Objective

To review the basic requirements and principal alternatives for storing germplasm

Introduction

The conservation of PGRs is not limited to the mere attainment and physical possession of materials (collection and storage) but must also ensure their existence under viable conditions and with their original genetic characteristics intact. For seeds or materials conserved *in vitro,* this is achieved by controlling storage conditions so that they inhibit or reduce the samples' metabolism; for planting materials, by maintaining them under optimal agronomic conditions.

After germplasm has been multiplied or regenerated, following all precautions to maintain its genetic identity, it is harvested and conditioned to conserve its physical and physiological integrity. Its viability and plant health status are then verified according to established procedures. The next step is to keep the germplasm under stable storage conditions so that it retains its viability over the longest period possible. To achieve this end, the required conditions must be established, including the determination of infrastructure and provision of necessary equipment and resources, as according to the magnitude of the collection and the bank's objectives.

As we develop this theme, some major topics will be described on storing germplasm as seed or maintaining it as planting material.

Alternatives for Storing Germplasm

Germplasm can be stored as **seed**, or maintained **in the field** or *in vitro,* or under **cryopreservation**, depending on how the species reproduces and reacts to storage. These characteristics, in their turn, determine the conditions under which it will remain viable. Planting materials may be conserved as complete plants in the field or as tissue cultured *in vitro.*

If a species reproduces by seed, its reaction to drying needs to be determined before storage to find out if it is orthodox, recalcitrant, or intermediate. This reaction will determine the form, time, and conditions under which the samples must be stored. If a species should possess orthodox seed, then it would be best conserved as seeds. If its seed is recalcitrant or intermediate, then it should be conserved in the field or *in vitro*, as these types of seeds can be conserved as seeds for only very short periods and under special conditions.

Seeds

For the storage of seeds in the collections conserved in germplasm banks, well-founded standards have been established (FAO and IPGRI 1994) (Box 1). These norms, in essence, consider storage conditions to be those that maintain seed viability by reducing respiration and other metabolic processes without damaging the embryo. The most important

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Box 1

Genebank standards: seed storage conditions

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Seed storage conditions for base collections

17. Acceptable: Sub-zero temperatures (<0ºC) with 3-7% seed moisture content (depending upon species). Preferred: -18ºC or cooler with 3–7% seed moisture content (depending upon species).

The above seed moisture content standard may need to be raised in exceptional cases where there is strong evidence that problems can arise at this moisture content (e.g. seed breakage during seed handling).

18. The preferred standards for storage of -18ºC or less with about 5% moisture content should not be relaxed. However, it should be emphasized that the choice of seed storage conditions by an individual genebank depends upon the species stored and the length of storage period envisaged before regeneration is likely to be required. Hence some flexibility is required with regard to what should be considered acceptable, particularly for those circumstances in which refrigeration to the extent required by the above preferred standard cannot be provided. Owing to the nature of the relation between seed longevity, storage temperature and seed moisture content, the same storage life can be achieved by different combinations of temperature and moisture.

19. The tendency to overemphasize the benefits of reduction in temperature compared to those in moisture content should be avoided. With regard to the effect of temperature, the relative response of longevity to reduction in seed storage temperature is very similar among diverse orthodox species, but the relative benefit of a given reduction in temperature becomes less as temperature is reduced (at least, that is, within the ranges usually investigated down to -20ºC). Thus, longevity is increased by a factor of almost 3 if storage temperature is reduced from 20ºC to 10ºC; by 2.4 from 10ºC to 0ºC; by 1.9 from 0ºC to -10ºC; but by only 1.5 from -10ºC to -20ºC.

20. In contrast, the relative benefit to longevity of reduction in moisture content: (i) varies among species; and (ii) becomes greater for each successive reduction in moisture content. This variation among species appears to be largely a function of difference in seed composition (which influences the equilibrium relation between seed moisture content and relative humidity).

