2. Acquisition

2. GERMPLASM ACQUISITION AND REGISTRATION

2.1 Germplasm acquisition

What is germplasm acquisition?

Germplasm acquisition involves obtaining genetic material of a species mandated for conservation in a genebank. It is the initial step in conservation of genetic resources.

Why is it done?

The main reason for acquiring germplasm is to ensure that sufficient diversity is available to meet current and future needs. Reasons for acquisition include:

- genetic erosion: when the threat of genetic diversity loss is present in a particular area and *in situ* conservation is not possible;
- gap-filling: when diversity is missing or insufficiently represented in an existing collection;
- need-based acquisition: when germplasm is needed for breeding, research or development work; and
- opportunistic acquisition: the unplanned, fortuitous collecting of non-target species as opportunities arise.

How is it done?

Germplasm is acquired by:

a. collecting it from farmers' fields, wild habitats or markets, particularly in known centres of diversity; and
b. securing materials of interest through correspondence and exchange with other plant-introduction centres, genebanks, individual scientists, private growers, seed companies or other germplasm suppliers.

Germplasm acquisition policy

Genebanks should have clear policies on acquisition so that the volume of material acquired is within the limits of each genebank's management capacity. When storage space or resources to maintain collections are limited, germplasm should be acquired based on priority.

Prioritization

Acquisition of germplasm should be based on its value or perceived threat of extinction. Value can be assessed as the usefulness of traits and adaptation to unique environments. Landraces, primitive cultivars and wild and weedy species should receive high priority for acquisition, followed by genetic stocks, elite breeding material and obsolete and modern varieties. Consider the availability of resources for management before acquiring wild taxa.

The Convention on Biological Diversity (CBD) and the International Treaty on Plant Genetic Resources for Food and Agriculture (PGRFA) provide the frameworks for acquisition and utilization of germplasm. Collecting is bound by the CBD, which covers access with prior informed consent under mutually agreed-upon terms and benefit-sharing. The International Treaty on PGRFA specifically refers to crop species listed in Annex I of the Treaty, which participating countries have identified as important for inclusion in a multilateral system of access. Access to germplasm under both these international instruments is now governed by the Standard Material Transfer Agreement (SMTA), which was adopted by the Governing Body of the Treaty; the terms of the SMTA cover both access to genetic materials and the benefits derived from them, and should be taken into account during collection and exchange of material.

A. Germplasm acquisition by collecting

Planning and implementation of germplasm-collecting has received in-depth coverage in publications by Guarino et al. (1995) and Smith et al. (2003). Genebank staff should refer to these publications for additional information.

Timing of seed collection

Ideally, seeds should be collected at optimum maturity when seed vigour, desiccation tolerance and longevity are expected to be highest. While it is difficult to monitor these traits in the field, changes in fruit colour, seed colour or black layer formation (in cereals) can be used as visual indicators to make preliminary assessments of optimal seed maturity. These changes correlate well with achievement of mass maturity, although not necessarily with maximum longevity. Nevertheless, they serve as useful indicators for germplasm collectors. Seed dispersal is also a good practical marker of seed maturity.

Fruit colour

In fleshy fruits, colour changes — usually from green to yellow, brown or red — occur with maturity.

- In tomato, red fruit colour indicates that most seeds are at maximum longevity. Seeds from green, yellow/pink or over-ripened fruits are likely to be immature or over-mature and of poor quality.
- In *Cucurbita moschata*, a change in fruit colour from green to yellow-brown and a straw-coloured peduncle indicate high seed vigour.
- In *Capsicum annuum*, seed vigour improves as fruit colour changes from green to red with a few green flecks and then to intense red.
- In *Brassica oleracea*, fruit (silique) colour changes from green to yellow, and in soya bean and many other legumes, it changes from green to yellow-brown and then to brown as the seed matures.

Seed colour

In many dry fruits, seed colour changes from green to yellow or brown as seeds mature:

- In soya bean, seed colour changes from green to yellow-green to yellow.
- In *Sesbania bispinosa*, seed coat colour changes from yellow to olive green and then to greenish brown.

