

NA	No anthocyanin
LA	Low anthocyanin
HA	High anthocyanin

Basal primary branch number: Number of branches emerging from the axils on the lower half of the main stem, average of 3–5 competitive plants from each accession at the time of harvest.

Apical primary branch number: Number of branches emerging from the leaf axils on the upper half of the main stem, average of 3–5 competitive plants from each accession at the time of harvest.

Basal secondary branch number: Number of branches emerging from the leaf axils of basal primary branches, average of 3–5 competitive plants from each accession at the time of harvest.

Apical secondary branch number: Number of branches emerging from the leaf axils of apical primary branches, average of 3–5 competitive plants from each accession at the time of harvest.

Tertiary branch number: Number of branches emerging from the leaf axils of basal and apical secondary branches, average of 3–5 competitive plants from each accession at the time of harvest.

Reproductive phase

Days to flowering: Number of days from sowing (first irrigation) to the stage when 50% of plants have begun to flower.

Flowering duration: Number of days from 50% flowering to the date when 50% of the plants of an accession stopped 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.

Pods plant⁻¹: Average number of fully formed pods plant⁻¹ from 3–5 representative plants at maturity.

Seeds pod⁻¹: Number of seeds pod⁻¹ — estimated by dividing the total number of seeds by the total number of pods harvested from 3–5 representative plants.

Seed color: Seed 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. 9C.2)

ANG	Angular, ram's head
OWL	Irregular round, owl's head
P	Pea-shaped, smooth round

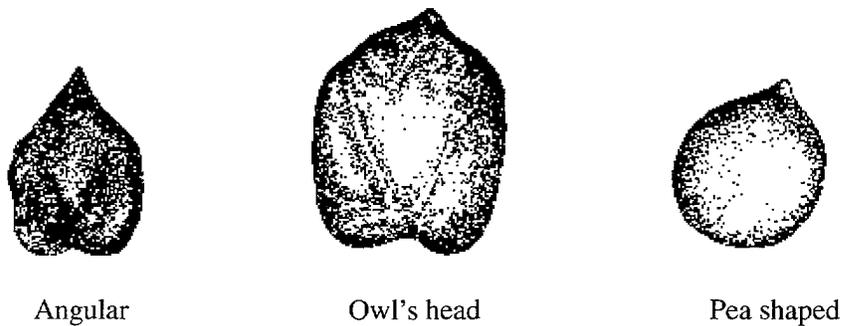


Figure 9C.2 — Seed shape in chickpea.

Seed surface: Seed surface observed from dry mature seed (Fig. 9C.3)

- R Rough — wrinkled with uneven surface
- T Tuberculated — sticky because of tiny projections
- S Smooth

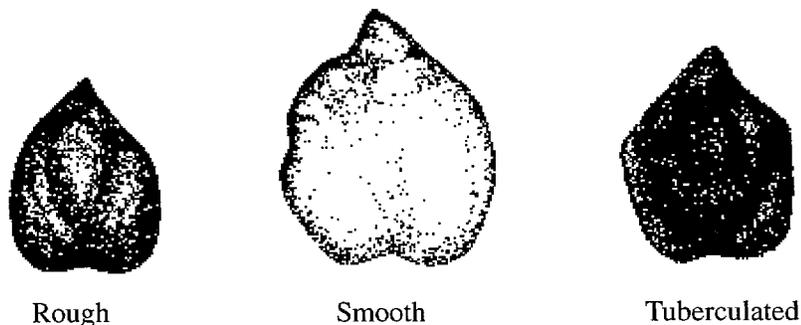


Figure 9C.3 — Testa texture in chickpea.

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.

Protein content (%): The percentage of crude protein estimated in the freshly harvested seeds using dye-binding method or automatic protein analyzer.

