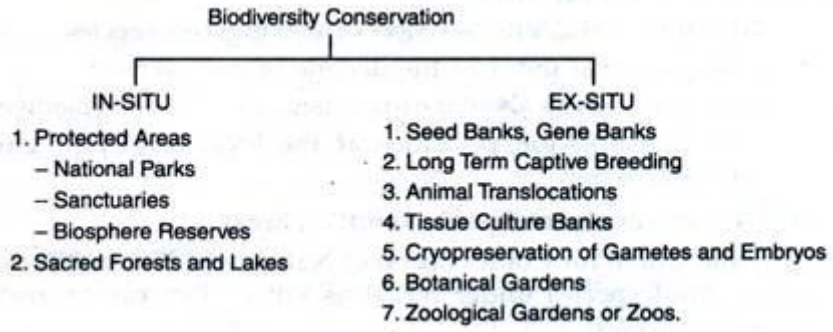
Conservation



Conservation of plant germplasm can be done on site (*in situ*) and off site (*ex situ*).

***In situ* conservation**

This type of conservation refers to the conservation of germplasm in ecosystems and natural habitats and the maintenance and recovery of viable populations of species in their natural surroundings. In the case of domesticated or cultivated species, it refers to their conservation in the surroundings where they have developed their distinctive properties. This is generally done in protected areas mostly for the conservation of wild relatives, and on-farm or in home gardens for the conservation of cultivated species. This type of conservation is not described further the Crop Genebank Knowledge Base.

***Ex situ* conservation**

This type of conservation is the storage of seeds or plant material under artificial conditions (other than their natural environment), to efficiently and effectively guarantee its longevity viability and availability. It is the type of conservation mostly used in genebanks. It covers a range of methods suitable for various types of seeds or plant materials. It ranges from cold storage of seeds or propagules, *in vitro* (tissue culture or cryopreservation), field, pollen or DNA conservation.
With *ex situ* conserveration two types of storage are recognized: storage of samples for long-term security – referred to as **base collections** – and storage of samples for immediate use – referred to as **active collections**. The storage conditions and distribution arrangements of these stores vary.

Base collections

A base collection is a set of accessions in which each is distinct and as close as possible to the original sample in terms of genetic integrity. Normally, material is not distributed from base collections directly to users. Base collections are only used to regenerate active collections (FAO, 2013). In seed banks, samples in base collections are stored for long periods at below 0°C

– usually at -18° to -20°C – to maintain seed viability and, in cryobanks, specially prepared *in vitro* culture samples are stored for long periods at -196°C, usually in liquid nitrogen. Engels and Visser (2003) introduced the term 'most-original sample' (MOS) to qualify the samples in base collections. A MOS consists of genetic material that has undergone the lowest number of regenerations since the material was acquired by the genebank; it may be a sub-sample of the original seed lot or a seed sample from the first regeneration cycle if the original seed lot required regeneration before storage or a cryopreserved sub sample of the first *in vitro* culture cycle.

Active collections

Active collections consist of accessions that are immediately available for distribution. These accessions are accessed frequently and storage of active collections can be in seed banks, vegetative banks, field banks and *in vitro* banks. Seeds are maintained in conditions that ensure at least 65% viability for 10-20 years (FAO, 2013) and *in vitro* cultures are maintained in slow growth conditions. Samples in vegetative banks are only stored for a few months but perennial living plants in field banks can be maintained for 20 years or more.

Cryo bank

Storage of germplasm using cryopreservation

Cryopreservation is becoming more widely used for long-term storage of seeds and *in vitro* cultures and is the method of choice for ensuring cost-effective and safe, long-term storage of genetic resources of species which have recalcitrant seeds or are vegetatively propagated. Storage is usually in liquid nitrogen (-196°C), whereby all metabolic processes and cell divisions are arrested.

Orthodox seeds

Seed conservation of orthodox seeds in liquid nitrogen at -196°C has been successfully achieved for a wide range of crop species. Once stored in liquid nitrogen, seeds can be kept for unlimited periods. Seed storage in base collections at -20°C is addressed under the seed conservation page.

Cryopreservation using liquid nitrogen storage tanks (photo: Bioversity/ILRI, by kind permission of RDA genebank, National Agrobiodiversity Center, Suwon, Republic of Korea)

Dessication intolerant seeds

Conservation of seeds with non-orthodox behaviour and those that are desiccation intolerant allows seeds which were previously considered as short-lived to be stored for long periods at ultra-low temperatures. This has been achieved for several species, including *Citrus*, coconut, coffee and areca nut through controlled desiccation of whole seeds, excised embryos or embryonic axes followed by cryopreservation.

Tissues

Preservation of dormant buds and *in vitro* cultures, including apical meristems and somatic embryos, at cryogenic temperatures is considered as the only suitable alternative that can ensure the long-term security of stored germplasm. Once stored in liquid nitrogen, tissues can be kept for unlimited periods. It is most appropriate for base collections of long-term storage

Advantages

The ultra low temperatures used during cryopreservation virtually stop metabolic deterioration during storage of tissues and seeds and therefore extends their longevity during long-term storage. The material is protected from contamination and very little maintenance is needed. The method relies on liquid nitrogen in self contained tanks and is not dependent on refrigeration or a constant electricity supply. It is also cost effective because of the reduced energy costs, limited space requirements and because there is no need for regular regeneration of plant material.

Disadvantages

There is no generic protocol which could be applicable to all types of explants. Therefore, each successive step of a cryopreservation protocol needs to be optimized for any new plant material to be cryopreserved.
Not all orthodox seeds are suitable for cryopreservation and hard seeded legume seeds may crack or shatter and some seeds with high oil contents loose viability. It is also a very costly method to store large seeds such as maize and beans.

Practical considerations

Until fifteen years ago, cryopreservation protocols for plant tissues were mainly based on slow freezing in the presence of cryoprotective mixtures containing dimethyl sulphoxide, sugars, glycerol and/or proline. Slow freezing results in a freeze-dehydration, leaving less water in the cells to form lethal ice crystals upon exposure to extreme low temperatures. While this method is very convenient for many plant tissues, especially undifferentiated callus or suspension cells, new methods had to be developed for plant species and tissues that were unresponsive.