Black layer formation

In cereals such as maize and sorghum, maturity coincides with the formation of a black or brown abscission layer, referred to as the 'black layer'. Maturity is also indicated by drying of the husks and lower leaves.

- The black layer is located on the kernel base at the point of attachment to the cob on the opposite side of the embryo (maize) or at the tip of the grain (sorghum and millet).
- The black layer may be found by gently scraping away the seed coat to expose the abscission layer.

Variation in seed maturity

Germplasm collectors often encounter variation in seed maturity as a result of differences in flowering time between plants and within a single inflorescence on individual plants. This can be overcome by collecting fruits of uniform maturity—provided some markers and sufficient time are available.

Containers for collecting samples

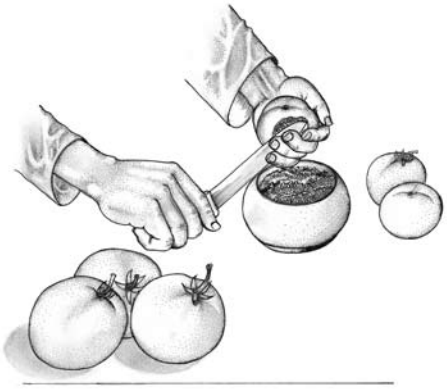
- Use paper bags for collecting seeds.
- Use cloth bags that allow circulation of air (such as muslin bags) for collecting panicles or dry fruits.
- Use open containers, such as baskets made of wire or bamboo or tubs to collect fleshy fruits.
- Ensure that fruits are not squashed.
- During transport, do not let fruits become too hot and ferment.

- Nylon-net bags are also very useful for collecting samples as they allow air to circulate freely. Besides their use for collecting seeds, pods and fruits, they can be used for seed extraction and for drying extracted seeds. They are available in a range of mesh sizes.

Processing of seeds in the field

Newly collected seeds often have high seed-moisture contents (10–20%) and are at risk of deteriorating from contamination with fungi and bacteria. Moist fruits and seeds have high respiration rates and if oxygen is depleted because of inadequate aeration, fermentation sets in. Both respiration and fermentation create heat, resulting in damage to the collected material. When collecting missions are particularly long, pre-cleaning, extraction of seeds and drying in the field becomes necessary in order to reduce bulk and weight during transportation, remove contaminants and bring seed moisture content to a safe level.

- Employ only manual methods for cleaning and seed extraction in order to maintain viability.
- If seeds are collected with surface moisture, dry them first in shade or a well-ventilated room by spreading them on newspaper or blotting paper before transferring them to cloth or paper bags.
- Seeds from dry dehiscent fruits (such as okra, rapeseed and sesame) can be extracted by spreading the fruits on a tarpaulin under shade.
- Mature fruits split open and release their seeds as they dry. Sometimes, additional impact such as raking or shaking is needed.
- Remove empty fruits and debris, and transfer the seeds into cotton, nylon-net or paper bags.
- With pulpy fruits (such as tomato and cucumber), extract the seeds carefully by hand, wash them under running water to remove pulp and mucilage, spread them in a thin layer to maximize aeration and allow them to dry in the shade (see Figure 2.1).
- Always maintain seeds in moisture-permeable containers such as cotton or paper bags, and ensure that air circulates freely between and through them.
- When conditions are hot and humid and collecting missions are long, further dry seeds using desiccants such as silica gel (the recommended ratio of seeds to silica gel is 3:2 to 1:1).
- Keep alternate layers of packed silica gel and packed seeds in a large airtight container to reduce seed moisture content.
- With small and many-seeded fleshy fruits (such as kiwi and strawberry), holding seeds inside fruits is the most practical option if collecting missions are short and logistics permit.
- Seed extraction should be avoided if fruits require after-ripening or if seeds are delicate or recalcitrant.



