A Training Module for the International Course on

Plant Genetic Resources and Genebank Management

Held at the Rural Development Administration, Suwon, Republic of Korea on 7-18 September 2009.

Sponsored by:

Bioversity International

RDA

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Bioversity International is an independent international scientific organization that seeks to improve the well-being of present and future generations of people by enhancing conservation and the deployment of agricultural biodiversity on farms and in forests. It is one of 15 centres supported by the Consultative Group on International Agricultural Research (CGIAR), an association of public and private members who support efforts to mobilize cutting-edge science to reduce hunger and poverty, improve human nutrition and health, and protect the environment. Bioversity has its headquarters in Maccarese, near Rome, Italy, with offices in more than 20 other countries worldwide. The Institute operates through four programmes: Diversity for Livelihoods, Understanding and Managing Biodiversity, Global Partnerships, and Commodities for Livelihoods.

Financial support for Bioversity's research is provided by more than 150 donors, including governments, private foundations and international organizations. For details of donors and research activities please see Bioversity's Annual Reports, which are available in printed form on request from bioversity-publications@cgiar.org or from Bioversity's website (www.bioversityinternational.org).

Rural Development Administration (RDA) of the Republic of Korea is responsible for extensive agricultural research and services in Korea. The missions of the RDA are research and development of agricultural technologies, dissemination of agricultural technologies, quality control of fertilizers, pesticides, and agro-machineries, and guidance and training for rural development and improvement. RDA's efforts are directed towards highly competitive agriculture and efficient rural development. It endeavors to support farmers to produce agricultural commodities with better quality; advancing low-input, labor-saving and environment-friendly cropping technologies; promoting modern and automated production facilities; and nurturing future farmers.

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Photo Credits: *From left to right.* a) Local varieties of rice on display at the CRRA Research Station in Sotuba, Bamako, Mali (P.Bordoni/Bioversity International); b) Long purple aubergines on sale in the Turgetreis Market, Turkey (A.Jarvis/Bioversity International); c) Barley (Bioversity slideshow); d) *Background photo:* Chillies, aubergines and tomatoes on sale in the Turgetreis Market,Turkey (A.Jarvis/Bioversity International).

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Acknowledgments

This module was developed by Bioversity International with support from Republic of Korea's Rural Development Administration (RDA) for the International Training Course on Plant Genetic Resources and Genebank Management held in Suwon, Republic of Korea on 7-18 September 2009. The purpose of the module is to serve as a guide for lecturers and practicum instructors. I also hope that it can serve as an easy reference for the trainees.

The training course is a joint collaboration of the Bioversity International and RDA with the purpose of contributing to capacity development on genebank management in the Asia Pacific and Oceania region. It aims to train genebank curators and genetic resource professionals on an array of good practices and new developments for effective *ex situ* conservation and sustainable utilization of genetic diversity.

The course brings together the best in the field to support the implementation of this module. The corps of trainers is composed of experts from RDA, Bioversity International, International Rice Research Institute (IRRI), Asian Vegetable Research Development Center (AVRDC), Food and Agriculture Organization (FAO) and the University of Tsukuba, Japan. Bioversity and RDA acknowledge the support of these international lecturers and their respective organizations.

We wish to thank Professor Nestor Altoveros and Professor Teresita Borromeo for drafting this module and Ms Katrina Borromeo for the cover design and layout. We also wish to acknowledge our Bioversity and RDA colleagues who contributed in the finalization of this module.

We hope that this module will not only be useful for this training course but also for implementing what our trainees learned afterwards. We encourage our trainees to echo what they have learned to their colleagues.

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Regional Director
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Contents

PART 2. Practicum

Introduction

This training module was prepared for the International Training Course on Plant Genetic Resources and Genebank Management. As the title suggests, the course is focused on technical procedures in genebank management and *ex situ* conservation for plant genetic resources. This module follows the order of the course, which is divided into discussions, lectures (PART 1) and practical hands-on sessions (PART 2).

Course objectives

The course aims to train genebank curators and genetic resource professionals in good practices for effective *ex situ* conservation and sustainable utilization of genetic diversity held in genebanks.