During the last two decades, several new cryopreservation procedures have been established. Among them, the protocols termed encapsulation/dehydration, encapsulation/vitrification and vitrification. All these new techniques involve the extraction of freezable water from the tissue cells before cooling. As a result vitrification of internal solutes takes place during cooling. Vitrification can be defined as the transition of water directly from the liquid phase into an amorphous phase or glass, whilst avoiding the formation of lethal crystalline ice The vitrification technique involves a treatment with cryoprotective loading solutions followed by dehydration with highly concentrated vitrification solutions. A modification of this technique, that further reduces the chance for lethal ice-crystal formation through the application of ultra-fast cooling and rewarming rates called “droplet vitrification” has now been developed for different vegetatively propagated tropical crops.

Each species requires specific protocols that must be carefully followed for preparation of samples to ensure maximum survival.

General considerations

- Tissues and recalcitrant seeds generally cannot be thawed and refrozen without damage.
- Orthodox seeds and pollen behave differently and can usually be thawed and refrozen
- Transfer cryopreserved material quickly between vessels to avoid rewarming.
- Ensure good air circulation in the room where LN storage tanks are placed because nitrogen gas is constantly boiling from the tanks. Oxygen monitors should be placed around the room for detection of oxygen content of the air.
- Use emergency fans that are triggered by the oxygen monitors or emergency buttons to increase air exchange in case of build up of nitrogen gas
-

Cryogenic vial rack containing frozen banana meristems taken out of the liquid nitrogen (photo: Bart Panis)

Controlling levels of liquid nitrogen at the cryo genebank at the National Bureau of Plant Genetic Resources, Delhi (photo: J Hanson, by kind permission of National Bureau of Plant Genetic Resources, India)

Storage containers

Storage container will vary with the size of the seeds or tissues. Store seeds and tissues in 1.8 and 2.0 ml cryogenic vials for cryopreservation:

For meristems and shoot tips, 10-25 tissues can be placed in one vial;

For small seeds from 1500 to 3000 can be placed in one vial;

Larger vials will be needed for storage of larger seeded orthodox seeds;

- Place multiple vials into aluminum cans or metal boxes for storage.
- Liquid nitrogen storage containers vary with tissue and size of collection:
  - Small 20-30 liter tanks with no vapor phase for direct tissue storage.
  - Five-foot-diameter steel tanks that allow a vapour phase for seed storage.
- Use well insulated containers to reduce loss of liquid nitrogen. Containers with narrow necks reduce loss of liquid nitrogen but are inconvenient for access.
- Use an alarm system to indicate low liquid nitrogen levels.
- Top up storage dewars once in a week.

Storage temperature

Cryogenic vials may be stored directly in the liquid nitrogen at -196°C or suspended in trays within the vapour phase of the liquid nitrogen at -160°C

Replication

The number of replications depends on the survival, crop type, speed of propagation, stability in culture and material available for storage. Probalistic tolls have been developed to assist in the establishment and management of cryopreserved collections (see Dussert *et al.* 2003)

Specific crop protocols

Storage protocols have been developed for several important vegetatively propagated crops, including banana, cassava, potato, sweet potato and yam.

Banana - Cryopreservation of *Musa* germplasm
Cassava - http://webapp.ciat.cgiar.org/asia_cassava/pdf/proceedings_workshop_02/136.pdf
Potato - http://www.cipotato.org/csd/Materials/Tissue/Capitulo4.pdf
Sweet potato - http://www.cipotato.org/csd/materials/Sweetpotato%202-4.asp
Yam - http://www.ejbiotechnology.info/content/vol1/issue3/full/2/bip/

SAK

Seed bank

Biodiversity Seed banks in India

Author – Thirumagal J

Our food and livelihood security depend on the sustained management of diverse biological resources that are economically important. The conservation of biodiversity in crop production systems is inherently linked to sustainable use and preservation, since the particular plant species would have been cultivated and nurtured for centuries.

Biodiversity is defined as the variety or differences in the living organisms on earth. It could mean differences in genetics, species, or in the ecosystem. All living organisms on the land, water and in air have their own diversity. Being the seventh largest country in the world, India is naturally major hub for biodiversity. Two of the 18 biodiversity hotspots in the world – the Himalayas and the Western Ghats – are present in the country. According to MoEF Report (1999), the country is estimated to have 49,219 plant species and 81,251 animal species, representing 12.5% of the world's flora and 6.6% of its fauna.

Agricultural biodiversity or agro-biodiversity is sub-set of biodiversity that has resulted from the natural selection processes and inventive developments of farming, herding and fishing by human over millennia. It consists of the diversity of genetic varieties, breeds and species used for food, fodder, fiber, fuel and pharmaceuticals. It also includes the diversity of non-harvested species that support production such as soil micro-organisms, predators, pollinators), as well as those in the wider environment that support agro-ecosystems.

Agro biodiversity plays an important role in agriculture, so local knowledge and culture can be considered as integral parts of agrobiodiversity, because it is the human activity of agriculture that shapes and conserves this biodiversity.

Agro-biodiversity can be divided into two categories:

- Intraspecific diversity covers the genetic variety within a single species – such as different sub-species of rice, Basmati rice, Thai Jasmine rice, Japanese Mochi rice, Sona Masuri, etc.
- Interspecific diversity refers to the number and types of different species – such as potatoes, carrots, peppers, lettuce etc.

Benefits of Agro-biodiversity

Our food and livelihood security depend on the sustained management of diverse biological resources that are economically important. The conservation of agro-biodiversity in crop production systems is inherently linked to sustainable use and preservation, since the particular plant species would have been cultivated and nurtured for centuries. So local knowledge and culture are integral parts of agro-biodiversity management. Further, Biodiversity provides critical support for drug discovery and the availability of medicinal resources, since a major proportion of drugs are derived from biological sources.

Agro-biodiversity helps in various ways such as:

- Increase productivity, food security and economic returns
- Reduce the pressure of agriculture on fragile areas, forests and endangered species
- Make farming systems more stable and sustainable
- Contribute to pest and disease management
- Reduce the spread of diseases to individuals and nations
- Improve human nutrition and provide sources of medicines
- Conserve ecosystem structure and stability of species diversity

A vast amount of agricultural biodiversity is being lost, as farmers abandon locally developed, centuries old seeds for the new hybrids. The UN's Food & Agriculture Organisation estimates that 75% of crop biodiversity has been lost from the world's fields. India is reckoned to have had over 100,000 varieties of rice a century ago; it now has only a few thousand.

