Best practices for regeneration of forage and pasture legume and range genetic resources

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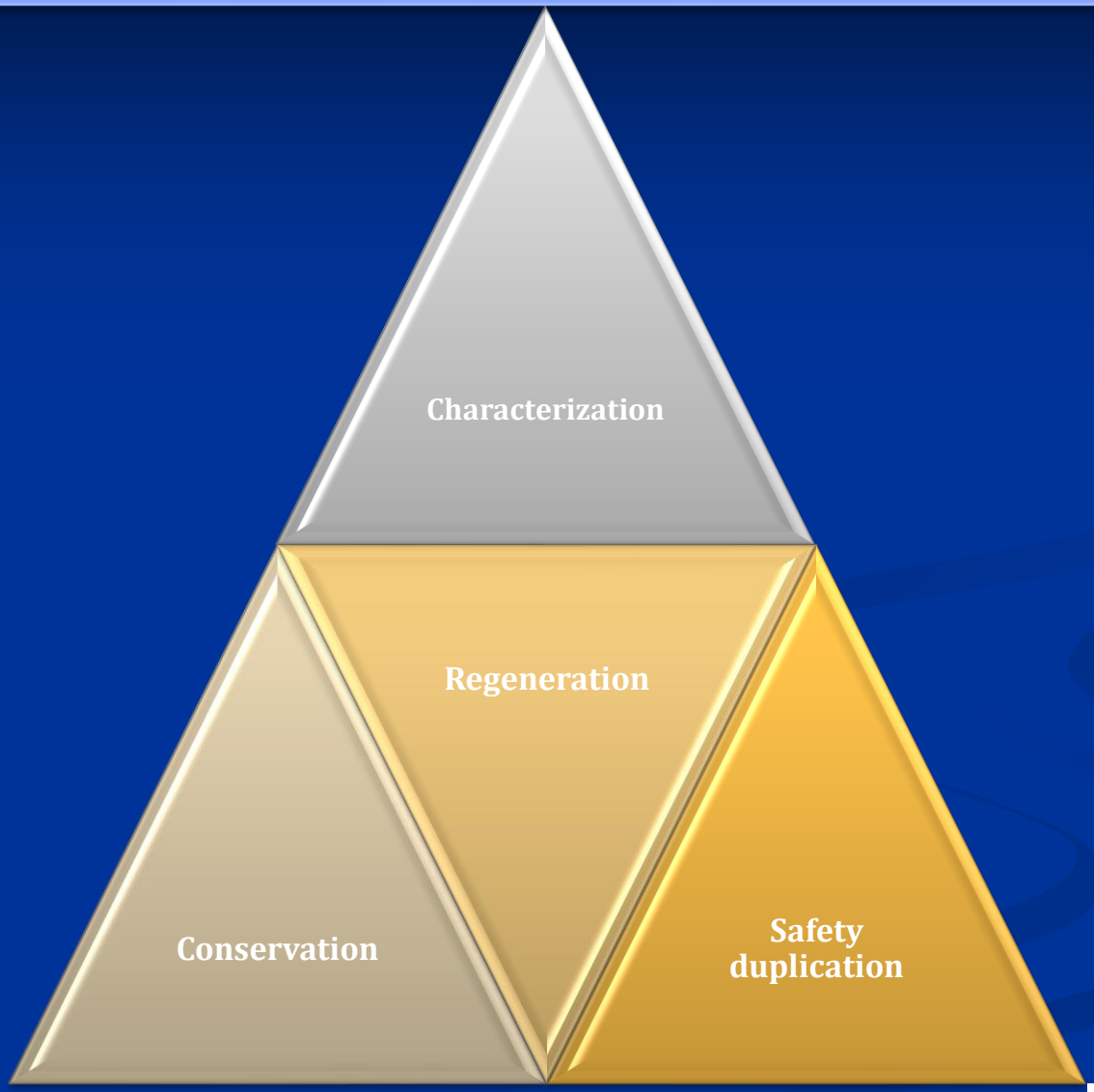
Best practice of genebank management



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Background

- Genebanks must ensure that their germplasm accessions are kept viable and at high quality for as long as possible. Even under the highest standards of management,
- However, germplasm deteriorates with time and needs to be regenerated.
- For many genebanks, maintaining collections at acceptable levels of viability and quality is difficult due to the costs involved and their limited capacity and technical expertise especially when faced with the complex regeneration procedures required by some species (FAO, 1998) .