Specific objectives include:

- ✓ To enhance the use of standard protocols for routine genebank operations including how to develop a genebank operation manual;
- ✓ To develop a practical understanding of the utilization of genetic resources and the interface between genebanks and breeders;
- ✓ To equip participants with essential knowledge in documentation and database management;
- ✓ To acquaint the participants with morphological andmolecular techniques to characterize and evaluate plant genetic resources; and
- ✓ To enhance awareness of international and national policy instruments and regimes concerning access to and exchange of genetic resources.

PART 1. Discussion and Lectures

Discussion 1. Policy Instruments

DISCUSSION 1. Policy Instruments and Regimes for Access and Exchange of Genetic Resources

Introduction

Over the last 20 years or so, technological advances particularly in molecular biology and genetic engineering, have given rise to a growing appreciation of the monetary and non-monetary value of genetic resources. This in turn has spurred increasing conflict over rights and responsibilities for these resources.

The current international debate on legal regimes for plant genetic resources has its origins in the late 1970s and early 1980s when developing countries became concerned over actions by the plant breeding industry in industrialized countries to extend intellectual property rights over 'improved' varieties. The concern focused in particular on the inequity of continuing the free flow of germplasm, which was seen as being a flow of resources from developing countries to industrialized countries. During this same period, efforts to collect and conserve plant genetic resources in genebanks heightened and the International Union for the Protection of New Varieties of Plants (UPOV) Convention was amended to admit non-European members. This led to expanded international cooperation in the recognition of plant-related intellectual property rights, which resulted in even greater attention being paid to questions over plant genetic resources ownership in various fora.

In the International Undertaking on Plant Genetic Resources (IU), Member States recognized that "plant genetic resources are a heritage of mankind to be preserved, and to be freely available for use, for the benefit of present and future generations." The IU made clear that this open availability was to apply to all plant genetic resources, including "special genetic stocks."

The Convention on Biological Diversity (CBD) reaffirmed the sovereign rights of countries over their own biological resources and established that States have the authority to determine access to genetic resources under their jurisdiction.

The assertions of sovereignty are most visible in Article 15, *Access to Genetic Resources.* Article 15 ultimately became a balancing act between traditional notions of sovereignty and the desire to ensure that access does not become so cumbersome as to make it effectively impossible. Despite its recognition elsewhere of a common concern for biodiversity loss, the Convention's provisions on access to genetic resources reaffirm national sovereignty over these natural resources and hence national authority to regulate access to genetic resources under a State's jurisdiction.

The International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) was negotiated to address the outstanding issues not resolved by the CBD. The ITPGRFA entered into force on June 29, 2004 after ratification by 40 countries. A Governing Body, composed of all Contracting Parties to the Treaty, will be convened. Until then, the FAO Commission on Genetic Resources for Food and Agriculture will act as the Interim Committee for the Treaty, and will oversee a number of tasks to be undertaken in the interim period.

The salient features of the ITPGR are:

- ✓ It is at the crossroads between agriculture, trade and the environment. It provides agriculture with a new, legally binding instrument that is at par with trade and environmental instruments, and promotes harmony and synergy across the sectors.
- ✓ It covers all plant genetic resources that are relevant to food and agriculture. Its objectives are the conservation and sustainable use of plant genetic resources and the fair and equitable benefits arising out of their use, in harmony with the Convention on Biological Diversity, for sustainable agriculture and food security. It aims at ensuring that the inherited capital they represent is conserved, and continues to supply the flow of services on which food security and development depend.
- ✓ It establishes a Multilateral System of Access and Benefit-sharing for plant genetic resources, for an agreed list of crops, established on the basis of interdependence and food security. The list currently covers 35 food crops, and 29 forage genera, representing more than 80% of the world's calorie intake. The genetic resources of these crops are pooled. The country of origin cannot therefore be the basis of benefit-sharing, which means that the benefits must also be shared on a multilateral, rather than on a bilateral, basis.
- ✓ It provides for benefit-sharing through information exchange, technology transfer, capacity-building, and the mandatory sharing of the monetary and other benefits of the commercialization of products incorporating material accessed from the Multilateral System. The primary focus is on farmers in the developing world, who conserve and sustainably utilize plant genetic resources for food and agriculture.
- ✓ It includes a Funding Strategy to mobilize funding for priority activities, plans and programs, in particular in developing countries and countries with economies in transition, taking into account the Global Plan of Action adopted in Leipzig in 1996.
- ✓ It provides for the realization of Farmers' Rights by national governments through:
    - the protection of relevant traditional knowledge;
    - equitable participation in sharing benefits derived from the use of plant genetic resources for food and agriculture; and
    - participation in national decision-making related to their conservation and sustainable use.