It has been estimated that 37% all living species may to vanish in the next century due to climate change. There are an estimated 8.7 million different living species on earth, of which 300,000 are plants. Many of them may vanish due to climate changes, human activities or due to natural disasters before we can understand their importance. Hence, it is important to not only know about biodiversity and also to take action to conserve them for our future generation. Here comes the role of seed banks.

Seed banks

Definition : A seed bank is a type of gene bank where seeds of different crops and rare plant species are stored for future use. Seed banks are created to maintain and protect biodiversity, where samples of all species are collected and stored. In case seed reserves elsewhere are

destroyed, the seed bank is opened to provide seeds to farmers at defined quantities for growing plants.

Most of the seeds can be stored for centuries without damaging their genetic properties. However, they should be replanted after a certain time period in order to avoid eventual DNA damage. The seeds are frozen at temperatures below -4 degrees centigrade and stored in seed vaults.

Seed banks primarily involve in selecting, collecting, and storing seed varieties. They also form seed exchange networks with government organizations, NGOs and community seed banks across the world. They also form ex situ storage facilities. They help in seed exchange, on farm conversation with experts and farmers, training and capacity building for farmers and continuous monitoring of cultivation. The Millennium Seed Bank and Svalbard Global Seed Vault are the largest seed banks in the world.

Well-known Seed Banks in India

The Indian government established the National Seeds Corporation in 1963 both at the national level and in every state. Working under the Ministry of Agriculture, NSC undertakes production, processing and marketing of agricultural seeds. It is also involved in formulation of seed certification standards done through seed testing laboratories by checking the compatibility of different seeds. State

agricultural universities and the Indian Council for Agricultural Research (ICAR) are involved in seed production and distribution. For each region or village there are community seed banks available for exchanging seeds.

Apart from government organizations, several private and voluntary organizations have also set up seed banks across the country.

- **Navdanya** is a leading NGO advocating for biodiversity conservation through a large network of seed keepers and organic producers. Led by Vandana Shiva, it has created a women centered movement for protecting biological and cultural diversity. Navdanya has helped set up 54 community seed banks, as well as the largest direct marketing, fair trade organic network in the country.
- **Annadana Seed and Soil Savers** led by Sangita Sharma works toward conserving food plant diversity and support sustainable natural farming. The Annadana Seed Bank conserves and distributes 101 varieties of organic open-pollinated vegetable seeds.
- **Green Foundation** is a community based organization started in 1996, which works on conserving local seed diversity and promoting biodiversity-based ecological agriculture. It has a network of farmer associations spread across 109 villages in Karnataka and Tamilnadu for preserving and promoting agro-biodiversity through community seed banks.
- **Deccan Development Society** is another NGO involved in conserving agro-biodiversity, which works with voluntary associations to help women and agricultural laborers in Andhra Pradesh. It has initiated a community gene bank project to preserve agro-biodiversity, networking with a number of small organizations involved in seed bank activities.
- **Sahaja Samrudha** is an organic farmers' collective that works for preserving India's traditional farming practices and the rich biodiversity of its indigenous crop varieties. It has created a seed savers group in Karnataka, which has done some remarkable work in identifying rare varieties of paddy, millets, lentils, vegetables, etc.

By preserving biodiversity, we can make farming systems more stable and sustainable, which would help farmers to lead a stable life and reduce the number of suicides of farmers due to

income loss and natural disasters. We can also increase the income for farmers by diversifying their produce, improve human nutrition level and protect ecosystem.

Factfile & Image Sources –

http://www.fao.org/docrep/007/y5609e/y5609e01.htm

http://www.economist.com/node/21549931

http://www3.telus.net/permaculture/spiralnews.html

http://www.motherearthnews.com

http://www.indiaorganic.net/products/sahaja.html

Storing seeds using cold storage

Storing genetic diversity as seed is the best researched, most widely used and most convenient method of *ex situ* conservation. Much is known about the optimum treatment of the seeds of most major crops. Requirements include adequate drying (seed moisture contents as low as 3% for oily seeds and 5% or more for starchy seeds); appropriate storage temperature (-18°C is recommended for long-term storage); and careful production of quality seed to ensure the greatest longevity (storing them in hermetically-sealed containers).

Packing seeds for cold storage at the USDA genebank (photo: L. Guarino, by kind permission of USDA genebank in Ames, Iowa, USA)

Orthodox seeds

It is the principal conservation method for species producing orthodox seeds that withstand desiccation to low moisture content and storage at very low temperatures. Most arable and forage species, and many tree species, produce seeds in this category.

A cold storage room (photo: ILRI)

Recalcitrant seeds

Several important tropical and sub-tropical tree species produce seeds that do not survive desiccation and cannot tolerate low temperatures, and which are therefore not easy to store; these are known as recalcitrant seeds. Techniques exist for storing some recalcitrant seeds, but the seeds are usually short-lived and each species requires its own method.

Intermediate seeds

A third category of seeds showing intermediate behaviour has also been recognized: these seeds tolerate combinations of desiccation and low temperatures. There is, in fact, a gradient from orthodox to recalcitrant, with no sharp boundaries between categories. Although research has been conducted to overcome problems associated with seed conservation, little progress has been made beyond short-term storage of non-orthodox seeds.

Principles of seed storage

The underlying principle of successful seed storage is to maintain genetic integrity of accessions as seeds with high viability for long periods. Seeds of the original sample should be stored under the best possible conditions to ensure safe long-term survival, while seeds of accessions that are frequently requested by breeders or other users should be stored in the active collection. Genebanks may maintain both base and active collections or focus on only one. Such decisions are based on the purpose and needs of the genebank and economics of conservation.

For orthodox seeds, low temperatures and low moisture content are used to extend longevity and reduce regeneration intervals with related risks to loss of diversity and genetic integrity. Genebank requirements differ for crops and it is important to select a combination of temperature and seed moisture content specific for the species that will retain high viability for many years of conservation (see specific crop regeneration guidelines).