Diseases

Wilt: Rating for fusarium wilt (causal organism: *Fusarium oxysporum*) resistance of 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: Rating for ascochyta blight (causal organism: *Ascochyta rabiei*) resistance. Ten day-old seedlings are inoculated in plant propagator and disease severity rated after 15 days incubation on a 1–9 scale:

- 1 No damage
- 9 Severe damage

Colletotrichum blight: Rating 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 rated 15 days after inoculation on a 1–9 scale:

- 1 No damage
- 9 Severe damage

D. Descriptors for characterization of pigeonpea

Vegetative stage

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 branch number: Average number of branches borne on the main stem, recorded from three plants at the time of harvest.

Secondary branch 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 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

- 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

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. 9D.1)

FS Few streaks
MS Medium streaks
DS Dense streaks
P Plain, uniform coverage
NO None

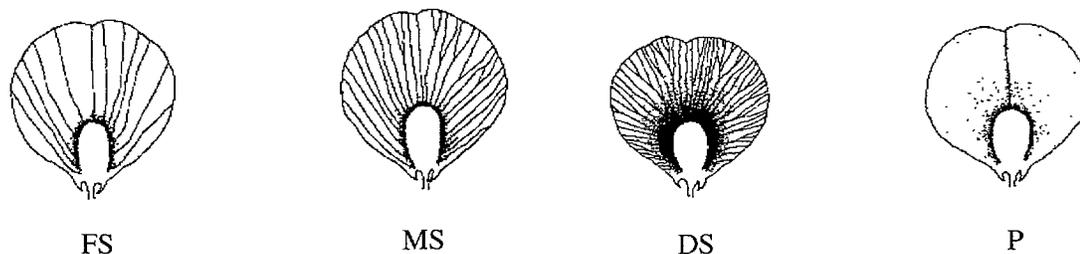


Figure 9D.1 — Pattern of streaks on standard petal in pigeonpea.

Raceme number: Average number of racemes plant⁻¹, recorded from three plants at the time of 50% flowering.

Days to 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

DP Dark purple
G Green
M Mixed green and purple
P Purple



Seeds pod⁻¹: Number of seeds pod⁻¹, determined from 10 pods randomly picked from three plants at harvest maturity.

Seed color pattern: Color pattern of seed coat recorded after drying (Fig. 9D.2)

- P Plain
- M Mottled
- S Speckled
- MS Mottled and speckled
- R Ringed



Figure 9D.2 — Seed color pattern in pigeonpea.

Primary seed color: Main color of the seed coat recorded after drying

- | | | | |
|----|--------|----|---------------|
| 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 | | |

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 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. 9D.3)

- O Oval
- P Pea (globular)
- S Square
- E Elongate



Figure 9D.3 — 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 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 (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.

E. 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. 9E.1)

- 1 Procumbent-1
- 2 Procumbent-2
- 3 Decumbent-1
- 4 Decumbent-2
- 5 Decumbent-3
- 6 Erect
- 7 Others