The main instrument of the ITPGRFA for the implementation of its access and benefit sharing provisions is the Standard Material Transfer Agreement (SMTA). In broad terms, the SMTA stipulates the conditions under which a provider of germplasm materials listed in Annex 1 of the ITPGRFA will grant access to a recipient. There are two options for the benefit sharing:

a) 1.1% of the sales of a commercialized product which incorporates material from the Multilateral System, when there are restrictions, such as patents, that result in the product not being freely available to others for research and breeding; and

b) a crop-based payment system, whereby user pays a lower rate, namely 0.5%, on all his commercialized products of a particular crop, regardless of whether material from the MLS is incorporated in those products, and regardless of whether or not they are freely available to others for research and breeding through the exercise of IPRs.

Objectives

1. Discuss the history of the development of policy instruments of access and benefit sharing of plant genetic resources for food and agriculture; and
2. Discuss the salient provisions of the ITPGRFA and the SMTA.

Description of lessons covered

The lecture will discuss the development of the present regimes for access and benefit sharing of PGRFA and the status of ITPGRFA implementation (2004-2009).

Lessons to learn

- ✓ The facilitated access and benefit sharing of PGRFA listed in Annex 1 can only be realized if Contracting Parties nominate their Annex 1 germplasm materials in the MLS.
- ✓ Contacting Parties should establish the legal mechanism for the implementation of the ITPGRFA.

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Lecture 1. Processing & Storage

LECTURE 1. Seed Processing and Storage

Introduction

Seeds put in the genebank are expected to be of highest quality. The length of storage that allows maintenance of high viability depends on the initial seed viability. Seed processing is necessary to ensure that seeds to be placed in storage should be of maximum quality (high initial viability) and quantity.

All factors that affect seed quality between collecting, harvesting and storage should be considered. The time between harvest and storage should be short to reduce the loss of viability and pest infestation.

Seed processing includes steps necessary to ensure that seeds are brought from the field to the storage room with minimal loss of viability and that seed purity and seed health are maintained. Seed processing is done after collecting, upon arrival in the genebank and after regeneration of germplasm materials. This involves seed cleaning, seed treatment if necessary and packaging.

Objectives

1. Describe the activities in seed processing after collecting, upon arrival in the genebank and after regeneration of germplasm materials;
2. Describe the activities to ensure seed purity, viability and health of each accession during processing; and
3. Discuss the storage conditions appropriate for long-, medium- and short-term storage of seeds.

Description of lessons covered

This lecture will discuss the best practices in seed processing and storage of germplasm for conservation. Specifically the topics will include seed cleaning, drying, viability testing and packaging; importance of reducing seed moisture content; the types of seeds and storage conditions; and factors affecting storage potential of seeds.

Lessons to learn

- ✓ Seed processing involves cleaning and drying.
- ✓ Moisture content determination and viability testing are necessary to assess the quality of seeds to be stored and insure optimum storability of seeds.
- ✓ Packaging and storage of seeds are necessary to maintain quality of seeds for long periods.

Seed Cleaning

Seed cleaning is the removal of debris, inert material, damaged and infested or infected seeds and seed of different species (e.g., weeds) to achieve clean and pure samples of seeds of high physiological quality for storage.

Seed cleaning is necessary to:

- ✓ Reduce bulk during transportation;
- ✓ Improve purity of the sample;
- ✓ Optimize storage space and reduce costs;
- ✓ Prevent seed from going moldy and help reduce 'damping-off' or fungal contamination after germination; and
- ✓ Allow precise regulation of seed moisture content during storage.

