International Course on Plant Genetic Resources and Genebank Management

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Lecture 2: Introduction to guidelines on regeneration of accessions in genebanks

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General guiding principles

Germplasm regeneration is the renewal of germplasm accessions by sowing and harvesting seeds, which will possess the same characteristics as the original sample when viability or plant numbers are low.

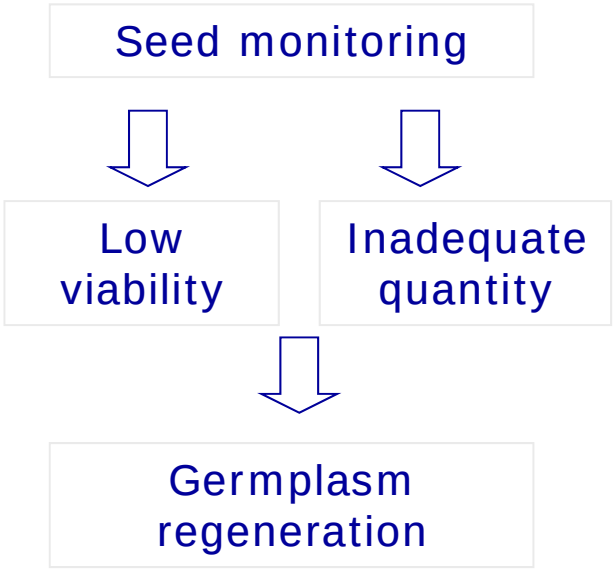
Germplasm regeneration is one of the most critical operations in genebank management

- Assuring genetic integrity (risks of selection pressure, out-crossing, mechanical mixtures, etc).

- Assuring efficiency (consider % germination, % establishment, seeds need to be collected, need for characterization and evaluation).

- Assuring quality (healthy, viable).

Germplasm regeneration



When you find out the following results after germplasm monitoring:

- Seed viability begins to decrease below the regeneration standard
- Seed stocks become low due to distribution from active collections and/or inadequate quantity

Or also when:

- New seeds need to be multiplied
- Need to meet special requests

proceed as soon as possible to **germplasm regeneration.**

Seed regeneration

Consider the following factors when regenerating germplasm accessions:

- Number of individuals needed to maintain genetic integrity
- Suitability of environment
- Special requirements if any to break dormancy and stimulate germination
- Correct spacing and sowing density for optimum seed set
- Breeding system of the plant and need for controlled pollination or isolation

Procedures for regeneration

An inappropriate biotic environment can be detrimental to plants, seed quality and genetic integrity of an accession.

- If possible, regenerate germplasm in the ecological **region of its origin**.
- Alternatively, seek a location with an **environment 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 such as a growth room.

Preparation of the regeneration plots

Use irrigation if rainfall is erratic

- 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).
- Free from weeds, pests and pathogens.
- Free from seeds/plants of the same species.

Cleanliness

Keep the plots clean from all seed and plants and reduce the risk of contamination with alien pollen of the same species during plot preparation by using isolation techniques (distance and mechanical barriers), intercultivation and hand weeding.

Wild Arachis species being regenerated in large cement pots

Hand weeding field plots of grasses

Prepare the regeneration plot considering:

- number of accessions to be regenerated;
- number of plants per accession;
- spacing between rows and between plants;
- mechanical access for weeding and harvesting.

Method of preparation depends on:

- soil structure;
- species to be sown or transplanted and its cultural requirement;
- need for plant supports, in case of climbers.

Preparation of seeds

Once you have prepared the regeneration plot, you can go on preparing seeds for regeneration. The minimum number of seeds for regeneration should be sufficient to:

- Provide enough plants for a **good genetic representation** of the accession
- To maintain **genetic integrity**
- To provide the **required number of seeds** for storage.

$$\textbf{Number of seeds for regeneration} = \frac{\text{Desired plant population for regeneration}}{(\text{Germination \%} \times \text{Expected field establishment \%})}$$