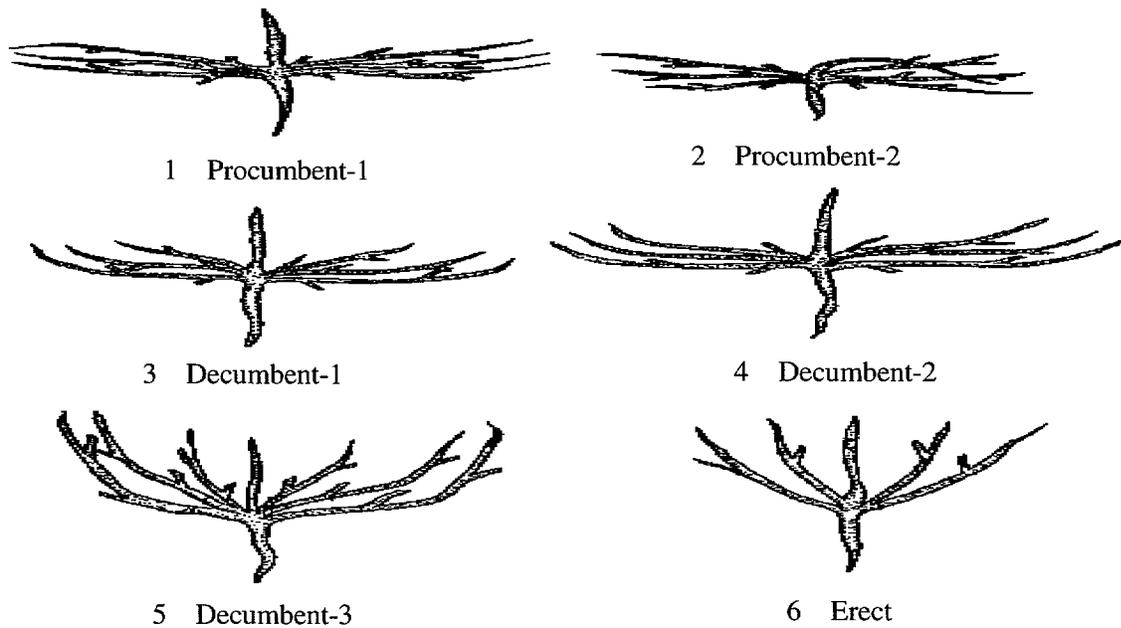


Figure 9E.1 — Growth habit in groundnut.

Plant height (cm): Height of main axis, measured from cotyledonary axil up to terminal bud, mean of 10 plants recorded 60–85 days after emergence.

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. 9E.2)

- 1 Alternate
- 2 Sequential
- 3 Irregular with flowers on main stem
- 4 Irregular without flowers on main stem
- 5 Others

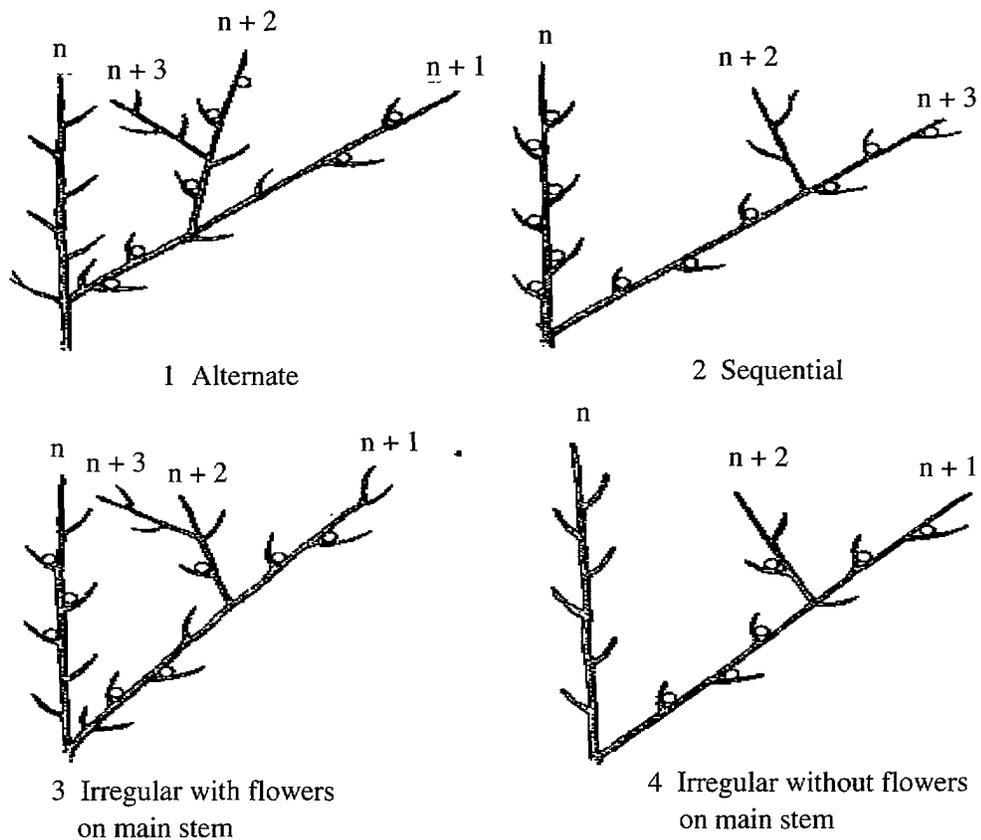


Figure 9E.2 — Branching pattern in groundnut.