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Introduction

- Over 1,500 species of legumes can be used as forage, although only about 60 species have been developed and widely used as forages. The wide range of species covers short-lived annuals to long lived perennial trees and small herbaceous species to large woody species, adapted both to tropical and Mediterranean areas. With such a wide range of diversity, specific conditions and methods are required.

- The term regeneration is taken to mean the re-establishment of samples genetically similar to the original collection when viability or plant numbers are low. Regeneration is also necessary for new accessions which need long-term storage if quantities are too low, and may be required for sanitary reasons to eliminate diseases.

- In the case of clonal crops conserved in field genebanks, re-establishment could either be on the same site or n a different site for security or o avoid diseases and pests.

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General principles

- Assuring genetic integrity
- Assuring efficiency
- Assuring quality

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Assuring genetic integrity

- Germplasm regeneration is a critical operation in genebank management, that which involves the greatest risks to the genetic integrity of germplasm, due to selection, out-crossing or mechanical mixing.
- The risk of losing genetic integrity is especially high when regenerating genetically heterogeneous germplasm accessions of out-crossing crops. PDF\breeding systems tropical forages.pdf
- The aim of regeneration is to maintain the original genetic diversity and structure of the accession or collection.

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Assuring efficiency

- The reproductive rate of the species and variety at the regeneration site should be considered in order to produce sufficient seeds or planting materials.
- The number of seeds, cuttings or other planting materials required for regeneration should be carefully calculated, taking into account the desired number of plants for regeneration,
- Germination and field establishment rates of the accession, the amount of seed required for characterization and evaluation are linked to regeneration.

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Assuring quality

- The aim of regenerating crops is to produce sufficient quantities of healthy, viable seeds, tubers or other vegetative plant propagules.
- Before distributing germplasm, genebanks must screen it for the presence of seed-borne or vegetative-borne pathogens and pests to prevent spread of diseases and pests.
- This germplasm should provide an uncontaminated basic stock for breeding programmes, multiplication or other projects.

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Type of collections

- The two types of genebank collections recognized for seed crops are active collections and base collections.
- Active collections should preferably be regenerated from original seeds taken from base collections. However, using seeds from an active collection for up to three regeneration cycles before returning to original seeds (base collection) is also acceptable (FAO/IPGRI 1994) .
- Base collections should only be regenerated using residual seed from the most original sample in the base collection.
- It is good practice to establish a duplicate collection as there will be no remnant seed to fall back on when plants fail, as in the case of seed-propagated crops.

When to regenerate

- Regeneration is a costly process and should only be carried out as often as is necessary to ensure accessions are available in sufficient quantity and adequate quality.
- For most seed crops, accessions are regenerated:
  - when seed viability falls below 85% of the initial germination percentage in active collections' s determined by viability monitoring (see FAO/IPGRI 1994; Rao et al. 2006; ISTA 2008 for more details). The initial viability before storage should not be less than 85%, Although in some genebank a lower percentage <75%)) is used, specially for wild species.
  - when he umber of viable seeds per accession s <1500 in active or base collections of populations and <250 seeds for inbred lines.
- Poor-quality (low viability) accessions are more important to regenerate than those with inadequate numbers of seeds.
- Accessions in base collections take priority over those in active collections. •

Choice of environment

- Germplasm accessions should be regenerated when possible in the same ecological region where they originated. Alternatively, select a location that minimizes selection pressures on genotypes or populations.
- If no suitable sites are found, collaborate with the institutions that can provide suitable sites or facilities for regeneration.
- Take care during regeneration and when handling seed or plant propagules to avoid contamination from adjacent plots, to minimize gene flow and to prevent inadvertent introduction of transgenes.
- Plants that can be clearly identified as contaminants and do not belong in the population should be removed.
- Accessions should not be planted in fields where the same crop has previously been grown to reduce the risk of volunteer plants, as well as the accumulation of soil pests and diseases.
- Regeneration plots should be as uniform as possible and fields should be well drained.
- Good irrigation should be available even for rain-fed crops to avoid any selection exerted by drought as well as to ensure good yields. A soil nutrient test is also advisable to determine what fertilizers are needed.