Practical considerations

Location

Choice of location of the seed store is important to maximize efficiency and minimize cost:

- Select a site with a cool, dry environment, if possible, to improve the efficiency of controlling the environment.
- Select a secure site with the best possible physical safety against theft, and avoid areas with natural threats such as earthquakes, volcanoes and hurricanes.
- Select a site with reliable electricity supply and adequate rainfall or water supply needed for associated laboratories.
- Construct the building with shade and North facing to minimize solar heating. In areas with erratic electricity supply, a standby generator is essential to maintain the controlled environment.

Type of store

The commonly available options for seed storage are walk-in cold stores, freezers and liquid nitrogen dewars. The choice depends on the number of accessions to be stored, seed size and storage temperatures selected.

- Select walk-in stores for large collections or large seeded species where space is needed.
- Select chest or upright freezers when collections are small and sub-zero temperatures are required.
- Select liquid nitrogen storage when collections are small and sub-zero temperatures are required for very long-tern storage.
- Assess the current collection size and probable number of accessions expected over the next ten years to determine the size and type of store needed.
- Use good insulation of about 200 mm thickness on coldrooms to reduce the electrical running costs.
- Monitor and log the temperature daily and check levels of liquid nitrogen weekly. An alarm system can be used to alert staff to malfunction of the cooling system and need to top-up liquid nitrogen.

Sample size

- The required minimum number of seeds for a genetically homogenous sample is 3000-4000 seeds and for a genetically heterogeneous sample it is 4000-12 000 seeds.
- More seeds should be stored for those accessions that are frequently requested or have long regeneration periods (some slow growing trees), to reduce regeneration requirements.

Organization of space

The organization of storage space depends on the type of storage facility and the type of containers used in the genebank. In view of the cost of maintaining cold storage, the space should be optimized so that a maximum number of seed accessions can be stored.

- For a walk-in cold store, use moveable racks that maximize storage space.

- Arrange the distance between each shelf according to the size of containers, leaving a gap of about 10 cm above the containers to allow cold air circulation.
- Arrange small containers or aluminium foil bags in boxes or trays to keep them together and save space on the shelves.
- Use a coding system based on the location for sample entry and retrieval.

Safety duplication

- Duplicate seed genebanks in more than one location as a safety backup. Accessions available at other genebanks may require fewer duplicates than unique samples.
- Select a site for safety duplication to minimize possible risks and provide the best possible storage facilities.
- A third safety backup can be kept in the Svalbard Global Seed Vault for added security.

For more information about safety duplication see the safety duplication page.

Almost all food begins with a seed. Even when people eat meat or other animal products, those animals were most likely fed on grasses or grains that began as seeds. Seeds are the basis of plant life and growth, and without them, the world would go hungry.

The world is home to hundreds of thousands of species of plants, and it requires a diverse variety of seeds to satisfy nutritional and environmental needs. Today, Nourishing the Planet takes a closer look at five seed banks that aim to protect biodiversity and help feed the world.

1. Kew's Millennium Seed Bank Project, Wakehurst, England

How many plant species can you think of? Of the roughly 400,000 known species, the Millennium Seed Bank aims to conserve 25 percent in the form of seeds by 2020. The seed bank is located on the grounds of Britain's Royal Botanical Gardens, which were constructed by King Henry VII and are now considered a UNESCO World Heritage Site. Focused on conserving seeds from plants that can be used for food production, the Millennium Seed Bank currently holds seeds from over 10 percent of all plant species.

Millennium in Action

The Royal Botanical Gardens has been collecting research on seed saving since 1898 and has had a formal seed bank for 40 years. In recent years, it has concentrated on collecting seeds from environments that are most vulnerable to climate change. In addition to developing new crop varieties that are more adaptable to changing environments, the Millennium Seed Bank Project has implemented an international education program in an attempt to preserve ecosystems worldwide. A large part of its educational outreach program has taken place in rural regions of

Africa, in countries including Kenya, Botswana, Burkina Faso, and Namibia. Promoting projects from nutrition to forestry to sustainable agriculture, the Millenium Seed Bank Project is working to feed the world and sustain the environment.

2. Navdanya, Uttrakhand, India

Since 1987, Vandana Shiva, who created Navdanya, has dedicated her life to protecting seed diversity. Navdanya is an agricultural research center that seeks to protect seed biodiversity and the livelihoods of small farmers. The organization believes that people should have a right to save and share seeds, and has created a seed bank that conserves only unpatented seeds.

Navdanya in Action

Since its creation, the Navdanya seed bank has conserved around 5,000 crop varieties, focusing largely on the preservation of grain species. The 54 community seed banks that Navdanya has piloted have preserved nearly 3,000 species of rice alone. In addition to protecting seed biodiversity, Navdanya aims to spread agricultural information through educational campaigns.

3. Svalbard Global Seed Vault, Svalbard, Norway

Preserving seeds for long periods of time requires extremely cold temperatures and low humidity. That's why Svalbard Global Seed Vault, located deep in the permafrost-covered mountains of Svalbard, was deemed the ideal site for a global seed bank. Funding for the seed bank, built from the remains of an abandoned mine, was provided largely by the Bill and Melinda Gates Foundation with the aim of permanently protecting agricultural and plant biodiversity. The vault has the capacity for 4.5 million seed samples and currently houses over 430,000 specimens, including samples from Armenia, Colombia, Costa Rica, and Tajikstan. Genetically modified organisms are allowed in the seed bank only after evaluation and approval and must be specially sealed to prevent the spread of genetic modification to other samples.

Svalbard in Action

Despite being nicknamed the "Doomsday Vault," Svalbard is a forerunner in global environmental problem-solving and innovation, and frequently hosts events on topics related to food security and climate change. In 2009, the seed vault held an international conference on climate change and the challenges of feeding the world's growing population. The vault also has hosted influential policymakers including United Nations Secretary-General Ban Ki-moon.