10

Primary branch 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 10 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 10 leaflets recorded from different plants.

Leaflet shape: Shape of fully expanded apical leaflet of the third leaf on the main stem (Fig.9E.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 below
- 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



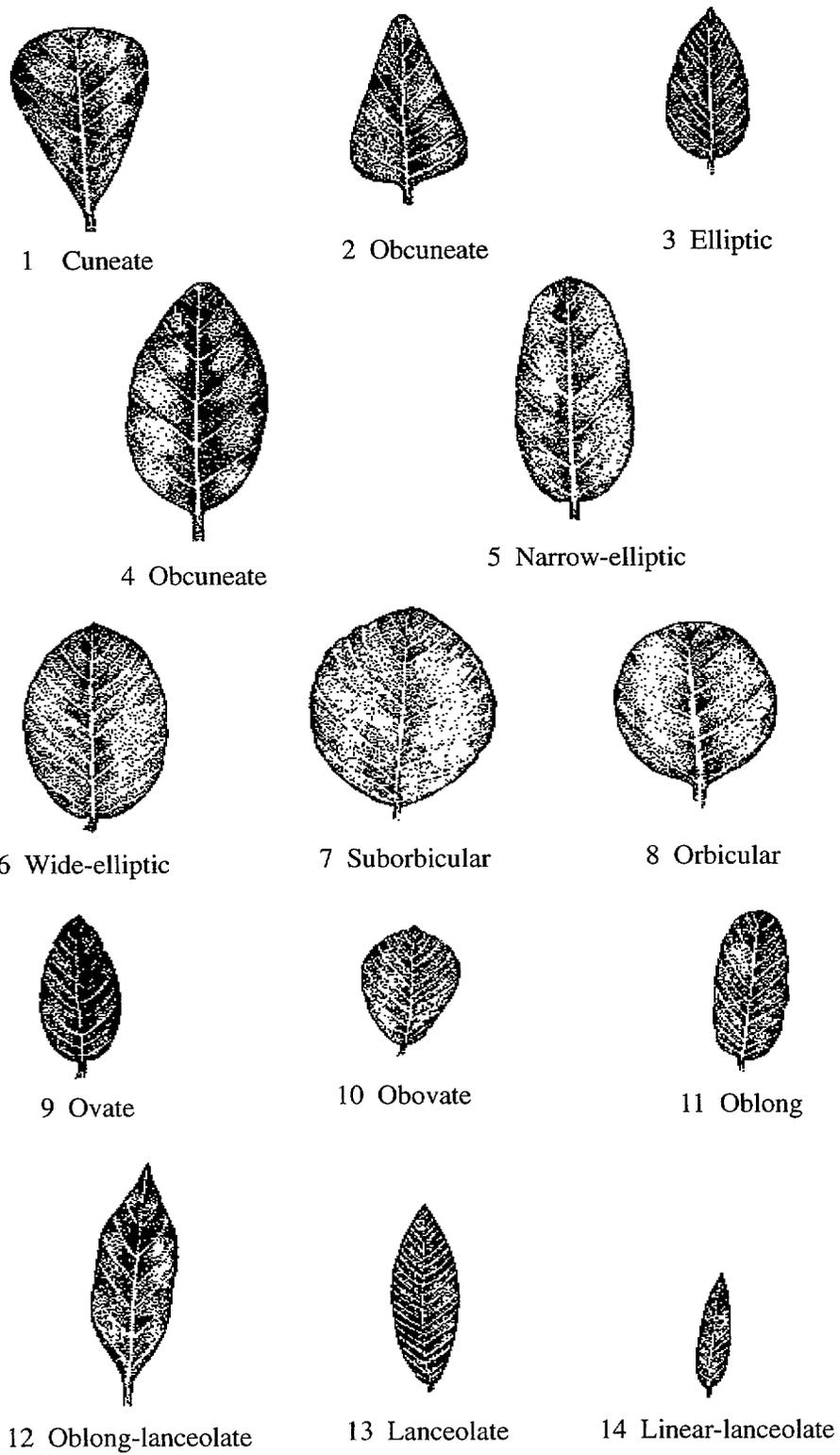


Figure 9E.3 — Leaflet shape in groundnut.

Reproductive phase