Where to regenerate?

- In the fields
- In the plastic or green houses
- In isolation cages for cross-pollinating crops or species

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Planting season

- Planting is often done at the start of the rainy season to aid establishment.
- Regeneration should be undertaken in the rainy season or under irrigation for perennial species.

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Preparation for regeneration

- When to regenerate
  - When seed stocks are less than 1,500 seeds.
  - When percent germination is reduced to 75% and 65% for cultivated crops and wild relatives respectively (FAO/IPGRI, 1994).

- Pre-treatments
  - Many of the wild species have hard seed and require scarification before planting to allow imbibitions.
  - Gently rub hard seeds between two pieces of sand paper until the seed coat is scarified or, using tweezers with well defined square edges, apply gentle but firm pressure to the seed coat with the arms of the tweezers open to about 2mm so that a small section of the seed coat will chip off.
  - A scalpel can also be used to chip the seed coat.

Field selection and preparation

- Select the environment and soil type best suited for the species
- Select the field based on appropriate rotation and infection history to avoid mixtures and infection/infestation with different pests.
- Soil should have good drainage and be well prepared for sowing.
- Soil should be ploughed and disked, weeds and grasses removed.
- The soil should be prepared by tillage to obtain a well prepared and level seed bed prior to planting.
- Deep plough to invert soil, followed by harrowing two or three times, to produce a fine and an even flat seedbed.
- The majority of forage and pasture legume species are small seeded on size. The depth of planting should not exceed more than 3cm.

- Other precautions
  - A population size of at least 100 plants should ideally be used for regeneration in order to maintain genetic variation.

Sample size

- The sample drawn from a seed accession for regeneration should be randomly chosen to represent the diversity within the accession or collection and to give a high probability of retaining low frequency alleles.
- As a rule of thumb, Crossa et al. (11993) estimate a range of 90 –210 seeds are needed to retain with a probability of 90 –95% alleles with a frequency of 0.003 to 0.05 for a number of loci ranging from 10 to 150.
- Cross-pollinating species usually require more plants to maintain the genetic variation that exists within the population than self-pollinating species (See Crossa (1995) for more details).
- However, this is not always the case and may depend on the degree of within-accession variation in sub-populations of self-pollinating species.

Sample size (cont.)

- **The minimum number of seeds for regeneration can be estimated from the standard sample size used for regeneration and the sample viability according to the following equation:**
- **Number of seeds required for regeneration = Desired plant population for regeneration /(percentage of germination [1] x percentage of expected field establishment [2]) (see Rao et al. 2006)**

1 Germination and field establishment percentage should be expressed as decimals: i.e. 95% expressed as 0.95.

2 Plant establishment is generally 5% less than the germination percentage in poor conditions and 1% less in good conditions.

Preparation of seeds/planting material

- When preparing seeds stored in a genebank for regeneration, it is necessary to remove the containers from storage and leave them to warm up overnight at room temperature before opening them, to avoid a rapid uptake of moisture.
- For clonally propagated crops, different parts of the plant may need to be used for regeneration, from tuberous roots to vines, stems, suckers or other plant parts.
- For each one, particular methods apply for selection, cutting, disinfestations and short-term storage or pre-conditioning before planting.

Planting layout, density and distance

- Aim for a final plant number of 100-150 in plots of approximately 25m^2 for out breeders and 25 plants in plots of 10m^2 for vegetative propagated grasses.
- Plant in up to 11 rows of 5m long, each row 50cm apart within row spacing of 50cm giving a density of 100 plants per plot. Smaller herbaceous plants can be planted closer together within the rows in smaller plots to avoid excessive weed growth on the large area of empty soil between plants.
- For accessions which are populations of genetically diverse material, such as landraces, it is recommended to plant 300 seeds in 2 rows, 2m length.
- Fodder trees should be planted 1-2m apart in larger plots or in rows.
- Forages vary in their breeding systems and species are planted at different isolation distances
- Although many forages are in-breeding they often have low percentages of out-crossing and some species have limited information, therefore it is recommended to use an isolation distance of at least 100m between accessions.
- Plant seeds of accessions of other species that do not hybridize or of other genera in between plots to increase the isolation distance.
- When spatial isolation is not possible, pollination cages can be used to isolate accessions and prevent insect pollination.