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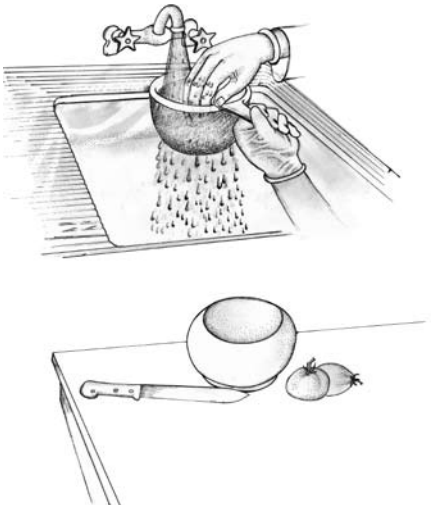

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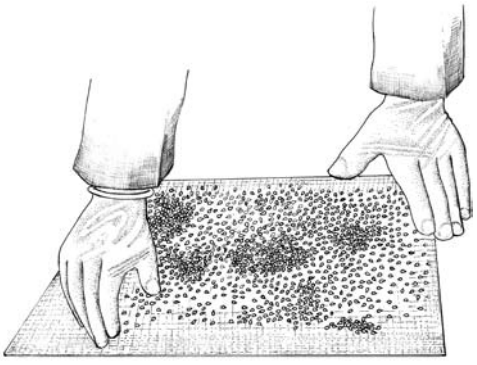

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Figure 2.1. Seed extraction from fleshy fruits.

Recruit couriers to accompany the team when collecting on long expeditions to remote areas and send perishable material or seeds with limited viability to the genebank as soon as possible.

Transporting the collected material to the genebank

The exploration team should ensure the safety of collected material until the time collecting ends and it is transported to the genebank. Exposing seeds to unfavourable environmental conditions during transport can be very damaging.

- Care must be taken to maintain the material at optimal temperature and safe moisture content even when transport distance is short.
- Ensure that the container holding seed samples is cushioned and that no damage is done to the seeds or fruits during transportation.

B. Germplasm acquisition by correspondence and exchange

Samples can be obtained by correspondence if it is known that collecting in the area of interest has already taken place. Genebanks may require documentation from countries or independent entities to certify that the consignment is free from genetically modified organisms (GMOs).

Identification of unique samples for acquisition

Maintaining samples in a genebank is expensive; genebanks should carefully check to ensure that samples do not already exist in their collections before acquisition. Since each genebank adopts its own numbering system, it is possible for the same accession to be recorded twice with different identification. Duplication in the collection is best identified by comparing relevant fields in the passport databases of donating and recipient genebanks.

Most errors are made during data entry, especially regarding spaces, hyphenation, case and spelling, which require careful checking when comparing databases to identify duplicate accessions.

Acquiring unique germplasm

Obtain complete passport information, including alternate names or identification numbers, pedigree and original source.

- Prepare a final list of unique accessions to be acquired.
- Send the final list of accessions identified for acquisition to the consignor to facilitate seed transfer.
- If material is being received from abroad, check the requirements of the host country's national phytosanitary authority and follow the procedures for seed import as described further below.

Germplasm accessions that have been 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 this material along with complete pedigree information.

Genebanks may also acquire elite germplasm generated in breeding programs for specific traits or with proven high yield. During acquisition, ensure that complete pedigree details and morphological data are included with the material.

Germplasm introduction and post-entry quarantine

Genebanks often acquire germplasm from areas where pests, pathogens and host species have co-evolved. The following classification will help genebank staff to assess the potential disease status of material to be acquired.

The risk of introducing new pests and pathogens is:

1. *low* for germplasm collected or produced in the area or country where the genebank is located;
2. *medium* for germplasm collected or produced in the same geographic region or continent where the host country is located; and
3. *high* for germplasm collected or produced in other continents and for vegetative material.

To reduce the risk of entry of pests, pathogens and weeds, some countries have legislation regulating the entry of exotic propagation material, including seeds. The importer must ensure compliance with all phytosanitary requirements of the destination country before importing any seeds.