Days to 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
- 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. 9E.4)

- 0 Absent
- 3 Slight
- 5 Moderate
- 7 Prominent
- 9 Very prominent

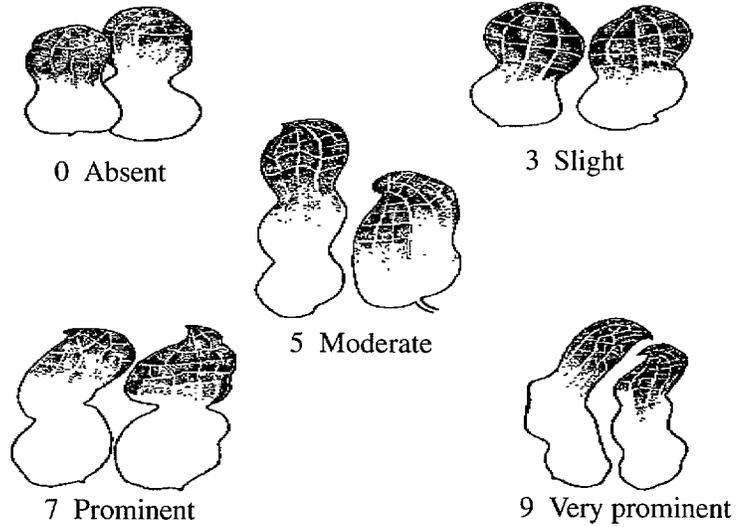


Figure 9E.4 — Pod beak in groundnut.

Pod constriction: Degree of pod constriction (Fig. 9E.5)

- 0 None
- 3 Slight
- 5 Moderate
- 7 Deep
- 9 Very deep

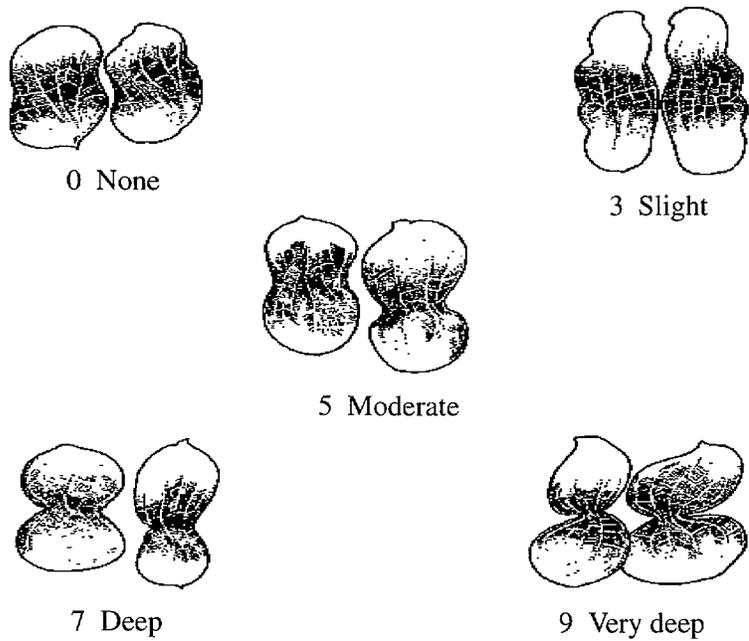


Figure 9E.5 — Pod constriction in groundnut.