Seeds should be cleaned immediately after harvest or soon after they arrive at the genebank. Cleaning methods vary according to the type of seed.

Seed Drying

Seed drying is the reduction of seed moisture content to the recommended levels (which should not be lower than the critical seed moisture content) for storage using techniques which are not detrimental to seed viability. Dry seeds retain viability for longer periods during storage.

Seeds are hygroscopic and absorb or desorb moisture depending on the relative humidity of the surrounding air or the gradient in water potential between the seed and surrounding air. If water vapor pressure of the seed is greater than the surrounding air, the seed will lose moisture and becomes drier (desorption). If the water vapor pressure of the seed is lower than the surrounding air, the seed will gain moisture (absorption). Absorption or desorption occurs until the water vapor pressure in the seed and the surrounding air is balanced.

The water content of seeds at equilibrium with the RH of surrounding air is referred to as equilibrium moisture content. Understanding the relation between equilibrium seed moisture content and relative humidity is important for the genebank technician to decide on the drying regime for seeds.

Several methods are available for drying seeds. Methods that minimize loss of viability during drying should be used. The most common and safe methods used for drying are dehumidified drying and silica gel drying.

Drying rate depends on seed size, shape, structure, composition, initial seed moisture content, amount of seeds and layers, air movement, temperature and relative humidity.

Seed Packaging

Seed packaging is the placing of a counted or weighed sample of seeds of an accession into a container which is then hermetically sealed for subsequent storage.

Seeds are packaged to:

- ✓ Prevent absorption of water from the atmosphere after drying;
- ✓ Keep each accession separate and avoid the mixing of accessions; and
- ✓ Prevent contamination of the seeds from insects and diseases.

The best time to package seeds is immediately after the moisture content has been determined and found to be within the required limits for safe storage. Dry seeds will reabsorb moisture from ambient air. Therefore, seeds should be packaged into containers and hermetically sealed without delay, soon after removal from the drying room or cabinet.

Different types of containers are available for packaging. The choice depends on storage conditions and species. The most important thing is that the packing material should be completely impermeable to water and suitable for long-term use. Some frequently used containers in genebanks are: glass bottles, aluminum cans, aluminum foil packets, and plastic bottles.

These different types of containers all have advantages and disadvantages. Glass bottles are good but fragile and can easily break. Aluminum cans are difficult to reseal once they are opened. Aluminum foil can be resealed and occupy less space in storage room. However seeds with sharp projection can pierce the packets and moisture can leak inside. Plastic bottles are moisture resistant but not moisture proof. They should be used with caution if relative humidity of the storage room is not controlled.

Packaging is best carried out in an air conditioned room where the relative humidity is controlled. It is important to ensure that seeds taken from the drying room are exposed to the ambient air for the shortest possible time so that they do not re-absorb water.

Seed Storage

Seed storage is the preservation of seeds under controlled environmental conditions (at low temperature and relative humidity) that maintain viability of the seeds for long periods.

Seeds are stored using appropriate methods and in the right environment to maintain their viability over time. Researches have demonstrated that seeds stored in ambient temperature and relative humidity lose viability faster.

Seeds need to be stored immediately after harvest. They need to be cleaned, dried to right moisture content, kept in the right container and put in cold storage rooms.

Seeds can be classified into the following types according to seed storage behavior:

1. **Orthodox** – seeds that can withstand low seed moisture content and can be stored at low temperature without losing viability.
2. **Recalcitrant** – seeds that lose viability if moisture content is less than 12-13%.
3. **Intermediate** – seeds that exhibit storage characteristics between orthodox and recalcitrant seeds.

For orthodox seeds, the storage potential is influenced by inherent and external factors, for example, some legumes that are hard seeded are long-lived while seeds with high oil content are short-lived. There is also observed variation at the ecogeographic races (e.g. indica vs japonica rice) and cultivar levels.

Seeds harvested at their physiological maturity generally store better. The environment (temperature, moisture, nutrition, light) under which the crop is planted can also affect storability.