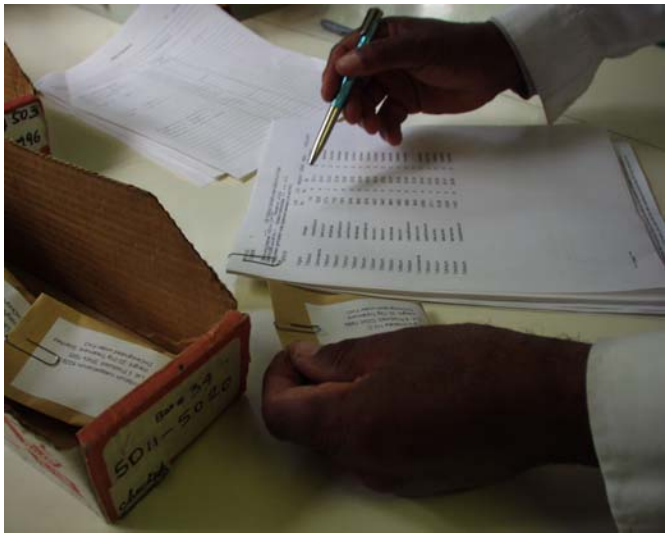
Germination and field establishment percentage are expressed as decimals: 95% is expressed as 0.95.

Plant establishment on average:

- Poor conditions = 5% (0.05) less than the germination percentage.
- Good conditions = 1% (0.01) less than germination percentage.

Preparation of seeds

Which seeds should be regenerated first?



- Regeneration of accessions with low viability should take priority over accessions with inadequate number of seeds to avoid loss of accessions.
- Regenerating accessions in base collections should take priority over accessions in active collections.

Seeds should be carefully removed:

- Allow the containers to warm up before extracting the seeds;
- 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 labelling the seed samples.

Seed pre-treatment

Specific pre-treatments may be needed to improve seed germination and establishment:

- If the seeds are very dry (e.g. moisture content <8%), raise the moisture content by humidification before sowing.
- Break dormancy of hard seeds to ensure good establishment (e.g., stratification, scarification, etc.).
- Apply seed dressings to reduce disease and insect damage.
- Inoculate with appropriate symbionts (*Rhizobium* treatment for legumes).

For accessions with limited seeds, germinate in controlled conditions and transplant the seedlings into pots with sterilized soil in a screen-house under close supervision.

In vitro techniques can also be used to rescue low viability seedlings. Rapid clonal propagation may be used to increase plant number from cultures. However, regeneration from few individuals reduces the genetic variability within the population and should be avoided if possible.

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From sowing to harvesting

Sowing

Sowing date

- Sow at an optimum time so that maturity and harvesting coincide with the most favourable weather conditions.
- If there is much variation between accessions in flowering time, sort on maturity (early and late) based on previous documentation and adjust the planting dates so that all accessions mature under a uniform favourable environment.
- Sow in uniformly spaced rows and with uniform and wide spacing between plants within rows.
- Thinning should normally not be undertaken. If required thin at random.

Sowing and crop management

Sowing and crop management

To avoid large losses of alleles and maximize seed yield:

- Use 100 or more plants in genetically heterogeneous accessions;
- Provide spatial or temporal isolation for those accessions which are outcrossing;
- Take special note of the day-length requirements of the species if any or they may not flower;
- Provide suitable conditions for growth to trigger abundant flowering;
- Ensure a stable source of water from irrigation if necessary for maximum survival;
- Ensure complete control of pathogens and pests using standard plant protection measures;
- To eliminate competition, ensure continued absence of alien plants in the vicinity throughout the regeneration cycle by hand weeding or intercultivation.

Plant growth

Sowing → Crop management

Verifying accession identity

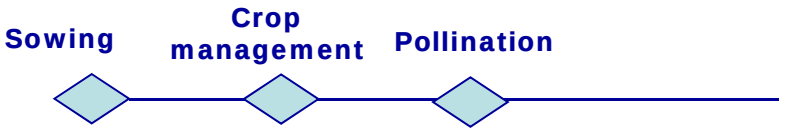
Accession identity should be verified while the plants are growing by comparing:

- Morphological data in the documentation system, or
- Reference material such as original herbarium specimens or seed.
- Rouging must be undertaken with caution and only when it is absolutely clear that the rogue plants are genuine mixtures from other accessions or varieties.
- When materials are grown in rows, plants growing off-row may be eliminated.

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

Pollination biology

Pollination



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

For out-breeders, elimination of alien pollen can be achieved through:

- Spatial isolation (This technique is very useful for dealing with limited number of accessions of many species).
- Temporal isolation.
- Natural or artificial barriers – growing accessions in standing crops of tall growing species like sunflower and hemp.
- Bagging selected inflorescence with pollen-proof or pollinator-proof bags made of linen or paper and erecting temporary pollen-proof or pollinator-proof nets around plots.