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.

Pod width (mm): Mean width of pod at widest point, recorded from 10 mature pods.

Seeds pod⁻¹: Number of seeds pod⁻¹. First number indicates most frequent number of seeds pod⁻¹, 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

- 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

- | | | | |
|----|---------------|----|-----------------------------|
| 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

- 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.

Seed width (mm): Width of seeds measured at mid-point.

Shelling percentage: Shelling percentage recorded with seeds at about 8% moisture as

$$\frac{\text{Mass of mature seeds} \times 100}{\text{Mass of mature pods}}$$

Fresh seed dormancy (%): Germination immediately after harvest and number of days to achieve 70% germination, e.g., 65/12 indicates that 65% seed can germinate immediately after harvest, and seeds require 12 days to reach 70% germination.

Section 10

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Taxonomic Classification

A. 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, pedicellate 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 of 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, some what contracted to loose with branches obliquely ascending or spreading, racemes 1–5 noded, fragile.

The cultivated sorghums (*bicolor* Subsp. *bicolor*) were classified into five basic races (Fig. 10A.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.

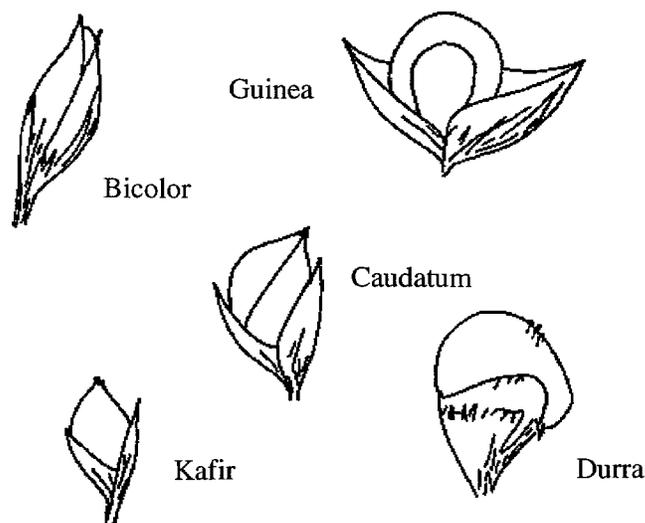


Figure 10A.1 — Glume coverage and racial classification in sorghum.

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. stipoidium*
 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 caduceus..... *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. matrankense*
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*

- 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 cuspidate-eristate; 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*

C. Key to the identification of *Cicer* species

(Source: van der Maesen 1987)

Annual species

1. Leaves with end leaflet, plant prostrate or erect 2
 - 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 3 3
3. Leaflets in 2–3 pairs with end leaflet 4
 - Leaflets more numerous 5
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 mm 6
 - Leaflets larger, 5–12 mm; flowers 8–12 mm; seeds 5–12 mm 7
- 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. judaic*
- 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 reticulate 8
- Seeds echinate *C. echinospermum*
 - Seeds reticulate *C. reticulatum*

Perennial species

1. Leaf rachis ending in a tendril or leaflet 2
 - 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 larger 3
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 mm 5
 - Flowers large, ca 20–27 mm 8
5. Leaflets spine-shaped, plant glabrous *C. subaphyllum*
 - Leaflets normal, flat; plant pubescent 6
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, curled 7
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 minute 9
 - Flowers (1) 2–5 per peduncle 10
9. Stipules flabellate-rounded, about as large as or larger than the leaflets, toothed 11
 - Stipules obliquely ovate or triangular, small or half as long as the leaflets, at some nodes sometimes nearly as large incised 12
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 foliolate 16
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) mm 13
 - Leaflets larger, up to 25(–27) mm, sometimes smaller 20
13. Leaflets in 2–4(–5) pairs, rachis with end leaflet or ending in a slender spine *C. paucijugum*
 - Leaflets more numerous 14