21. A calculation which was made some years ago (but which, like many calculations involving extended periods of longevity, is to some extent based on extrapolation) to put the relative benefits of reduction in each of storage temperature and moisture content in context concerns the crop sesame *(Sesamum indicum* L.). The effect of a reduction from 5% to 2% seed moisture content provides about a forty-fold increase in longevity. This is about the same relative benefit as a reduction in temperature from +20ºC to -20ºC. However, in most crops the benefit of desiccation to longevity does not extend to such low moisture content values.

22. There is a low-moisture-limit to the increase in longevity observed to occur with reduction in seed storage moisture content. The value of this limit varies among species, but it is thought that this variation is also related to differences in seed composition such that equilibrium relative humidities at the critical moisture content are similar for different species. One estimate of this value is moisture contents in equilibrium with about 10–12% r.h. at 20ºC. It is reasonable to maximize the benefit of desiccation to subsequent longevity by drying seeds to 10–12% r.h. at 20ºC and then storing hermetically at ambient, but preferably cooler temperatures, if the storage temperature could not be controlled, or where the reduction in temperature provided by refrigeration is not adequate to meet the preferred standard for temperature. This approach has been previously described as "ultra-dry storage". However, in some species this standard is actually slightly greater than the original 5% standard (e.g. 6–6.5% moisture content in pea).

(Continued)

Box 1. (Continued.)

23. Whether seeds are stored dry or ultra-dry, it is essential that all seeds be "conditioned" or "humidified" (by placing in a very moist atmosphere, usually overnight but occasionally slightly longer in the case of very large seeds) prior to testing for germination or growing out.

Seed storage conditions for active collections

24. Active collections should be kept in conditions which would ensure that accession viability remain above at least 65% for 10 to 20 years, being the only standard which should be provided. The precise storage regimes used to fulfil this objective will vary depending upon the species stored, the prevailing ambient environment and the relative local costs of (principally) electricity and labour. As indicated in the preceding section, different combinations of storage temperature and moisture can provide the same longevity. However, it could be emphasized that, in most locations, the reduction and control of seed storage moisture content will be a more cost-effective approach than controlling temperature.

SOURCE: FAO and IPGRI (1994).

conditions for achieving these results are seed moisture content reduced to appropriate levels, low temperatures, and modified storage atmosphere. Usually seeds are stored for variable times after harvesting.

For orthodox seeds to remain viable under conservation, they must maintain **low and constant moisture content**. To ensure this, as described in *Module 3, Submodule B, Lessons 1* and *2*, on conditioning, the seeds are subjected to drying, and then kept in hermetically sealed containers to prevent their coming into contact with atmospheric humidity and becoming rehydrated (FAO and IPGRI 1994). The reaction of seeds to drying for storage has been studied for many species, and information exists for more than 2000 genera of about 250 families (Hong et al. 1996). When insufficient information is available on the species of interest, research is needed to classify its seeds (Figure 1) and determine their characteristics (Hong and Ellis 1996).
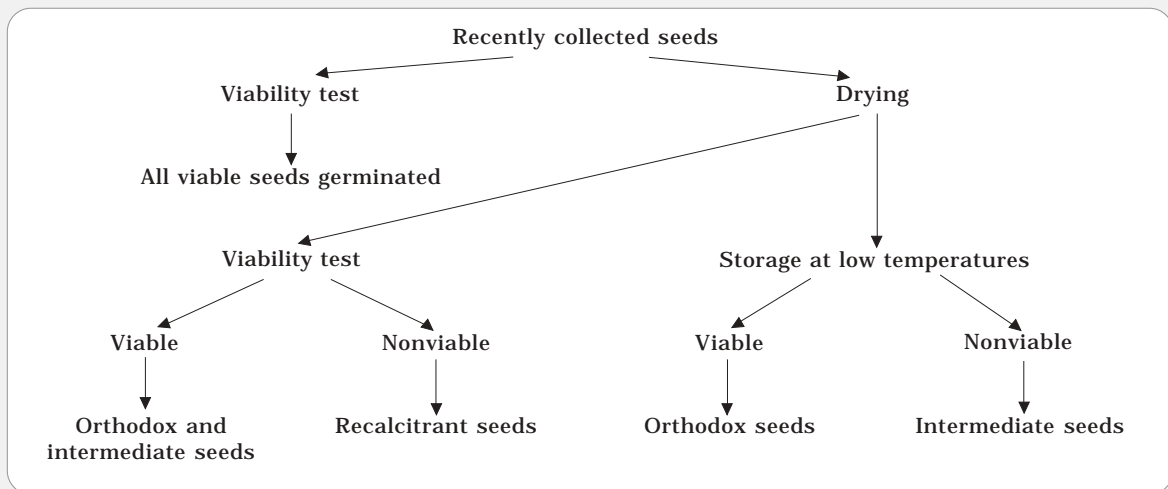