4. National Center for Genetic Resources, Fort Collins, Colorado

Located on the campus of Colorado State University, the National Center for Genetic Resources (NCGR) is home to one of the world's largest gene banks. The center is unique in that it does not simply host seeds, but various types of germplasms, or collections of genetic information, including plants, animals, insects, and microorganisms. In its plant division, the center contains

pollen, meristem tissue, and cell cultures. The NCGR strives to ensure that its germplasms maintain the same genetic properties over time, so that traits do not change as reproduction occurs.

NCGR in Action

One of the goals of the NCGR, a part of the U.S. Department of Agriculture'sAgricultural Research Service, is to conduct genetic research for the development of new cultivar varieties. The center frequently publishes briefs on nutrition and agricultural methods, and also tracks ecological trends. In August 2012, scientists from the center created two new blueberry varieties known as Gupton and Pearl that are praised for their high yields and flavorful fruit. Also within the department, horticulturalist Joseph Albano of the Agricultural Research Service has recently developed an ecologically cleaner alternative to fertilizers that contribute to heavy metal watershed pollution. In addition to hosting over 8,000 species of seeds, the NCGR contributes greatly to genetic research and agricultural development.

5. Vavilov Research Institute, Russia

Russia has a rich history of botanical studies that have included plant management, disease control, and the recording of plant varieties. The Vavilov Research Institute (VRI) was opened in 1924 and has since expanded into 12 research stations throughout Russia. The stations' seed banks house a combined total of some 60,000 seed varieties, and their herbariums contain some 250,000 plant specimens. Specializing in berries and other fruits, the VRI holds over 1,000 types of strawberries alone. According to journalist Fred Pearce, nearly 90 percent of seed and plant specimens at the VRI's Pavlovsk station are not found in any other seed or gene bank in the world.

Vavilov in Action

Seed banks around the world are continuously at risk, and Vavilov is no exception. The VIR is probably most well known for its Pavlovsk station, which during World War II was put under siege by the Axis powers. During the siege, 12 scientists protected the station's seed bank from destruction, and out of respect for the value of seeds, they starved instead of eating them. Today, this station is once again under attack, but this time the threat comes from developers who wish to build on 227 acres, or three-quarters of the field station's property. Another station in Krasnodar, Russia, was recently exposed to extreme flooding.

Seeds, not frequently the subject of public discussion, hold the potential to regenerate species, promote biodiversity, and enable ecosystems to adapt to an ever-changing world. Biodiversity in seed varieties is essential to the maintenance of human, plant, and animal life as we know it. Seed banks around the world provide a valuable service by protecting these small but important resources.

Field genebank

The use of field genebanks

In field genebanks the plant genetic resources are kept as live plants that undergo continuous growth and require continuous maintenance. They are often used when the germplasm is either difficult or impossible to conserve as seeds (i.e. when no seeds are formed, seeds are recalcitrant or seed production takes many years, as for many tree species) or the crop is reproduced vegetatively.

Advantages

Field genebanks provide an easy and ready access to the plant genetic resources, for characterization, evaluation or utilization, while the same material conserved in the form of seeds, *in vitro* or cryo must be germinated or regenerated and grown before it can be used. They are also useful for conserving vegetatively propagated genotypes that commonly produce variants (genetic variation) since these can be more easily identified and rouged out in the field than *in vitro*.

Disadvantages

Field genebanks however, are generally more expensive to maintain, requiring more labour, more inputs and more space (land) than other methods of conservation. They also have higher levels of risk from natural disasters and adverse environmental conditions like drought, floods or attacks from pests and diseases.

Field genebank at the USDA genebank (photo: L. Guarino, by kind permission of USDA genebank in Ames, Iowa, USA)

Practical considerations

Maintaining plants in field genebanks is costly and risky and this method of conservation is usually used when there are no available alternatives or the storage period of other alternatives is very short and not practical. Field genebanks are mostly used for the conservation of clonal crops, often complementary to other conservation methods such as *in vitro* and cryo banks. Field genebanks are particularly sensitive to germplasm health issues and regular monitoring and testing together with application of disease control measures is essential to maintain plants free of diseases. Although field genebanks may not be the most secure method of germplasm conservation, often they are the only practical and cost effective way to conserve germplasm of clonal crops, especially when resources and skills are limiting.

When field genebank conservation is the only viable alternative, careful planning and field management can help to mitigate the risks.

Some best practices for establishment and management of a field genebank include:

Location

- Duplicate field genebanks in more than one site or in an *in vitro* genebank as a safety backup. Accessions available at other genebanks may require fewer duplicates than unique samples.
- Select a site with environment and soil type best suited for the species to reduce the risk of poor adaptation.
- Select a secure site with the best possible physical safety for theft and avoid areas with natural threats such as volcanoes and hurricanes if possible.
- Select a site some distance from fields of the same crop to reduce threats from pests and diseases.
- Select the field based on appropriate rotation and infection history to avoid mixtures and infection/infestation with different pests.
- Select a site with adequate rainfall or water supply for supplementary irrigation to avoid drought stress.

Establishment

- For those reproduced vegetatively, one plant is sufficient to maintain the genetic variation but more plants are needed for security and it is recommended that a minimum of five plants are maintained for each accession.
- For tree species that are slow growing and maintained in the genebank until, eventual seed production, more plants are needed to capture the variation at the time of eventual seed production. Up to 100 plants per accession is preferred with a minimum of 25 plants.
- Use adequate spacing and plant density in the plot to avoid competition that will result in weak plants or allow rapid spread of disease or insect pests.
- Avoid selecting only strong plants to retain in the field genebank, as that would reduce genetic variation. Select the planting material carefully so that only healthy material and vigorous parts of the plant are used when planting new fields or replanting empty plots.
- When material is transferred from other sites or countries, follow the specific regulations for the safe transfer of germplasm for that crop.
- Correct and clearly written labels are extremely important in germplasm collections. Field labels should be in indelible ink and as indestructible as possible; field maps are essential as a backup to labels that are easily lost or destroyed.
- When new plots are established, allow an overlap of a few months before destroying the older field to ensure the plants establish well in the new field.

Field management

- Keep the plots well weeded to avoid competition.
- Eliminate plants growing off-row. Rogue plants that are genuine mixtures.
- Irrigate the field after establishment of the plants and when subsequently needed.
- Do not allow the leaves to wilt at any stage.
- Apply fertilizer at the recommended rate for the crop in that area.
- Inspect the field regularly and preferably daily to ensure that the plants and plots are in good condition and take action quickly if any problems are noted.