Planting method

- Direct sowing for large seeded species:
  - Count the number of seeds to be planted per row and place in separate envelopes/bags.
  - Allow 2 seeds per hole if there are sufficient seeds because not all seeds will germinate. If there are few seeds, plant 1 seed per hole.
  - Label the plot with the accession number and plot number as a minimum (taxonomic identification and origin of accession are also recommended).
  - Lay out the plots at the chosen row spacing.
  - Mark rows about the depth of the size of the seeds.
  - Check that the accession number is correct and place one envelope/bag on the end of each row.
  - Open the envelope and plant directly into a flat seedbed at a depth of 3cm.
  - Place 2 seeds per hole at 50cm along the row by hand sowing for large seeds and spacing from 1 to 2cm for smaller seeds.
  - Cover with soil and lightly compact the soil in the row.

Planting method (Cont.)

- Seedling transfer for accessions with few seeds:
  - Germinate seeds in Petri dishes in an incubator. The conditions vary with species.
  - As soon as the radicle start to emerge, plant the young seedlings individually in seedling trays or pots using sterilized compost or forest soil.
  - Keep the pots away from direct sun but with good light intensity or in a greenhouse.
  - Water carefully so the pots remain moist but not wet.
  - Once seedlings are strong and growing well, place the pots outside for the seedlings to harden off for one week, watering carefully.
  - Label the plot with the accession number, planting date and plot number. Taxonomic name and origin of accessions are also recommended.
  - Peg out the plots at the chosen row spacing and make holes at 50cm along the row.
  - Transplant the seedlings carefully to the field, one seedling per hole, taking care not to damage the roots when the seedlings are transferred from the pots.
  - Water after transplanting.
  - Maintain few plants or weak seedlings in Jiffy pots for regeneration in the greenhouse.

Crop management

- Thinning
- Weed management
- Irrigation
- Fertilization
- Pest and disease control
- Harvesting
- Threshing
- Post -harvesting

Thinning

- If direct sown, thin to one plant per hole at 4-6 weeks after establishment when plants are growing well to keep plant density at about 100 plants per plot.
- Avoid competition that will result in weak plants and low seed yields.
- Avoid selecting only strong plants that would reduce genetic variation.
- Thinning can be done simultaneously with the first weeding.

Weed management

- Early growth can be slow and hand weeding 4 weeks after establishment is recommended.
- Cultivate between rows twice during early stages of plant growth.
- Eliminate plants growing off-row. Rogue plants that are genuine mixtures.

Irrigation

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

Fertilization

- Although it is possible to grow the crop without fertilizer, it is recommended to apply Phosphorous at planting using DAP at 100kg per ha.
- An additional application of 50-60kg N per ha as a top dressing at early flowering stage will ensure good seed quality.
- Apply appropriate *Rhizobium* strains when needed.

Pest and disease control

- Coordinate periodic field inspections with pathologists and virologists during the growing season.
- Spray with appropriate chemicals when necessary. Spray with fungicide to control mildew during the rainy season or when using irrigation and with insecticide at the first sign of insect damage.
- Rogue out any infested material to eliminate all parasites before seed production and burn the residues.

Isolation cages

For Lathyrus and grasspeas

For pastures and rangeland species:

- *Medicago sativa*
- *Trifolium*
- *Onobrychis, Lotus, Coronilla* and others

Harvesting

- Harvest by hand picking when the pods start to turn brown and begin to dry but before fully ripe pods start to dehisce and shatter.
- For non-dehiscent pods, cut the stems close to the ground and loosely roll the plant to allow final ripening and air drying before threshing to separate the seeds from the pods.
- Label all bags clearly with accession number, plot number and date of harvest on both the outside and on an inside label.
- Collect the pods from each plant in labeled cloth or paper bags with labels inside and outside each bag. Paper bags should only be used in dry climates.
- Harvest un-shattered and non-dropped pods by vacuum machine from large scale plots, otherwise by hand. Vacuum machine should be meticulously cleaned after harvesting each accession.