14. Plant densely pubescent, 10–30 cm; leaves imparipinnate; leaves in 9–18 pairs, obovate to oblonglanceolate, top toothed, flower 1 per peduncle *C. multijugum*
 - 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 *C. microphyllum*
 - 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 *C. nuristanicum*
 - 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 *C. mogoltavicum*
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 *C. montbretii*
 - Leaves ending in a tendril, ramified or not, at lower leaves a top leaflet; flowers blue or purple; plant climbing. *C. graecum*
19. Leaflets oblong-obovate, spiny toothed, 7–24-mm long, 5–15-mm wide; bracts 1–2 mm; flowers white *C. isauricum*
 - Leaflets oblong-elliptical, finely toothed, 8–15 mm, 3–8-mm wide; bracts 2–3 mm; flowers blue-violet *C. qoribundum*
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) *C. anatolicum*
 - 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 *C. flexuosum*
 - 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. *C. baldshuanicum*
 - 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 *C. grande*
 - 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 *C. korshinskiyi*
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 *C. pungens*
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 *C. macracanthum*
 - Stipules shorter, vertical spinelets, up to 8 mm, or foliate 26
26. Leaflets mostly spine shaped *C. stapfianum*
 - Leaflets foliate 27
27. Stipules horizontal lanceolate perules, 2–5-mm long; leaflets in 3–7 pairs *C. acanthophyllum*
 - 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 *C. rechingeri*
 - 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 *C. tragacanthoides*
 - Taller, slender forms from Kopet-dagh var. *turcomanicum*

D. Key to the identification of *Cajanus* species

(Source: van der Maesen 1990)

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. rugosus</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*

- 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. Ellipsoidal 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 orange *A. lignosa*

- 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 vicious.....*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 vicious *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. kuhlmanii*
 42 Pod without segment, with 1–5 seeds. Peg tenacious*A. hypogaea*
- 68 Main stem without flowers and n+l branches in plants that alternate with regularity two vegetative branches and two reproductive (alternate branching) subsp. *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 fruitification.
 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. 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*



- 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. pseudovillosa*
- 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*

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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.

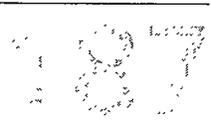
Average germination (%) of replicates		Probability (%)			
		5	2.5	1	0.1
99	2	3	4	4	5
98	3	4	5	5	7
97	4	5	6	6	8
96	5	5	6	7	9
95	6	6	7	8	10
94	7	7	8	9	11
93	8	7	8	9	12
92	9	7	9	10	13
91	10	8	9	10	13
90	11	8	9	11	14
89	12	9	10	11	15
88	13	9	10	12	15
87	14	9	11	12	16
86	15	9	11	13	16
85	16	10	11	13	17
84	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%; 1.0%, 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 requirements of different countries in respect of ICRISAT mandate crops

List of the countries for Import permit requirement.