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 harvesting any plant material and burn the residues.

Monitoring accession identity

- Distinctive traits are specific for each species. For accessions compare traits with previous passport or morphological data.
- Digital images are useful for identification and comparison.
- Herbarium specimens are important to compare specimens to verify accession identity.

For more detailed information about when to store material in a field genebank see Reed *et al*., 2004.

References and further reading

Engels JMM, Visser L, editors. 2003. A guide to effective management of germplasm collections. IPGRI Handbooks for Genebanks No. 6. IPGRI, Rome, Italy. Available in English (1.4 MB) and Spanish (1.5 MB).
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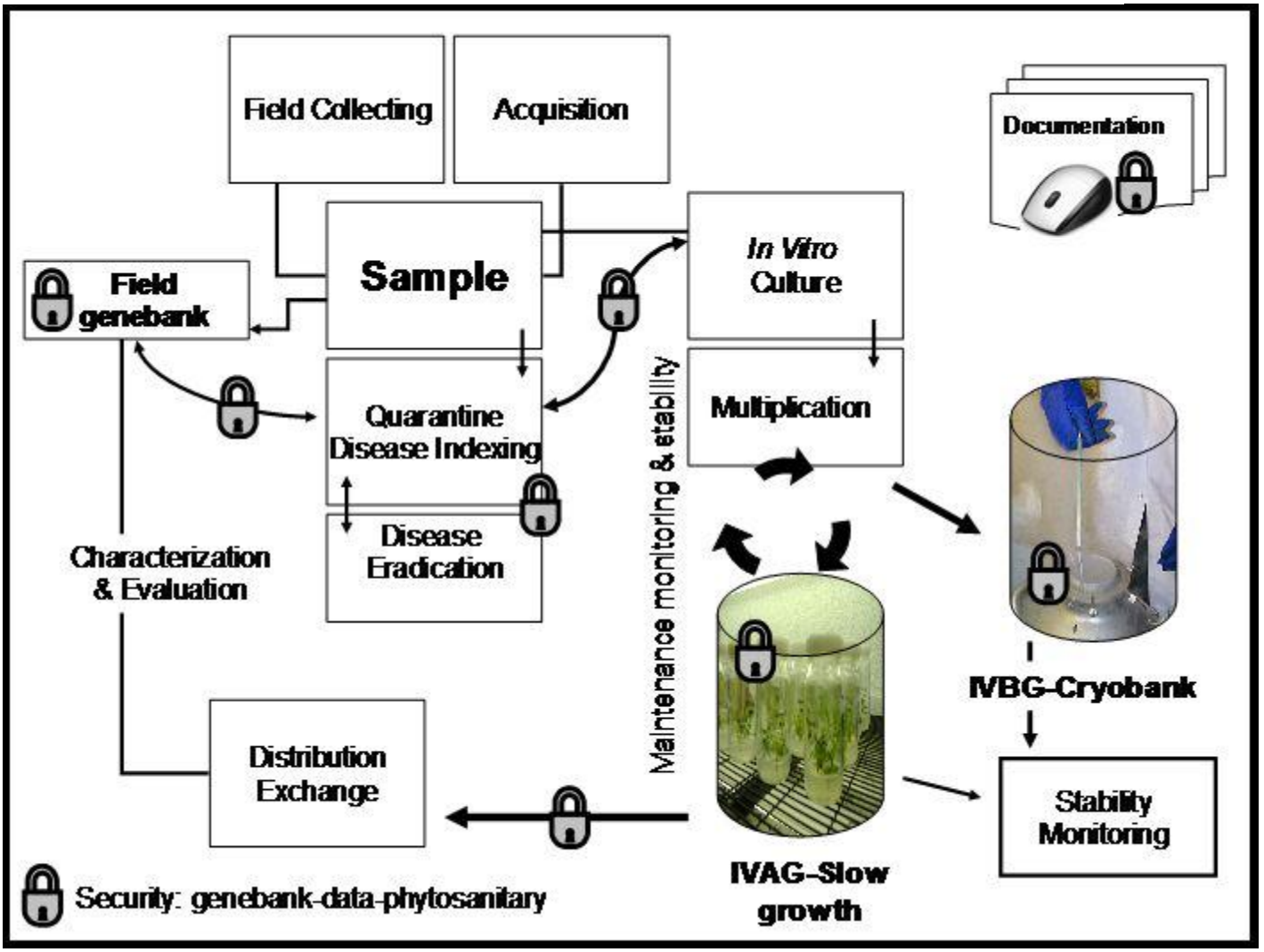
Reed BM, Engelmann F, Dulloo ME, Engels JMM. 2004. Technical guidelines for the management of field and *in vitro* germplasm collections. IPGRI Handbook for Genebanks No.7. IPGRI, Rome, Italy. Available here.
Saad MS, Ramanatha Rao V, editors. 2001 Establishment and management of field genebank, a Training Manual. IPGRI-APO, Serdang. Available here.

In vitro bank

Tissue culture conservation

Seed storage is the preferred conservation method. However, it is not feasible for germplasm from clonal crops which are either vegetatively propagated and/or do not produce seeds, or for species with short lived recalcitrant seeds. For some genotypes, elite genetic combinations are only preserved through clonal means. Their conservation is dictated by breeding strategy as heterozygosity does not permit the maintenance of desired characteristics. Clonally propagated plants incur special needs for their conservation. Common options for storage include maintenance in field genebanks, and for species producing dormant vegetative propagules, conservation in cold stores (Reed, 2001) calledvegetative banks. These approaches have limitations regarding efficiency, costs, security and long-term maintenance.*In vitro* conservation, which involves maintenance of explants in a sterile, pathogen-free environment is therefore preferentially applied to clonal crop germplasm and multiplication of species that produce recalcitrant seeds, or do not produce seeds. It also supports safe germplasm transfers under regulated phytosanitary control. This modern technique has already been applied for multiplication, storage and collection of germplasm of more than 1000 species.