Threshing

- Thresh the pods on a tarpaulin by gently beating or thresh small quantities by hand and return the seeds to their labeled bag.
- Use appropriate methods for mechanical threshing:
  - *Medicago* spp: A mechanical thresher designed specially for medics is used. In case of small quantities use hand rubber thresher.
  - *Trifolium* spp: A hand rubber thresher is recommended to release the seeds from the fruiting heads.
- For all other species: A hand rubber thresher is recommended.
- Clean seed thresher between each accession to ensure that seed mixing does not occur during threshing.

Post-harvest management

- Clean the seeds of debris by hand picking, hand winnowing or using a seed blower.
- Hand pick over the seeds in trays to remove any shriveled, discolored, infected or damaged seeds from each plant. Incinerate the waste to avoid spread of seed borne diseases.
- In order to have equal replications of all individuals in a population take equal quantities of seeds from each plant and mix in one paper bag labeled inside and outside. Once you are sure you have all the seeds needed, the plants can be removed from the field or screen house.
- However, in practical terms for forages with indeterminate growth habit that are twining and climbing, individual plants cannot be easily distinguished in the plots. In these cases the best practice is to ensure the seeds are harvested from all parts of the plot thoroughly mixed and kept as a bulk sample.
- Retain the bags of each accession in temporary storage until seed drying.
- Take a sample of the seeds for seed health testing. If the fresh seeds are infected with seed borne pathogens and more original seeds are available for a second regeneration, destroy the seeds by incineration. If no original seeds are available, schedule the seeds for a further regeneration in controlled environmental conditions using agrochemicals to obtain clean seeds.

Post-harvest management (Cont.)

- If the seeds are free from pests and diseases, dry the seeds in low relative humidity at 15°C until they reach between 3-7% moisture content. Seed moisture determination.docx
- Remove the seeds from the drying room, weigh and pack directly into storage containers. Options for containers for medium term storage include using plastic containers or cans with sealed lids for storage in environments with humidity control or laminated aluminum foil packets for storage in environments without relative humidity control. Use of laminated aluminium foil packets is more suitable for long-term storage. Seal the containers or packets immediately.
- Sample and test the viability of the seeds and record the results following standard germination methods (ISTA, 2008). If viability is high, proceed to storage, if viability is low, reschedule the accession for a further regeneration from the original seeds.
- Store seeds in the genebank at 0-4°C in medium-term storage or at -21°C in long-term storage.

Monitoring accession identity

- **Comparisons with previous passport or morphological data**
  - Distinctive traits are specific for each species. In general flower color, flower parts, seed shape, color and seed coat texture, number of leaf pinnate in compound leaves, pubescence and stem traits may be useful for comparison.
  - Herbarium specimens are important to compare specimens to verify accession identity.

Documentation of information during regeneration

- The following information should be collected during regeneration:
  - Regeneration site name and map/GPS reference.
  - Name of data collector.
  - Field/plot/nursery/glasshouse reference.
  - Accession number; population identification.
  - Source of seed.
  - Generation or previous multiplication or regeneration (if generation is not known).
  - Preparation of planting materials (pre-treatments).
  - Sowing date.
  - Field layout and density used.
  - Field management details (watering, fertilizer, weeding, pest and disease control, stresses recorded, others).
  - Environmental conditions (altitude, precipitation, soil type, others).
  - Emergence in the field or green house (number of plants germinated).

Documentation of information during regeneration (cont.)

- Number of plants established.
- Pollination control method used.
- Harvest date and method.
- Days from sowing to flowering.
- Number of plants established and harvested.
- Quantity of seeds harvested/plant.
- Comparisons with reference materials (record any identification numbers or references of any samples or herbarium specimens taken from this regeneration plot).
- Agronomic evaluation; agro-morphological traits recorded.
- Taxonomic identification.
- Post harvest procedures.