Figure 1. Procedures for determining the behaviour of seeds in terms of their tolerance of drying (from Vázquez Y et al. 2004).

Orthodox seeds can be dried to very low moisture content without being harmed—at least, to a level of constant moisture that is kept in balance with an environmental relative humidity of 10%. Longevity increases when moisture content is reduced and storage is kept at low temperatures in a quantifiable and predictable way (Sandoval S 2000; Vázquez Y et al. 2004).

Recalcitrant seeds cannot be dried below a relatively high level of moisture content without being damaged. Although species vary greatly in the critical moisture content below which viability is reduced, some species will die rapidly, even if in balance with an environmental relative humidity of 98%–99%. Most recalcitrant seeds die when their moisture content is in balance with an environmental humidity of 60%–70% (corresponding to a seed moisture content of 16%–30%, fresh weight). Despite considerable research, no good method has been found for maintaining the viability of such seeds in storage, particularly those of tropical origin (Sandoval S 2000; Vázquez Y et al. 2004).

Intermediate seeds are sensitive to drying to a relatively low level of moisture content (7% to 10%, in balance with an environmental relative humidity of 30%–50%). The conditions regarded as ideal for long-term storage of orthodox seeds (5% moisture content and -18°C) are potentially harmful for intermediate seeds and should not be used, as they will be killed within few months. Even so, intermediate seeds can be stored for as long as 10 years if they are dried to 7%–10% of their moisture content and maintained at laboratory temperatures (Sandoval S 2000; Vázquez Y et al. 2004).

Another requirement for maintaining seed viability is storage at low temperatures in environments that are poor in oxygen. **Low temperatures** invariably extend the life of seeds in storage and can generally counteract the adverse effects of high moisture content. **Modifying the atmosphere** in which seeds are stored by vacuum packaging, increasing levels of carbon dioxide, or replacing oxygen with nitrogen or other gases can, according to various studies, benefit the very short-lived seeds of some tropical plants. For example, rubber *(Hevea brasiliensis)* seeds can be kept in sealed containers filled with 40%–45% of carbon dioxide. Sugar-cane seeds can also be dried in the open air and packaged in sealed tins in which the air is displaced by carbon dioxide, and 9 g of calcium chloride per litre of capacity are added. The whole is then stored at temperatures close to freezing point (Hartmann and Kester 1971).

The combination of low moisture content, sealed containers, low temperatures, and, in some cases, modified storage atmosphere help prevent the ageing and degeneration of cellular tissues, phenomena that occur over time as substances accumulate from the metabolism the organism uses to stay alive. These substances are believed to inactivate enzymes and nucleic acids, preventing cellular membranes from fulfilling their function as selectively permeable barriers in the exchange of compounds and thus resulting in the accumulation of not only metabolically inert materials but also mutagenic substances.