CIP In vitro genebank for potato and sweet potato (photo: A Jorge)



In vitro genebanks and their relationships with other operations. IVAG = *In vitro* active genebank, utilising slow growth for medium term storage (MTS) and IVBG = *In vitro* base genebank, utilising cryostorage. Critical points of security are indicated. Based on IBPGR (1986).

Conservation in tissue culture in *in vitro* genebanks is often combined with cryopreservation. Cultures in the active genebank are maintained by successive subculturing allowing culture renewal and distribution. For medium term storage, sub-culture intervals are extended, reducing processing costs by arresting growth using cold treatments, adapted light conditions, culture medium modifications (osmotic active compounds, growth retardants). This increases efficient use of resources and staff time and offsets selection risks and contamination

Advantages

One of the major advantages of *in vitro* conservation of genetic resources is that tissue culture collections can be cleaned to provide a source of disease-free material. *In vitro* cultures are free of fungi and most bacteria while viruses can still be present. Therefore careful virus indexing procedures need to be applied to ensure material is disease free. Tissue culture storage also allows the conservation of germplasm in a protected environment, aseptic plant production, safe and easy international exchange of plant material and lower conservation costs. It is most appropriate for rapid multiplication purposes, dissemination and active collections.

Dissemination of vegetatively propagated material (photo: IITA)

Disadvantages

Maintaining material as shoot tip or meristem cultures, even when applying reduced growth conditions, remains labour intensive. It also involves the risk of losing valuable germplasm through accidental contamination of cultures and human error. Another major impediment of tissue culture storage under slow growth conditions is the possibility of genetic instability due to somaclonal variation (mutations that occur spontaneously *in vivo* or *in vitro*, whose frequency is generally increased during *in vitro* culture).

Musa in vitro (photo: Bioversity)

Practical considerations

Security

Security measures should be compliant with safety and ethical authorities regulations and guidelines, including observance of: (a) the Convention on Biological Diversity, (b) the Material Transfer Agreement, with respect to genetic resources exchange and (c) the International Plant Protection Convention. Security should ensure:

- Purity: freedom from contaminating organisms.
- Authenticity: correct identity.
- Stability: fit-for-purpose and trueness-to-type.

Good laboratory practices, application of aseptic techniques with careful containment strategies, clear and accurate documentation and avoiding practices that increase risks of genetic variation are all essential to ensure security of cultures.

Culture facilities

Research may be necessary to determine the appropriate environment to successfully culture and grow materials of different species in *in vitro* cultures. Some general guidelines are:

Potato *In vitro* collection at International Potato Centre (CIP), Lima, Peru". (photo: M.E. Dulloo)

- Use culture growth rooms with temperature control, lighting and shelving.
- Aim for a room where the humidity is 40–50%. High humidity increases fungal growth, while low humidity dries cultures and creates dust problems.
- Use an isolated growth room for *in vitro* explants of materials taken directly from the field to allow time to detect insect infestations and prevent their spread to other cultures.
- Ensure a light intensity in the range from 10 to 1000 μmol S-1 m-2. Most plant cultures require 50–200 μmol S-1 m-2.
- Use ventilation systems or air-conditioning units to regulate temperature. Air should not flow directly onto the cultures. Common growth room temperatures range from 22ºC to 28ºC, depending on species requirements.
- Back-up generators are advisable for areas with frequent power cuts to control temperature and light.

Genetic stability during storage

Somaclonal variation, while a problem with plants regenerated from single cells, callus or adventitious buds, is not common in plants micropropagated from axillary buds. The frequency of somaclonal variation occurring, gross chromosomal aberrations and *in vitro* selection are enhanced in prolonged tissue culture. Exposure to minimal growth conditions over long periods of time can also be expected to lead to genetic change. It is significant that asexually propagated species for germplasm conservation may display a higher frequency of somaclonal variation as compared to those where the propagule is a seed. Great care should be taken to select culture practices to reduce this variation and ensure genetic integrity.

Preferred practices are:

- Avoid using germplasm propagated via dedifferentiated and adventitious routes for conservation.
- Select germplasm from young cultures because somaclonal variation increases and totipotency decreases during prolonged culture.

Medium term storage using slow growth

The objective of slow growth (or minimal growth) is to reduce the sub-culture interval to a critical level which does not impose a long-term deleterious effect on the germplasm, or the stability of regenerated/regrown plants. However, slow growth treatments incur some level of

stress and it is essential to optimise regimes for each species for timing of sub-culture and regeneration. Minimal growth storage is achieved via several treatments, applied singularly or in combination:

- Physical growth limitation
  - Low temperature
  - Low light/restricted photoperiod
  - Minimal containment
  - Minimal O2
  - Osmotic (water) stress
- Chemical growth limitation
  - Growth regulator retardation
  - Growth inhibitors
- Minimal nutrition
  - Low macro nutrient levels
  - Low micro nutrients levels

Choice of treatment is largely species-dependent and dictated by the ability of specific cultures to withstand the stresses incurred.

Culture and storage protocols have been developed for several important vegetatively propagated crops, including banana, cassava, potato, sweet potato, yam.

Banana - http://cropgenebank.sgrp.cgiar.org/index.php?option=com_content&view=article&id=545&Itemid=740&lang=english
Cassava - http://webapp.ciat.cgiar.org/asia_cassava/pdf/proceedings_workshop_02/136.pdf
Potato - http://www.cipotato.org/csd/Materials/Tissue/Capitulo4.pdf
Sweet potato - http://www.cipotato.org/csd/materials/Sweetpotato%202-4.asp
Yam - http://www.ejbiotechnology.info/content/vol1/issue3/full/2/bip/

Vegetative bank

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Storing plant propagules using cold storage

Vegetative propagules of several tuber crops, including potato, sweet potato, yam and cassava can be conserved under cold conditions of 4-20°C for several months between one harvest and

the next planting season. Cold storage avoids deterioration following the harvesting of fresh tubers or stem cuttings and consequent losses caused by both physiological changes within the plant by reducing respiration and infection by pathogens and pests during storage.

Pretreatment

Storage propagules should be free of damage caused by insects and nematodes and any other visible symptoms of diseases before storage. Any type of wounds, scraping and peeling can affect the storage life of vegetative material by allowing fungi and bacteria to enter the plant and causing rotting. The storage propagules should be disinfected as soon as possible after harvest using either a 5% sodium hypochlorite solution or an insecticide and fungicide solution and soaking the propagules inside mesh sacks or storage containers for 10 minutes.