General features of import regulations may include provisions such as the following:

- Consignments of plants and seeds may need to be imported through specific entry points, as determined by the national plant protection authority in the importing country.
- Seeds and planting material may need to be grown in isolation, or contained in a certified post-entry quarantine facility for a specified period of time, or to meet certain conditions.
- Additional provisions may be required for shipments consisting of plants or plant products.
- Importing soil, earth, sand, compost and plant debris accompanying seeds or planting material is usually prohibited.

Consignments will probably be inspected, and if necessary disinfected, by authorized phytosanitary officials before clearance, provided all other requirements of the importing country have been met. Failure to meet these requirements can cause unnecessary delays and may result in the consignment being destroyed.
Seed material requiring isolation may be planted under pest-proof glass, in screenhouses or in field plots. Phytosanitary officials carry

out periodic inspections during the growing period in which plants affected by seed-associated pests are destroyed while seeds collected from healthy plants are released to the genebank.

Procedure for seed import

At the planning stage, pay attention to the pests encountered on the target species and to the phytosanitary requirements for germplasm introduction.

1. Collect information on pests that are likely to be encountered in the country or area of seed collection or production.
2. Determine what plant parts these pests are found on.
3. Check the requirements for seed import with the national phytosanitary authority. If required, obtain a plant *import permit*[1] from the appropriate authority and send it to the consignor prior to import. All phytosanitary applications should be submitted to the national phytosanitary authority of the host country for approval.
4. If post-entry quarantine is required, grow each new accession under containment or isolation.
5. Plants should be observed periodically and those suspected of infection by seed-associated pests should be destroyed by incineration.
6. All symptom less plants should be tested for latent infections by viruses known to occur in the place of origin and in the country of maintenance; infected plants should be incinerated.
7. Seeds should be collected from healthy plants only.

Genetically modified organisms (GMOs)

Genebanks should be conscious of the dangers inherent in the inadvertent introduction of transgenes or genetically modified crops during germplasm assemblage, and must take measures to minimize these introductions (see Annex I and also section 5.3 of this manual). When planning for collecting or acquiring new accessions by other means, genebanks should conduct a risk analysis to determine:

1. whether transgenic events (commercial and research) in the relevant taxa are likely to be present in the area of collection or acquisition;
2. the distance between the collecting site and areas where transgenic events are situated; and
3. whether germplasm suppliers can provide adequate documentation of their management practices with respect to the material in question.

[1] An import permit is a written authorization by national plant protection services to import regulated items, including plants and plant products.

Further reading

Ebbels, D.L. 2003. Principles of plant health and quarantine. CAB International, Wallingford, UK.

FAO (2006). http://www.fao.org/waicent/FaoInfo/Agricult/AGP/AGPS/pgr/ITWG3rd/docsp1.htm

Guarino, L., Rao, V.R. and Reid, R. (eds.). 1995. Collecting plant genetic diversity. CAB International, Wallingford, UK.

International Treaty on Plant Genetic Resources for Food and Agriculture. FAO, Rome Italy. http://www.fao.org/ag/cgrfa/itpgr.htm. (Last visited: 11 October 2006)

Hay, F.R. and Smith, R.D. 2003. Seed maturity: when to collect seeds from wild plants. Pp. 97-133 in Seed conservation: Turning science into practice. (R.D. Smith, J.B. Dickie, S.H. Linington, H.W. Pritchard and R.J. Probert, eds.). Royal Botanic Gardens, Kew, UK.

2.2 Germplasm registration

What is registration?

Registration is the assignment of a unique identification number called an *accession number* for tracking each seed sample received by a genebank in order to distinguish it from other samples.

Why is it done?

Registration is carried out in order to allow genebanks to keep accurate records of samples and to produce inventory lists for conservation, distribution, and other aspects of germplasm management.

When is it done?

Registration is done when the sample first enters the genebank. For efficient management and use of the collections, register the samples if they meet the conditions described below.

How is it done?