The organelles within cells, the cells themselves, and organs may possibly become ineffective through constant use. In other cases, mutations may increase with age. Over time, an organism becomes more inefficient as mutations increase in number, leading to the production of defective proteins that affect the organism's deoxyribonucleic acid (DNA). During storage, seeds accumulate genetic damage, which manifests as chromosomal

aberrations occurring during the first phases of cellular division in germination. Thus, many effects of ageing will appear, not so much during germination, but during the cellular differentiation and formation of the seedling (Moreno C 2004; Roos 1982).

Types of storage

Open storage (no control of temperature or humidity) is not recommended for germplasm conservation. Seed longevity depends largely on the relative humidity and temperature of storage atmosphere. It also depends on the class of seeds and their condition at the beginning of storage. Maintaining the viability of stored seeds therefore depends on the region's climatic conditions, with the most adverse occurring in warm humid regions and the best in cold dry regions. In the latter areas, the most preferred seeds are those with hard coats, provided they have been dried appropriately. Fumigation or applications of insecticides may be needed to control insect infestations (Hartmann and Kester 1971; Sandoval S 2000).

Warm storage with humidity control is a better technique than the previous one, provided seeds are stored in sealed bags that ensure the minimization of fluctuations in humidity and remain in rooms with controlled temperatures. For vegetable seeds, the following recommendations (Hartmann and Kester 1971) serve as a general guide:

• The environmental air of seeds exposed to 27°C (80°F) for more than a few days must have a relative humidity of no more than 45%;
• Seeds exposed to 21°C (70°F) should be kept at a humidity of no more than 60%; and
• Very short-lived seeds (e.g., onion and peanut) should be conserved at even lower humidity levels.

Dried seeds may also be stored in sealed containers made of materials resistant to moisture. Many types of containers are used that vary in duration, resistance, cost, protective capacity against rodents and insects, and ability to hold or transmit humidity (Hartmann and Kester 1971; Sandoval S 2000).

Cold storage (with or without humidity control) is the best method as seeds are kept with low moisture content, in sealed containers, and at low temperatures, thus prolonging their longevity to the utmost. After conditioning and packaging, seeds can be stored in rooms for the long, medium, or short term, according to goals (Figure 2). Storage conditions for maintaining the samples viable are determined according to species, the reason for conserving it, and the planned storage time.

Conservation temperatures depend on the storage period envisaged. Most species with orthodox seed can be conserved for indefinite periods at temperatures between -10°C and -20°C, with a moisture content of 3%–7% and a viability value of no less than 85%. Seeds conserved under these conditions can be kept for 70 to 100 years. It should be remembered that the benefit of reducing temperatures is less than the benefit of reducing seed moisture content.

Studies have suggested that greater benefit is obtained when temperatures are reduced considerably. That is, longevity triples when the temperature drops from 20° to 10ºC; multiplies by (×) 2.4 when it drops from 10° to 0°C; × 1.9 from 0° to 10°C;

5°C
35% RH
Short-term
cold room

-20°C
Long-term
cold room

Figure 2.   Short-term (left) and long-term (right) storage rooms for seeds kept at low temperatures (photographs by B Pineda, GRU, CIAT).

and × 1.5 from -10° to -20°C. Hence, standards suggest that acceptable temperatures for conservation are those that are below 0ºC and preferably below -18ºC (FAO and IPGRI 1994).

Toole (cited by Hartmann and Kester 1971) recommends that, for vegetable seeds stored at 40°–50°F (4.5°–10°C), relative humidity should be no more than 70% but preferably no more than 50%. When taken out of storage with a humidity of more than 50%, seeds should be dried to a safe moisture content unless they are planted immediately. Storage temperatures kept as low as freezing point may be desirable if the need justifies the additional cost. Temperatures below freezing point may be used for prolonged storage and for conifers such as the silver fir (*Afoles*) or spruce (*Picea* sp.). If seeds are to be conserved for the medium term (10–20 years, maximum 30), they can be maintained at temperatures between 0° and 15°C (usually 1°–4°C), with a moisture content between 3% and 7% and a viability value of no less than 65%. For short-term conservation, seed can be stored in air-conditioned rooms (Cromarty et al. 1985; Engle 1992; Towil and Roos 1989).