The longevity of seeds depends on the initial seed quality, moisture content and temperature during storage. In general, low moisture content and low temperature reduce the loss of seed viability.

Harrington's rule of thumb can be applied as rough estimate of length of storage in reference to temperature and seed mc. The rule states that beginning at 14% mc, for every 1% decrease, the lifespan of the seeds doubles and beginning at 50^{o}C, for every 5^{o}C decrease in storage temperature, the lifespan of the seeds doubles.

Types of storage

Two types of seed stores are used for conservation of genetic resources: those holding seed samples for long-term and future use (referred as Base collections) and those holding seed samples for immediate use (referred as Active collections). The temperature, relative humidity (RH), seed moisture content, containers and distribution arrangements vary between these stores.

1. Base collections

A base collection is defined as a set of accessions which should be distinct and in terms of genetic integrity, as close as possible to the sample provided originally, and preserved for future use. Normally, seeds will not be distributed from base collections directly to users but only used to regenerate active collections (FAO/IPGRI, 1994). Base collections are held under long-term storage conditions at below 0°C and usually at -20°C.

2. Active collections
Active collections consist of accessions which are immediately available for multiplication and distribution for use. Because these are accessed frequently, they are maintained under medium-term conditions, which ensure that the viability of these accessions remains above at least 65% for 10–20 years FAO/ IPGRI 1994).

It is recommended that the seeds to be kept under long-term storage should be stored in a room with low temperature (-10 to -18^{o}C) and low RH (40%). Seeds in the medium-term storage should be stored at 0-5^{o}C and 60% RH, medium-term. Under these conditions it is predicted that seeds will be viable for 10-25 years. Seeds kept at short-term storage (1-10 years), are stored in cold rooms with 15^{o}C temperature.. An ordinary air conditioned room may provide such conditions. Cold rooms must be sealed especially in tropical conditions. If there is no cold storage room, ordinary refrigerators or freezers and rooms with air conditioning units may be used.

Safety duplication

Safety duplication means a genetically identical duplicate accession sample is stored at another location (preferably outside the country) in a base collection for safety reasons. Safety duplication provides insurance against loss of material. Safety duplication also includes both the duplication of the material and the documentation process. Specific agreements should be entered with the recipient institute for holding the duplication of the collection.

Several types of duplication exist:

1. **Black box** – when the sole responsibility of the recipient's institute is to maintain the duplicates in adequate storage facilities without handling the samples. It is the originator's responsibility to monitor seed viability and when necessary regenerate the collection. For black box duplication outside the country, special permissions are required to export seeds without Phytosanitary Certificates from the originating country. Similarly, Plant Quarantine Authority in destination country needs to permit the importation of seeds by the recipient bypassing the routine quarantine examination.
2. **Active** – when the duplicate collection is incorporated into the recipient's collection thus being subject to regeneration, multiplication and distribution by the recipient.
3. **Base** – maintained under suitable condition for long term seed storage and is incorporated into the recipient's collection.

Number of seeds to be stored

The number of seeds to be stored will depend on the species being conserved. FAO/IPGRI Genebank Standards (1994) recommends that for material which shows little morphological variation (genetically homogeneous) 3000 seeds are acceptable, but 4000 seeds are preferable to represent each accession.

For materials showing large amount of morphological variation (heterogeneous) an accession should consist of at least 4000 seeds, but 12000 seeds are preferred.

It is easier in a genebank to work in weights. However, seeds numbers can easily be converted from weights. An inter-conversion of numbers and weights is possible, if we know the 100-seed or 1000-seed weight.

For example:

Equation 1.

$$\text{Number of seeds in the sample} = \frac{\text{Sample weight (g)} \times 100}{\text{Weight of 100 seeds (g)}}$$

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Lecture 2. Germplasm Regeneration

LECTURE 2. Germplasm Regeneration

Introduction

Seeds in storage will lose viability or their quantity will become depleted over time. It is therefore imperative that seeds in storage will be replenished to insure the continued conservation of the germplasm materials. The decision whether or not to regenerate conserved accessions in storage is made when the seed quality or the seed quantity, or both, fall below a certain critical level.

