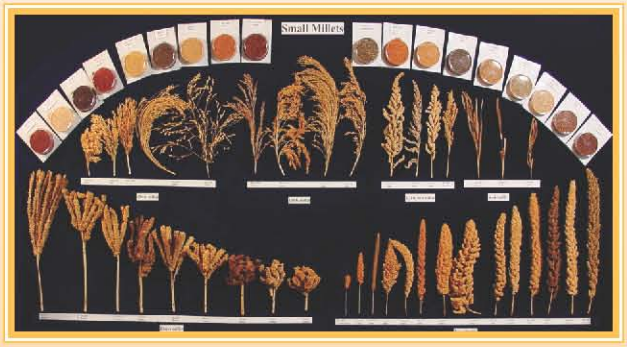
Technical Manual no. 10

Managing and Enhancing the Use of Germplasm – Strategies and Methodologies



ICRISAT
Science with a human face

International Crops Research Institute for the Semi-Arid Tropics

Technical Manual no. 10

Managing and Enhancing the Use of Germplasm – Strategies and Methodologies

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2009

Germplasm Regeneration

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

9A. Reasons for regeneration

Germplasm is regenerated for the following purposes:

Initial seed increase

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

Long-term conservation

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

Replenish seed stocks in active and base collections

Seed increase of accessions that have

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

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

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

Meet special requirement

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

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

Consider the following factors when regenerating germplasm accessions:

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

9B. Procedures for regeneration

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

Selection of accessions

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

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

Preparation of field

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

Solarization

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

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

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

Cleanliness

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

Fertilizers

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

Weeds

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

Preparation of seed

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

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

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

Seed pretreatments

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

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

Sowing and crop management

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

To maximize seed yield and avoid losses of alleles:

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

Regular inspection of plants is mandatory to achieve these objectives.

Sowing

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

Irrigation

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

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

Verifying accession identity

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

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

Elimination of alien pollen can be achieved through:

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

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

Harvesting and post-harvest management

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

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

Seed drying and processing

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

Initial drying

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

Options for initial drying include:

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

Threshing and cleaning

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

Final drying

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

Options for final drying include:

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

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

Seed health

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

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

Initial viability testing

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

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

Seed packing and storage

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

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

9C. Sorghum regeneration

Season

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

Sowing time

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

Field

Vertisols (black soil) are used for seed regeneration.

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

Fertilization

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

Field layout

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

Sowing method

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

Irrigation

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

Labeling

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

Thinning

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

Cultural practices

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

Weed management

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

Pest and disease control

Follow normal cultural practices to control diseases and pests.

Pollination control

Genetic integrity of sorghum accessions is maintained by selfing.

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

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

Verification of accession identity

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

Rogue the plants that are genuine mixtures.

Harvesting

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

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

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

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

Seed health

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

Wild species

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

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

9D. Pearl millet regeneration

Season

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

Sowing time

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

Field

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

Fertilization

Apply diammonium phosphate @ 150 kg ha^{-1} as basal dressing and urea @ 100 kg ha^{-1} as top dressing.

Field layout

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

Sowing method

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

Irrigation

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

Labeling

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

Thinning

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

Cultural practices

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

Weed management

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

Pest and disease control

Follow normal cultural practices to control diseases and pests.

Pollination control

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

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

Cluster bagging

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

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

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

Selfing

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

Sibbing

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

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

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

Verification of accession identity

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

Harvesting

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

Seed health

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

Wild species

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

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

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

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

9E. Chickpea regeneration

Season

Postrainy season is best suited to chickpea.

Sowing time

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

Field

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

Fertilization

Apply a basal dose of Diammonium Phosphate (DAP) @ 100 kg ha^{-1}.

Field layout

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

Sowing method

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

Irrigation

Irrigate the field after sowing. Subsequently irrigate when necessary.

Labeling

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

Thinning

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

Weed management

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

Pest and disease control

Follow normal cultural practices to control diseases and pests.

Verification of accession identity

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

Harvesting

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

Seed health

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

Wild species

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

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

Raising seedlings

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

Transplanting

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

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

9F. Pigeonpea regeneration

Season

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

Sowing time

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

Field

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

Fertilization

Apply a basal dose of Diammonium Phosphate (DAP) @ 100 kg ha^{-1}.

Field layout

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

Sowing method

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

Irrigation

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

Labeling

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

Thinning

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

Cultural practices

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

Weed management

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

Pest and disease control

Follow normal cultural practices to control diseases and pests.

Pollination control

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

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

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

Verification of accession identity

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

Harvesting

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

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

Seed health

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

Wild species

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

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

Raising seedlings

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

Transplanting

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

9G. Groundnut regeneration

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

Season

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

Sowing time

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

Field

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

Fertilization

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

Field layout

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

Sowing method

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

Labeling

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

Irrigation

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

Thinning

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

Weed management

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

Verification of accession identity

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

Harvesting

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

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

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

Groundnut harvesting consists of two operations:

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

Lifting of vines is done manually.

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

Stripping pods from vines

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

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

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

Seed health

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

Wild species

Seed propagated species

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

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

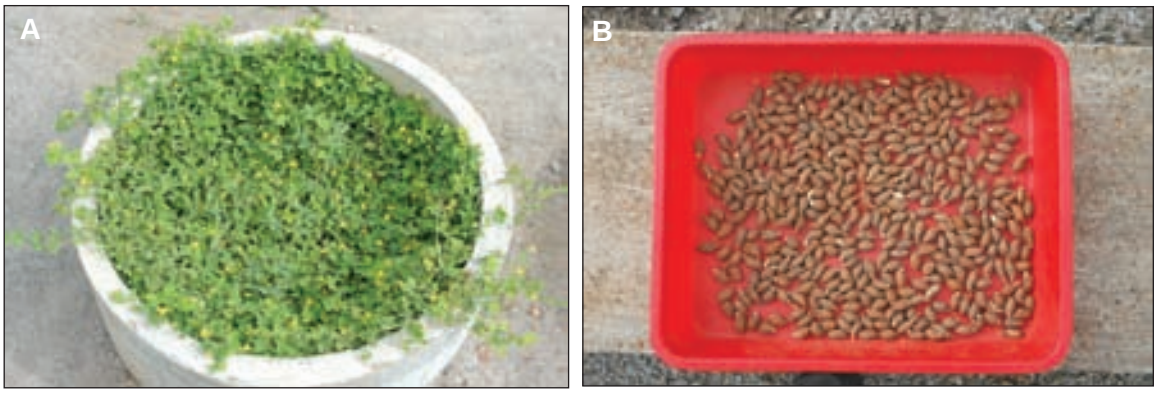

Figure 9G.1. Groundnut wild relative grown in cement ring in a special facility for regeneration (A) and harvested pods (B).

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

Rhizomatous species

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

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

9H. Small millets regeneration

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

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

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

9I. Documentation on regeneration

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

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

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

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

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

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

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

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

Method of drying: Methods used for drying seed samples are

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

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

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

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