The equipment used for cold storage usually consists of appropriately designed cold rooms, which should be hermetic and able to maintain as constant temperatures, relative

humidity, and light intensity. Machines for refrigeration, dehumidification, and control of light hours are used. In particular, the rooms should be designed for the samples they will store, the period over which they will remain in the rooms, and the area's climate where the rooms are established. Usually, cold rooms should be built with prefabricated panels of galvanized steel, joined by polyurethane foam, and insulated to protect the germplasm from outside conditions. Each room must have two independent refrigeration systems, a constant and stable energy supply, and verification instruments such as mercury and wet- and dry-bulb thermometers. Information on the infrastructure and equipment required can be found in the manual by Cromarty et al. (1985) for designing seed storage installations.

The cold rooms for seed storage can be modified considerably in terms of size and complexity, depending on the needs for each storage installation. Spacious underground chambers, constructed with thick concrete walls, are ideal for very large installations. They would be isolated from atmospheric changes in temperature, require less energy to function, and, especially, can resist environmental catastrophes or wars (Vázquez Y et al. 2004).

The simplest system, when operating with few resources and personnel, is to store orthodox seeds in a domestic freezer, whether horizontal or vertical. Care must be taken to prevent prolonged changes of temperature during electrical faults by using a generator that turns on automatically when a fault occurs.

Seeds can also be stored in glass vials sealed under heat. This method consists of first placing predried seeds in the vials and plugging with cotton wool. The air is then replaced with carbon dioxide and the vials sealed under heat. A desiccator can also be added, placing it between the cotton wool plug and the vial opening before sealing (Figure 3). This method facilitates the management of seeds when no special cold room is available in which to open the containers. That is, the vials can be left to gradually take up the room temperature before opening them. Thus, only a small sample of seeds is exposed, whenever seeds must be extracted from the bank.

In all cases, whether dealing with a small seed bank or one of national importance, a stable conservation policy for the germplasm is indispensable, together with an administrator and personnel, who are well prepared and interested in the project. Also necessary is continuous economic support to ensure the perpetuation of installations and their effective operation (Vázquez Y et al. 2004).

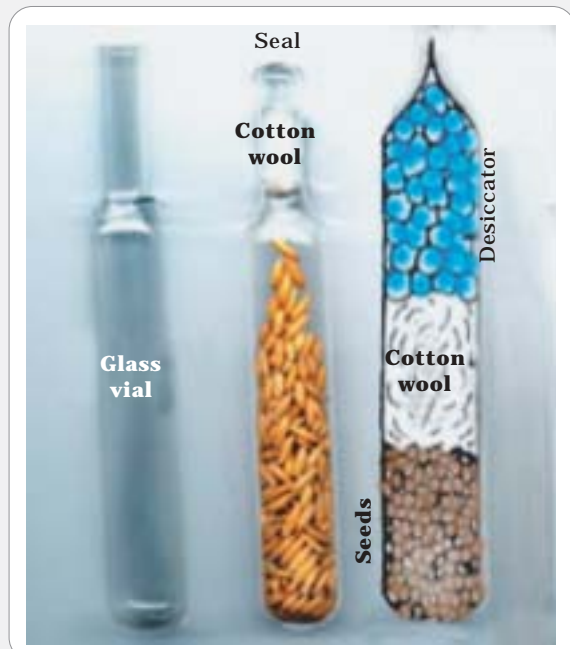***Cold and humid storage*** involves placing seeds in containers that maintain humidity,

Figure 3. Glass vials are used to store seeds (left and centre, photographs by B Pineda, GRU, CIAT; right, sketch from Vázquez Y et al. [2004] and redrawn by B Pineda).