The regeneration involves the activities from seed preparation to processing of materials for conservation. The regeneration program should aim to maximize seed production to minimize regeneration cycles, maintain the genetic integrity of the population and maximize seed quality.

When regenerating, the number of individuals that make up the regeneration population, breeding system of species being regenerated, specific environmental requirements when practical (e.g. temperature, moisture, photoperiod), and the number of accessions that can be effectively managed during regeneration should be considered.

Objectives

1. To describe the decision criteria for regeneration;
2. To discuss the considerations (biological, environmental and institutional) in regeneration;
3. To discuss the procedures to maintain population structure and accession purity; and
4. To describe the procedures in regeneration.

Description of lessons covered

The lecture will discuss the best practices in maintenance of genetic integrity, maintenance of population structure, cultural management practices, and harvesting.

Lessons to learn

✓ Germplasm regeneration is the most critical operation in genebank management, because it involves risks to genetic integrity of germplasm accessions due to selection pressures, out-crossing and mechanical mixtures, among other factors.

Why is Regeneration Necessary?

Germplasm is regenerated for the following purposes:

1. Initial seed increase or multiplication

In case of new collections or materials received as donations, the quantity of seeds received by the genebank is often insufficient for direct conservation. It is also possible that the seeds are of poor quality due to low viability or infections. All such materials need multiplication for the first time.

2. Long-term conservation

Seed accessions that are not yet in the base collection and needed for long-term conservation should be regenerated first to produce good.

3. Replenish seed stocks in active and base collections

Seed increase should be done for accessions that have:

- ✓ Low viability identified during periodical monitoring (regeneration), and insufficient stocks for either distribution or conservation (multiplication).
- ✓ The FAO/IPGRI Genebank Standards (1994) recommends that the initial germination value should exceed 85% for most seeds and regeneration should be undertaken when viability falls below 85% of the initial value. Further, regeneration should be undertaken when the number of seeds in base collection falls below the number required for at least three cycles of regeneration.
- ✓ Active collections should preferably be regenerated from original seeds in base collection. This is particularly important for outbreeding species. However, using seeds from active collection for up to three regeneration cycles before returning to original seeds (base collection) is also acceptable (FAO/IPGRI 1994).
- ✓ Base collections should normally be regenerated using the residual seed in that same sample.

4. To meet special requirement

Special requirement for seed multiplication may arise for accessions that are often requested or with special traits that breeders and researchers frequently use (high yielding, pest and disease resistant accessions, genetic stocks, etc.) or accessions requiring safety duplication and repatriation.

The following factors should be considered when regenerating germplasm accessions:

- ✓ Suitability of environment to minimize natural selection;
- ✓ Special requirements to break dormancy and stimulate germination (e.g., scarification);
- ✓ Correct spacing for optimum seed set;
- ✓ Effective population size;
- ✓ Appropriate cultural management practices; and
- ✓ Breeding system of the plant and need for controlled pollination.

Procedures for regeneration

Regeneration involves the following management practices:

1. Selection of accessions;
2. Preparation of regeneration plots;
3. Preparation of seeds;
4. Sowing and crop management;
5. Verifying accession identity;
6. Determining and using appropriate pollination control for outbreeders;
7. Harvesting and postharvest management;
8. Seed drying and processing;
9. Seed health testing;
10. Initial viability testing; and
11. Packaging and storage.

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Lecture 3. Characterization & Evaluation

LECTURE 3. Characterization and Evaluation

Introduction

Characterization is the recording of characters or traits which are highly heritable or can be easily seen and are expressed in all environments. It makes possible the easy and quick discrimination between accessions or phenotypes. Characterization is normally the responsibility of genebank curators.

Evaluation is usually done for traits such as yield, agronomic performance, biotic and abiotic stress susceptibilities and biochemical and cytological traits. The expression of these traits is usually influenced by the environment and therefore may require special experimental designs and techniques. Evaluation is usually done by a multidisciplinary team of scientists which usually includes a breeder and specialists (e.g. entomologist for insect resistance, physiologist for stress tolerance, pathologist for disease resistance). In practice, characterization and preliminary evaluation are usually done during the initial seed increase or the first regeneration cycle.