Registration is carried out in several steps (see Flowchart 2.1).

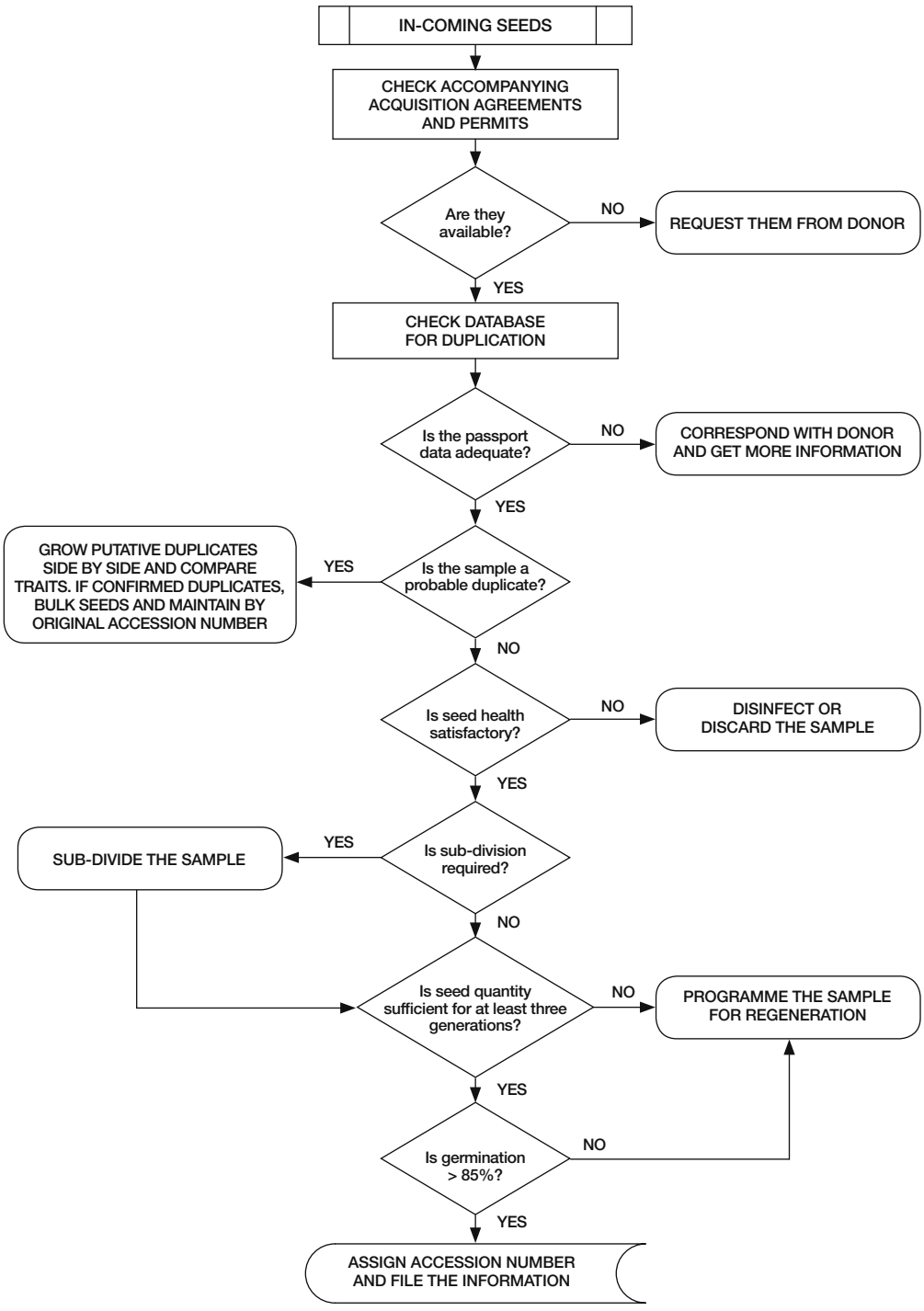
Step 1: Before registration

Prior to registration, the status of the samples should be verified to ensure that the following minimum conditions are met before acceptance in the genebank.