or mixing them with materials that hold moisture such as damp sand. This method is applied to some recalcitrant seeds for a short period, together with an oxygen supply for respiration. This procedure is similar to that described for seed stratification (see *Module 3, Submodule C, Lesson 2*). Examples of plants whose seeds require this treatment are *Acer* spp. (maples, especially *A. saccharinum*), *Aesculus* spp. (buckeyes or horse-chestnuts), *Carpinus caroliniana* (American hornbeam), *Carya* spp. (hickories), *Castanea* spp. (chestnuts), *Corylus* spp. (hazelnuts), *Citrus* spp. (citrics), *Fagus* spp. (beeches), *Juglans* spp. (walnuts), *Nyssa sylvatica* (tupelo), and *Quercus* spp. (oaks) (Hartmann and Kester 1971).

Many tropical tree species produce seeds with high moisture content and fast metabolic rates, and which behave as recalcitrant. They are characterized by their inability to reorder the structure of their cellular components as water exits from the cells during dehydration. That is, the protoplasm loses its functional structure and does not recover it on rehydrating. The presence of free water in the cells eliminates the protective effect of freezing because ice crystals form that damage the cells. However, recent studies show that some dehydration treatments (Table 1) carried out on these seeds (e.g., papaya) permit storing them for longer (Vázquez Y et al. 2004).

Table 1. Treatments that can be carried out with recalcitrant seeds for their storage.

Treatment	Observations	Effect
Gradual and careful dehydration under controlled conditions of temperature, aeration, and air humidity	The degree of dehydration that seeds can tolerate at different temperatures without losing their viability must be determined	Increases longevity briefly
Dehydration in the presence of substances that protect the cellular ultrastructure	The level of dehydration at which the cellular ultrastructure is protected when conducting treatments with proline, betaine, saccharose, and other products must be determined	Increases the possibility of dehydrating seeds to a greater degree
Dehydration at very low temperatures in the presence of cryoprotectants or through the use of respiratory inhibitors	The levels of dehydration tolerated, and the effects of temperature, cryoprotectants, and inhibitors used must be determined	Duration of seed reserves increases and, as a result, viability is lasts longer when the seeds are stored under suitable conditions

SOURCE: Vázquez Y et al. (2004).

Quantity of seeds to store

The first concern to resolve is how many seeds would be used within a period, averaging 15 years. Norms for germplasm banks recommend that a base collection should be represented by a minimum of 1000 viable seeds (FAO and IPGRI 1994). An active collection can be represented by 1500 (presuming 100 seeds per year are distributed for use), and 500 seeds destined for testing viability—one initial and at least four periodic tests—and plant health, to total 3000 as a minimum for each entry.

This quantity of seeds should be taken as a suggestion for the minimum number of seeds to conserve rather than as a magic number. This is because variations should be taken into account, such as the species' reproductive mode. Allogamous or cross-pollinating species should be represented by a larger number of seeds, as they represent a wider genetic heterogeneity of individuals. Similarly, species with very long life cycles such as woody species must be represented by the largest possible number of seeds, because the plants' regeneration cycle is reached only after a growing period of several years. Likewise, species that have an abundant and easy production can be conserved, using a larger number of seeds.

Those international germplasm banks that are responsible for conserving the germplasm of several countries must keep a larger number of seeds, as they must repatriate seeds to the country of origin and maintain one or two duplicates for safety reasons to conserve in other institutions.

Plant materials

Conservation in the field. This method is applied to species that are perennial, arboreal, wild, semi-domesticated, or heterozygous, and to those with vegetative reproduction, short-lived seeds, or seeds that are sensitive to drying. Many important varieties of field, horticultural, and forestry species are either difficult or impossible to conserve as seeds (i.e., seeds do not form or, if they do, they are recalcitrant). Hence, they are conserved in field germplasm banks (Rao 2001). Field conservation thus involves conditioning the material where needed, multiplying it, selecting and preparing a site, planting the materials, and recording information on the accessions' location.