Characterization and evaluation start with the adoption of a descriptor list. A descriptor list is a collation of all individual descriptors used for a particular species. Many species have a standardized, agreed descriptor list. A descriptor is an identifiable and measurable trait or characteristic of a plant accession (e.g. height; color) used to make classification, storage, retrieval and use, more uniform. Each descriptor has variants of expression known as descriptor states.

Several important species have a standardized descriptor list published by the former International Board for Plant Genetic Resources (IBPGR) and International Plant Genetic Resources Institute (IPGRI), now Bioversity International.

Good characterization and evaluation data in a well organized documentation system leads to enhanced utilization of germplasm.

Objectives

1. Describe the methods of characterization and evaluation of germplasm; and
2. Discuss the importance of germplasm characterization and evaluation.

Description of lessons covered

This lecture will discuss the best practices in characterization and evaluation. Specifically the topics will include basic information on the different approaches used in PGR characterization with focus on morphological and molecular techniques.

Lessons to learn

- ✓ Complete characterization and evaluation of accessions in the collection ensures the effective utilization of the germplasm collection.
- ✓ Knowledge of the taxonomy, morphology and genetics of the crop species is essential for characterization and evaluation.
- ✓ Characterization and evaluation are necessary to assess the extent of diversity of the germplasm collection and determine whether there are gaps in the collection.
- ✓ If resources permit, morphological and molecular characterization should be done to obtain a total picture of the diversity to the found in the germplasm collection.

Methods of characterization and evaluation

The methods on the description of the germplasm include morphological (morphometric), cytological, cytogenetic, biochemical and molecular approaches. Morphological traits are evaluated based on the descriptor list. This can be supplemented by studying plants on the cellular level using cytology and cytogenetics. These are useful in the establishment of the chromosome number and genome composition of the genetic material. Another approach is the direct study of the genome using biochemical and molecular markers.

Characterization and evaluation using morphological markers

Morphological characterization is the description of an accession based on morphological markers taken at the various stages of growth (seedling, vegetative, inflorescence, fruit and seed). Descriptors used in characterization of germplasm accessions may include morphological/botanical features, which maybe mono/oligogenic, highly heritable, and expressed within acceptable limits of deviation over a range of agroclimatic conditions (e.g. leaf shape, flower color, seedcoat color).

Descriptors used in evaluation are generally useful to crop improvement. They are highly affected by the environment, involve complex biochemical or molecular processes, and may include yield, agronomic performance, stress reactions, etc.

Characterization using molecular markers

Molecular characterization is the description of an accession using molecular markers. Molecular markers are readily detectable sequence of DNA or proteins whose inheritance can be monitored. There are several methods that can be employed in molecular characterization, which differ from each other in terms of ease of analysis, reproducibility, level of polymorphism, number and genome distribution of loci. The most commonly used techniques and their advantages and disadvantages are presented below.

DNA-Based Molecular Markers

1. Restriction Fragment Length Polymorphism (RFLP)

A **restriction fragment length polymorphism**, or RFLP, is a variation in the DNA sequence of a genome that can be detected by cutting the DNA into pieces with restriction enzymes and analyzing the size of the resulting fragments by gel electrophoresis. RFLPs are detected by fragmenting a sample of DNA using a restriction enzyme, which can recognize and cut DNA wherever a specific short sequence occurs. The resulting DNA fragments are then separated by length through gel electrophoresis, and transferred to a membrane using the Southern blot. Hybridization of the membrane to a labeled DNA probe then determines the length of the fragments which are complementary to the probe. Fragment lengths vary depending on the location of the restriction sites along the DNA. Each fragment length (band) can be used in the characterization of genetic diversity

RFLPs are generally found to be moderately polymorphic. In addition to their high genomic abundance and their random distribution, RFLPs have the advantages of showing co-dominant alleles and having high reproducibility.