Acquisition agreements and permits

The samples should have been acquired from collectors, genebanks or other sources with appropriate material acquisition or transfer

Flowchart 2.1. Germplasm registration.



agreements and permits in line with national and international regulations regarding conservation, distribution and use (see Annex I for more information).

Passport information

Samples should be accompanied by adequate passport information, especially cultivar name, collector number and pedigree (for genetic stocks and improved material) to ensure that each sample does not already exist in the genebank. The minimum required passport data may include the following:

A. Samples from collecting missions:

- Common crop name and/or genus and species
- Collecting number
- Location of collecting site
- Country of origin
- Collecting date
- Phenology
- Collecting source
- Number of plants sampled

B. Samples received as donations:

- Common crop name and/or genus and species
- Accession name and/or other identification associated with the sample
- Pedigree information and breeding institute's details (for breeding lines)
- Phenology
- Acquisition source
- Country of origin
- Donor accession number (if applicable)

Distinctiveness

New samples should be genetically distinct from any other accessions already registered in the genebank. Two samples may have identical or very similar names and identical grain characteristics but may be genetically distinct, while samples with very different names may be genetically similar.

Morphological, biochemical and molecular approaches can be used to identify duplicates, depending on the facilities and resources available in the genebank. The following tests can be performed:

Morphological

- The suspected duplicates are grown side by side in the field or in a greenhouse and differences between morphological

characteristics such as plant height, flowering time, leaf and flower size, and shape and colour are compared.
- The candidate accession is defined as distinct when it is found to differ significantly in at least one characteristic from existing registered accessions.
- Morphology-based distinctness tests can be similar to the crop-specific set of characteristics that comply with guidelines set by the International Union for the Protection of New Varieties of Plants (UPOV, 1991). If necessary, these characteristics can be assessed over two or three seasons. This may not be practical in landraces with high within-accession variation, however.
- The statistical procedure to assess distinctness is the t-test.

Biochemical

When phenotypic comparison does not provide enough evidence of distinctness, biochemical methods such as electrophoresis of seed proteins and isozymes can be used for improving the comparison of morphological traits and to discriminate the samples.

Molecular

DNA markers such as AFLPs, SSRs and SNPs offer powerful discriminating tools and can be successfully applied in checking genetic relatedness between samples, provided that this approach is feasible and cost effective. For more details on molecular methods, see de Vicente and Fulton (2003).

If the samples being compared are confirmed to be duplicates, genebanks are recommended to bulk the seeds and treat them as one entity. If the sample is identical to an existing accession, maintain it under the original accession number.

Seed health

- Each sample should be accompanied by a *phytosanitary certificate* and additional declarations as required under the host country's phytosanitary regulations (see Chapter 7 for more details).
- Seed samples should be inspected by visual examination under a stereoscopic microscope. They should be free of pathogens, fungal growth, bacterial and viral infections, and insects.

Seed quality and quantity

Seeds should be of the highest quality and in adequate numbers for storage.

- In general, the percentage germinated should not be below 85% for cultivated species or below 75% for the wild species (for more information on germination testing, see Chapter 5).

Box 2.1. Base unit for registration.

The minimum number of seeds for registration (*base unit*) 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 registration = Desired plant population for regeneration x minimum number of regenerations / (Germination % × Expected field establishment 1∗%)[†]

Example:

Desired plant population for each regeneration = 100
Germination = 95%
Expected field establishment = 90%
Minimum number of regenerations (safety factor) = 3

Base unit or minimum number of seeds for registration = $\frac{(100 \times 3)}{(0.95 \times 0.90)}$ = 351 seeds

[†] Germination and field establishment are expressed in decimals; for example, 95% is expressed as 0.95. Plant establishment is generally 5% less than the germination percentage in poor conditions and 1% less in good conditions.