As mentioned before, vegetative propagation implies the use of parts taken directly from plants. As a result, during multiplication, there is a risk of propagating pathogens and other pests that associate with such plant material. Consequently, any programme aiming to maintain propagative disease-free clones that are genetically identical to the original materials must undertake the following three steps:

1. Initial selection from sources of planting materials that are characteristic of the species and free of serious pathogens;
2. Maintenance of such materials in sites with adequate protection against re-infection or genetic change; and
3. Establishment of a propagation and distribution system by which such materials are disseminated without becoming infected before reaching the user.

To conserve the material in the field, it must first be conditioned. The collected plant material is washed and disinfected before propagating it and taking it to the conservation site. Disinfection may be carried out with bactericides, fungicides (bulbs and rhizomes), or thermotherapy (stakes). The planting material, once disinfected, is propagated in the field, greenhouse, or *in vitro*. In the field and greenhouse, samples are planted on seedbeds or in pots, and left to grow until plants are obtained from which new samples can be collected. The procedure is repeated until the number of plants needed to establish the collection in the definitive site is reached.

If propagation is to be *in vitro*, then samples are planted in the greenhouse, in soils of optimal nutritional quality. From the resulting plants—preferably the youngest—explants

are extracted and micropropagated *in vitro* until complete plants are obtained. These are taken again to the greenhouse, planted in sterilized soil, and, after 2 or 3 weeks, transferred to the definitive site in the field.

Micropropagation consists of (a) disinfecting the explants in a solution of sodium or calcium hypochlorite, mercuric bichloride ($HgCl_2$), or ethanol, (b) planting them in an *in vitro* culture medium until new shoots develop, and (c) rooting the shoots until entire plants are obtained (George 1996; George and Sherrington 1984; IPGRI and CIAT 1994; Jaramillo and Baena 2000; Roca and Mroginski 1991).

Propagation in the field and greenhouse is simple but requires time and space. It does not guarantee that the plants obtained are healthy and genetically identical to the originals. *In vitro* propagation solves these problems, making the propagation of many species possible and more convenient, even for those that reproduce by seed. The site selected to conserve materials in the field should be safe and favour the plants' development. It should also be isolated to prevent attacks from pests and diseases but easy to access for management.

The physical and chemical preparation of the planting site depends on the species' requirements and the number of accessions expected to be planted in the field. Taking vigorous plants to the field in a number that represents the accessions' genetic variability will ensure the continuity of the conserved materials. The plants are so arranged in the field that they do not exchange pollen and the populations do not lose their original genotype. The exact site where each accession was planted should remain recorded on a map; and the accessions identified both in the field and on the plants.

In vitro *conservation and storage.* This system is gaining ever-growing importance as a tool of conservation and germplasm exchange because it permits the maintenance of a wide range of species, with a diversity of healthy samples, in a small space, and permits their easy exchange. However, such a system requires technology and knowledge that is still developing, protocols for each species, and considerable resources. This means that alternative conservation options should be evaluated before deciding on *in vitro* conservation. It is best applied for those species that are difficult to conserve as seed or in the field.

Tissue culture permits the *in vitro* conservation of a broad range of species in various types of samples such as complete plants, seeds, sprouts, buds, cauline apexes, meristems, ovules, embryos, cells in suspension, protoplasts, anthers, pollen, and DNA. The *in vitro* conservation of germplasm focuses on controlling the normal growth of viable explants— either reducing it or stopping it—by managing the constitution of the culture medium and/ or storage conditions.

As with conservation in the field, materials are conditioned, planted—*in vitro* in this case—and taken to the conservation site (Figure 4). Conditioning consists of disinfecting samples and washing them in distilled water to eliminate excess disinfectant. The solutions most used are sodium hypochlorite (NaOCl) at 1%–3%, calcium hypochlorite ($Ca(OCl)_2$) at 6%–12%, and ethanol at 70%. Explants (the smaller, the better) are extracted from the cleaned sample and planted in culture media placed in glass containers. They are subjected to one of two ways of *in vitro* conservation: slow growth or cryopreservation. In both cases, the medium and conservation environment must be sterilized and storage conditions controlled (George 1996; Jaramillo and Baena 2000; Roca and Mroginski 1991).