Date: 20-04-1999

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	Not required
4	Botswana	Required	4	Bhutan	Not required
5	Burundi	Not required	5	Cambodia	Not required
6	Burkina Faso	Not required	6	Hong Kong	Not required
7	Cameroon	Not required	7	Indonesia	Not required
8	Canary Is.	Not required	8	Iran	Not required
9	Cape Verde Is.	Not required	9	Iraq	Not required
10	Central African Rep.	Not required	10	Israel	Required
11	Chad	Not required	11	Japan	Not required
12	Comoro Is.	Not required	12	Jordan	Not required
13	Congo	Not required	13	Korea DPR	Not required
14	Côte d'Ivoire	Not required	14	Korean Rep.	Not required
15	Djibouti	Not required	15	Kuwait	Not required
16	Egypt	Not required	16	Laos PDR	Not required
17	Eritrea	Required	17	Lebanon	Not required
18	Ethiopia	Required	18	Malaysia	Required
19	Gabon	Not required	19	Maldives	Not required
20	Gambia	Not required	20	Micronesia	Required
21	Ghana	Not required	21	Myanmar	Not required
22	Guinea	Not required	22	Nepal	Not required
23	Guinea Bissau	Not required	23	Oman	Not required
24	Kenya	Required	24	Pakistan	Not required
25	Lesotho	Not required	25	Philippines	Required
26	Liberia	Not required	26	PR China	Not required
27	Libya	Not required	27	Qatar	Not required
28	Malagasy Rep.	Not required	28	Rep. of Palau	Not required
29	Malawi	Required	29	Saudi Arabia	Not required
30	Mali	Not required	30	Singapore	Not required
31	Mauritania	Not required	31	Sri Lanka	Not required
32	Mauritius	Required	32	Syria	Not required
33	Morocco	Not required	33	Taiwan	Not required
34	Mozambique	Not required	34	Thailand	Not required
35	Namibia	Required	35	UAE	Not required
36	Niger	Not required	36	Uzbekistan	Not required
37	Nigeria	Required	37	Vietnam	Not required
38	Rep. Fr. Mayotte	Not required	38	Yemen	Not required
39	Rwanda	Not required			
40	Senegal	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	Not required			
50	Zaire	Not required			
51	Zambia	Not 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	Required
7	France	Not required	7	Colombia	Required
8	Germany	Not required	8	Costa Rica	Not required
9	Greece	Not required	9	El Salvador	Not 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	Honduras	Required
14	Portugal	Not required	14	Mexico	Not required
15	Romania	Not required	15	Nicaragua	Required
16	Russia	Not required	16	Panama	Not required
17	Slovakia	Not required	17	Paraguay	Not required
18	Spain	Not required	18	Peru	Required
19	Sweden	Not required	19	Puerto Rico	Not required
20	Switzerland	Not required	20	Surinam	Not required
21	The Netherlands	Not required	21	Uruguay	Not required
22	Turkey	Not required	22	USA	Required
23	UK	Not required	23	Venezuela	Not required
24	Ukraine	Not required	24	Virgin Is.	Not required
25	Yugoslavia	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	New Zealand	Not required
5	Dominican Rep.	Not required	5	Papua New Guinea	Required
6	Granada	Not required	6	Solomon Is.	Required
7	Haiti	Not required	7	Tonga Is.	Required
8	Jamaica	Required	8	Vanuatu	Not required
9	St. Helena	Not required	9	Western Samoa	Required
10	St. Kitts & Nevis	Not required			
11	St. Lucia	Not required			
12	Trinidad & Tobago	Required			

About ICRISAT

The semi-arid tropics (SAT) encompasses parts of 48 developing countries including most of India, parts of southeast Asia, a swathe across sub-Saharan Africa, much of southern and eastern Africa, and parts of Latin America. Many of these countries are among the poorest in the world. Approximately one-sixth of the world's population lives in the SAT, which is typified by unpredictable weather, limited and erratic rainfall, and nutrient-poor soils.

ICRISAT's mandate crops are sorghum, pearl millet, finger millet, chickpea, pigeonpea, and groundnut; these six crops are vital to life for the ever-increasing populations of the SAT. ICRISAT's mission is to conduct research which can lead to enhanced sustainable production of these crops and to improved management of the limited natural resources of the SAT. ICRISAT communicates information on technologies as they are developed through workshops, networks, training, library services, and publishing.

ICRISAT was established in 1972. It is one of 16 nonprofit, research and training centers funded through the Consultative Group on International Agricultural Research (CGIAR). The CGIAR is an informal association of approximately 50 public and private sector donors; it is co-sponsored by the Food and Agriculture Organization of the United Nations (FAO), the United Nations Development Programme (UNDP), the United Nations Environment Programme (UNEP), and the World Bank.