- Seed quantity should be sufficient to conduct at least three regenerations. This will ensure that seeds are still available for another planting even if the first attempt to regenerate fails (see Box 2.1).

What if minimum conditions are not met?

If the sample does not meet the required conditions, assign a *temporary number* until the sample is ready to receive a permanent registration number. The temporary number should be easily distinguishable from other accession numbers.

Agreements and permits

Contact the collector or donor for the necessary agreements defining the status of samples with regard to conservation and further use.

Duplicate accessions

Confirm duplication and assign the seeds as a new seed lot under the original accession number.

Missing passport information

Write to the collector or donor of germplasm to request missing information.

Poor seed health

If seeds contain pathogens or insects, send the sample to a phytopathologist or entomologist for treatment. If it is possible to acquire a replacement sample, immediately incinerate the sample and make note of the action taken and the justification; request a fresh sample from the donor.

Inadequate seed quality and quantity

Regenerate the sample immediately.

If samples are registered without adequate passport data, their identities and biological status will remain unknown, hampering their use. Failure to regenerate samples with low viability or very few seeds may result in loss of the accession, leaving gaps in inventory.

Restructuring samples

In self-pollinating crops, if a sample comprises of a physical mixture of two or more distinct lines or species, they may be subdivided and maintained as distinct accessions. In this case, subdividing the sample into its components helps in effective maintenance of genetic integrity. *Note that subdivision should not be undertaken if variation in the original sample is continuous, as in highly cross-pollinating crops.*

Step 2: Procedure for registration

If the sample meets the minimum conditions described above, it may be accepted for registration and assigned an accession number using the following procedure:

1. Arrange the material in alphabetical order by variety name or in numerical order by collection number, depending on the identification provided.
2. Check all packets against the list accompanying the samples.
3. If no list is provided or seeds do not correspond to the data, prepare a new list. Check again to confirm that all packets have been included.
4. Check the passport data file to determine the last accession number given.
5. Assign the next ascending accession number to the first sample on the list and consecutive numbers to succeeding samples.
6. Write the accession number clearly on the packet using a permanent marker and on the list of new samples.
7. Enter the details in the passport data files of the genebank's documentation system. For each accession, record all passport data, original identification data and registration date in the designated fields of the passport data file.
8. If data are missing, leave the field blank and contact the donor to supply the missing data.

Numbering procedures for new genebanks

A genebank numbering system should be simple and practical to use.

- Use a strictly numeric system that is sequential (1, 2, 3). Assigned numbers are usually preceded by an acronym (such as GBK for Genebank of Kenya) to identify each sample with its registered genebank. Additional information such as year of acquisition and crop code should not be incorporated into an accession number.
- If large collections of germplasm are maintained, separate but sequential accession numbering may be given for each crop. This approach is not recommended if the genebank is small or has many crops, however.

- Avoid assigning 'reserved' numbers for particular crops (for instance, 1 to 500 for maize, 501 to 1000 for cowpea) or for wild species when using a single numbering system.

Documentation

Documenting the information received along with a sample is an important aspect of registration. Information documented at registration consists of passport data providing basic information for identification and general management of individual accessions.

Much of this information is either recorded when the sample is collected or accompanies the sample if it is received from other sources. The use of internationally accepted descriptor lists to document passport information simplifies data exchange between genebanks. The standard Multi-crop Passport Descriptor (MCPD) list developed by FAO and IPGRI is available at www.bioversityinternational.org/publications/pdf/124.pdf.

Further reading

Engels J.M. and Visser, L. (eds.). 2003. A guide to effective management of germplasm collections. IPGRI Handbook for Genebanks No.6. IPGRI, Rome, Italy.

de Vicente, C. and Fulton, T. 2003. Using molecular marker technology in studies on plant genetic diversity: Learning module Vol 1. IPGRI, Rome, Italy.

International Union for the Protection of New Plant Varieties (UPOV). 1991. International Convention for the Protection of New Varieties of Plants. UPOV, Geneva. (http://www.upov.int)