Slow growth. Slow growth consists of reducing the explants' development by modifying the culture medium and/or conditions under which they are maintained. Through the culture medium, growth can be reduced by increasing the osmotic potential (adding mannitol, proline, glycerol, or sucrose), adding growth inhibitors (abscisic acid), and reducing or suppressing nutrients that the explants need for growing (carbon and nitrogen). Growth is also limited by controlling the conditions under which the samples are stored, either using small containers or reducing the temperature, light, and partial oxygen pressure.

Reducing the temperature is the most effective way of controlling the explants' growth by reducing metabolic activity. However, it is equally important to ensure and maintain a slow growth rate to keep the explants viable for the longest possible time. Hence, a combination of methods should be used. Samples in slow growth are kept in rooms with low temperatures for periods that may vary from some months to usually two years. The temperature will depend on the species and variety, although most *in vitro* crops are kept at temperatures between 20° and 30°C. Lower temperatures can reduce even further the growth of some species but will negatively affect others. Some *Prunus* species, for example, conserve well at -3°C, whereas temperatures below 15°C will quickly destroy explants of *Musa* spp. (Pérez-Ruíz 1997), and those below 18°C will damage many cassava varieties (Roca and Mroginski 1991).

Figure 4.   *In vitro* germplasm bank for cassava (*Manihot esculenta*) germplasm, GRU, CIAT.

Materials conserved in slow growth need to be renewed every so often, even though they have continued growing, albeit slowly. Samples are micropropagated and transferred to a fresh medium for recovery and strengthening. When new explants have been established, they are propagated again, and taken again to the conservation medium. An example of the successful application of this methodology is cassava at CIAT, Colombia, where about 6017 accessions are conserved (IPGRI and CIAT 1994; Jaramillo and Baena 2000; Roca and Mroginski 1991).

Cryopreservation consists of placing explants in liquid nitrogen (-196°C) to stop growth while conserving viability and genetic and physiological stability. This technique is recent, with good prospects, as it allows the storage for indefinite periods any species that can tolerate and outlive freezing. Hence, it is particularly useful for conserving species with

non-orthodox seed or vegetative reproduction, and difficult to conserve in rooms or in the field (Ashmore 1997; Benson 1999; Engelmann and Takagi 2000; Jaramillo and Baena 2000).

Cryopreservation consists of (a) growing the explants *in vitro* (pregrowth), (b) drying them to the permissible minimum according to species, (c) treating them with cryoprotectants (glycerol, sucrose, mannitol, proline, polyethylene glycol) to prevent crystallization of intracellular liquids, (d) freezing in liquid nitrogen, (e) storing, (f) thawing, and (g) treating to recover viable plants (Jaramillo and Baena 2000; Pérez-Ruíz 1997; Rao and Riley 1994; Wang et al. 1993).

The success of cryopreservation depends on the species' reaction to freezing, which means that each species requires specific protocols. Various techniques exist such as encapsulation/dehydration, vitrification, encapsulation, desiccation, pregrowth, pregrowth-desiccation, and droplet-freezing (Ashmore 1997). But studies in this field, such as those carried out by CIP (Peru) with potatoes that tolerate freezing and CIAT with cassava, are still based on trial and error (Rao and Riley 1994). The methodology has limitations, the principal ones being the difficulty and time required to regenerate entire plants from the conserved structures.

Evaluating the Lesson

After this lesson, you be familiar with the basic requirements and main alternatives and procedures for storing plant germplasm.

Before going on to the next *Module 4*, briefly describe the type or types of storage used in your bank. If your work does not involve storing germplasm, describe that mode of storage that would be the most suitable according to the resources available to the bank of your institution.

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Next Lesson

In the next *Module 4*, you will study aspects of germplasm characterization.