Storage

The propagules selected for storage are usually stored in mesh sacks, or open boxes made of wood, or plastic to allow air circulation with duplicated labels with the identification number, both inside and outside the container. Stems are often more robust and can be stored in bundles or in polythene bags with the cut ends covered with wax to prevent excessive drying during storage. The material should be monitored weekly for signs of rotting, insect damage or rodent damage.

Storage of potato tubers in a cold room at CIP (photo: A Jorge)

Reported methods for storage include:

Sweet potato storage tubers may be cured by placing them in a high temperature chamber (25-30ºC) with a high relative humidity (RH) (85-90%) for 4-7 days in order to heal all wounds and then stored under a controlled temperature of 12ºC and a high relative humidity for up to 7 months.

Potato tubers are usually stored at temperatures from 2 to 14 ºC and a relative humidity around 70% in the dark for up to 6 months.

Cassava stems with viable buds can be stored at 20–23ºC and 70–80% RH in bundles or in polythene bags in drier areas during the dry season.

Yam storage tubers should be allowed to dry in the shade for 4 to 6 weeks and can then stored at 18–20°C in a dry cool area for 3 to 4 months. Chilling damage occurs when tubers are stored below 10°C.

DNA bank

The use of DNA banks

Molecular techniques are becoming increasingly important in the study and management of genetic resources. DNA has been routinely extracted and stored from the nuclei, mitochondria and chloroplasts of many plant species, together with derivatives such as RNA, cDNA and genes. Technologies are available to allow all these to be stored quickly and at low cost in DNA banks as an insurance policy against loss of crop diversity. DNA storage has so far been undertaken with objectives other than conservation in mind; usually to allow genetic material to be made readily available for molecular applications, for distribution or training.

DNA banks can now be considered as a means of complimentary conservation. DNA storage is particularly useful for those species that cannot be conserved in traditional seed or field genebanks and nor conserved *in situ* due to high risk in that area. Although to date there are no cases where DNA banks have been assembled to specifically replace traditional methods of conserving genetic resources, the potential for DNA storage is promising due to the small sample size for storage of genetic information and the stable nature of DNA in cold storage. However, use of DNA banks in conservation is limited as whole plants cannot be directly reconstituted from DNA nor are the original genotypes recovered. The genetic material must first be introduced artificially, through transformation or transduction using plasmids or liposomes, back into somatic cells that can then be grown into whole plants in *in vitro* culture.
The field of molecular biology is advancing rapidly. As new techniques are developed DNA banks are likely to become a more feasible option for conservation of crop diversity in the future. For more information on the options for DNA banking see DNA Banks.

A DNA bank (photo: Bioversity/ILRI, by kind permission of RDA genebank, National Agrobiodiversity Center, Suwon, Republic of Korea)

Advantages

DNA banking is an efficient, simple and long-term method to conserve the genetic information.

Disadvantages

There are problems with subsequent gene isolation, cloning and transfer of DNA back to a plant and it currently does not allow the regeneration of the same genotype as the original sample.

Vials used to store DNA samples at low temperature (photo: Bioversity/ILRI, by kind permission of RDA genebank, National Agrobiodiversity Center, Suwon, Republic of Korea)

Practical considerations

Storage strategy

Determining what to store and for how long is an important consideration, used to determine sample size, capacity of the DNA bank, preparation of samples and documentation. Long-term needs and expected volume and number of samples to be stored will determine organization and repository design. The International Society for Biological and Environmental Repositories (ISBER) has developed a set of best practices for management of biological samples for research that provide useful guidance for DNA banks athttp://www.isber.org/Pubs/BestPractices2008.pdf. Although the specifics vary with type of biological sample, many of their recommended best practices on choice of location, storage design, risk mitigation and documentation follow similar considerations to those for seed genebanks.

Processing of samples

DNA preserved in DNA banks will be stored either within cells and extracted upon retrieval from storage or extracted from cells and purified before storage. The quality of the DNA is expressed through yield, purity, molecular weight, amplification efficiency and authenticity of sequences. The quality of DNA extracted from plant specimens is dependent on the condition of the specimen before storage, the storage environment and the duration of storage. Rapid drying of plant samples with silica gel or lyophilisation helps to preserve the DNA. Plant samples can also be frozen but DNA should be extracted immediately when the samples are retrieved from frozen storage using protocols that inhibit nuclease activity. Careful and clean extraction methods are critical during processing. DNA banks should determine whether the collector or the curator is responsible for extracting DNA for storage as part of the quality control process.

Storage

Once extracted DNA is a stable biomolecule, although it can easily be degraded during extraction and storage. Quality declines within days in hydrated samples held at room temperature or in refrigerators. Drying the sample or storing it in freezers or liquid nitrogen achieves better preservation of DNA molecular size. For this reason, DNA is better conserved in a form that is close to the original state and most DNA banks store cells or tissues and extract DNA upon request.

There is little information on the long-term stability of extracted DNA during frozen storage, but most repositories consider several years to decades as realistic. Information on the stability of purified DNA dissolved in buffer suggests that the overall fragment size decreases with storage time, and that the usefulness of the specimen for PCR-based assays may be 1–2 years when stored at 4 ºC, 4–7 years when stored at -18 ºC and greater than 4 years when stored at -80 ºC (Madisen et al. 1987; Visvikis et al. 1998). The choice of temperature usually depends on the moisture level within the sample.

It is proposed that replicated DNA samples can be maintained at -20ºC for short- and mid-term storage (up to 2 years), and at -70ºC or in liquid nitrogen for longer periods. For rice, DNA clones, such as ESTs, full-length cDNAs, BACs, PACs and YACs, are maintained in labelled 96-well microplates or 384-well microplates stored in -80°C ultra low temperature freezers. These clones are preserved in duplicate or triplicate to reduce risk of loss from equipment failures. For cDNA clones, plasmid DNA is extracted from the host *Escherichia coli*, and stored at -30°C in labelled 1.5 ml Eppendorf tubes.

Standard storage protocols should be developed and used and any risks and changes in environment within the storage period should be documented