Pollination biology

Pollination

Sowing → Crop management → Pollination

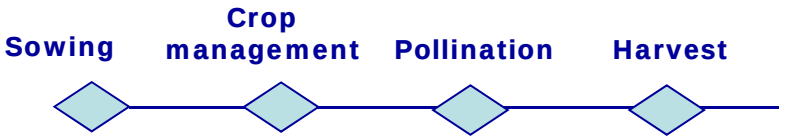
Insect pollinated crops may be grown in net or nylon screened cages with specially designed hives for insect pollinators like bees.

Supplemental hand pollination may be necessary to improve seed set (e.g. wild species of tomato and eggplant). Isolation cages can be expensive and shading may affect plant growth.

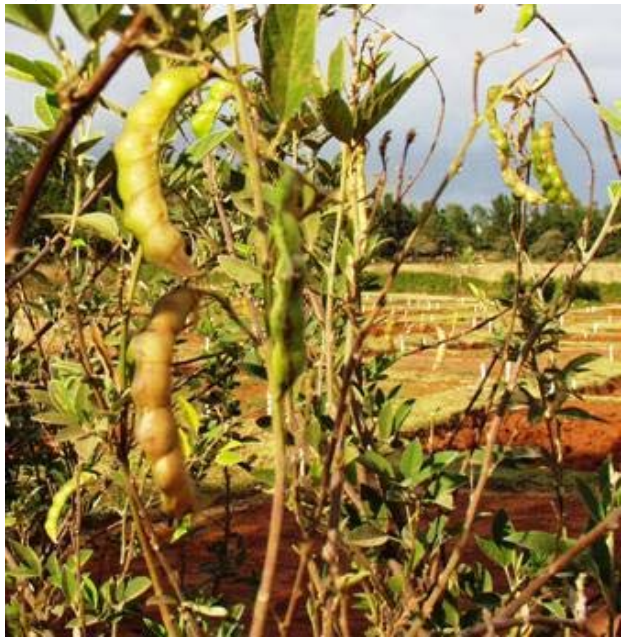
An effective solution could be bagging and controlled pollination by hand, where feasible. However, if plants flower during or at the end of the wet season, pollination bags may be damaged by the rain. Excess moisture and humidity in the bags around the flowers can also lead to increased infection with bacteria and fungi.

In wet or humid conditions, it is best to tag the flowers and remove the bag as soon as pollination is complete, so the fruits develop under normal field conditions.

Pollination biology



Harvesting



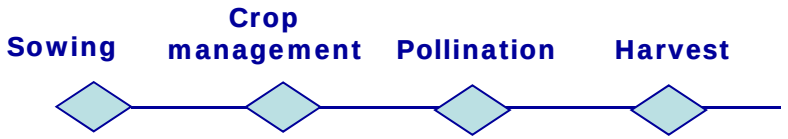
Harvest at optimum maturity:

- When maximum number of seeds are ripe;
- Seeds become tolerant to desiccation and can be threshed without mechanical injuries;
- Before deterioration sets in and natural dispersal occurs (e.g., through shattering).

Harvest individual plants (at different times) within an accession when there are differences in flowering and maturity.

Collect an equal number of seeds from the largest possible number of maternal plants.

Pollination biology



Harvesting

Options for harvesting depend on the crop:

- Harvest plants individually, preferably by hand. If machine harvested, use purpose-built machinery because commercial machinery cannot be cleaned adequately between regeneration plots.

- Initiate seed drying immediately after harvesting to prevent seed deterioration or place seeds in a temporary holding area under a controlled environment.

Documentation

As regeneration methods vary according to species, the types of descriptors to record information also vary. In general, the following descriptors will help in documenting the data:

- Regeneration site
- Collaborator (where applicable)
- Plot reference
- Sowing date
- Germination in the field
- Number of plants established
- Days from sowing to flowering
- Breeding system
- Pollination control method used
- Harvest date
- Number of plants harvested
- Quantity of seeds harvested

Summary

Important to monitor accessions during storage to avoid excessive deterioration of seed quality and reduction of seed quantity.

- Germplasm accessions identified with low viability and inadequate quantity during the course of monitoring or registration should be regenerated as soon as possible.
- Germplasm should be regenerated in the ecological region of its origin.
- Regeneration plots should be accurately prepared, through the elimination of weeds, pests and pathogens.
- The number of seeds to establish, the minimum number of plants for regeneration to ensure genetic integrity and sufficient seeds for storage can be estimated from the standard population size used for regeneration and the sample viability.
- Seeds should also be properly pre-treated before sowing.
- Suitable conditions should be provided for proper growth and flowering and isolation should be used to ensure genetic integrity of out-breeding accessions.
- Harvest should be carried out when the maximum number of seeds are ripe so as to obtain high quality seeds for storage.