There are several disadvantages. The methodological procedures for RFLPs are laborious and technically demanding, and highly expensive. In general, if research is conducted with poorly studied crops or wild species, suitable probes may not yet be available, so considerable investments are needed for development. The procedure also requires large quantities of purified, high molecular weight DNA for each digestion. Larger quantities are needed for species with larger genomes, and for the greater number of times needed to probe each blot. RFLPs are not amenable to automation and collaboration among research teams requires distribution of probes.

2. Random Amplified Polymorphic DNA (RAPD)

The method termed **random amplification of polymorphic DNA (RAPD)** uses a polymerase chain reaction machine (PCR) to produce many copies (amplify) random segments of DNA, called **random amplified polymorphic DNA (also RAPD)**. In the method, several arbitrary, short primers (8-12 nucleotides) are created and applied in the PCR using a large template of genomic DNA, hoping that fragments will amplify.

By resolving the resulting patterns in agarose gel and ethidium bromide staining, a semi-unique profile can be gleaned from a RAPD reaction.

No knowledge of the DNA sequence for the targeted gene is required, as the primers will bind somewhere in the sequence, but it is not certain exactly where. This makes the method popular for comparing the DNA of biological systems that have not had the attention of the scientific community, or in a system in which relatively few DNA sequences are compared. Because it relies on a large, intact DNA template sequence, it has some limitations in the use of degraded DNA samples. Its resolving power is much lower than targeted, species specific DNA comparison methods, such as short tandem repeats.

Unlike traditional PCR analysis, RAPD does not require any specific knowledge of the DNA sequence of the target organism: the identical 10-mer primers will or will not amplify a segment of DNA, depending on positions that are complementary to the primers' sequence. For example, no fragment is produced if primers annealed too far apart or 3' ends of the primers are not facing each other. Therefore, if a mutation has occurred in the template DNA at the site that was previously complementary to the primer, a PCR product will not be produced, resulting in a different pattern of amplified DNA segments on the gel.

Selecting the right sequence for the primer is very important because different sequences will produce different band patterns and possibly allow for a more specific recognition of individual strains.

The main advantage of RAPDs is that they are quick and easy to assay. Because PCR is involved, only low quantities of template DNA are required, usually 5-50 ng per reaction. Since random primers are commercially available, no sequence data for primer construction are needed. Moreover, RAPDs have a very high genomic abundance and are randomly distributed throughout the genome.

RAPDs have several limitations. Nearly all RAPD markers are dominant, i.e. it is not possible to distinguish whether a DNA segment is amplified from a locus that is heterozygous (1 copy) or homozygous (2 copies). Co-dominant RAPD markers, observed as different-sized DNA segments amplified from the same locus, are detected only rarely.

Additionally, since PCR is an enzymatic reaction, the quality and concentration of template DNA, concentrations of PCR components, and the PCR cycling conditions may greatly influence the outcome. Thus, the RAPD technique is notoriously laboratory dependent and needs carefully developed laboratory protocols to be reproducible.

Finally, mismatches between the primer and the template may result in the total absence of PCR product as well as in a merely decreased amount of the product. Thus, the RAPD results can be difficult to interpret.

RAPDs is used to characterize, and trace, the phylogeny of diverse plant and animal species. RAPDs permit identification of most cultivars, since the probability of obtaining the same pattern between two different cultivars is very low ($3.9x1^{-5}$). RAPDs have also been applied in gene mapping studies to fill gaps not covered by other markers.

3. Amplified Fragment Length Polymorphism (AFLP)

Amplified Fragment Length Polymorphisms (AFLPs) are DNA fragments obtained by using restriction enzymes to cut genomic DNA, followed by ligation of adaptors to the sticky ends of the restriction fragments. A subset of the restriction fragments are then amplified using primers complementary to the adaptor and part of the restriction site fragments. The amplified fragments are visualized on denaturing polyacrylamide gels either through autoradiography or fluorescence methodologies.

There are many advantages to AFLP when compared to other marker technologies. AFLP-PCR is a highly sensitive method for detecting polymorphisms in DNA. AFLP has higher reproducibility, resolution, and sensitivity at the whole genome level compared to other techniques. It also has the capability to amplify between 50 and 100 fragments at one time. In addition, no prior sequence information is needed for amplification.

AFLP is widely used for the identification of genetic variation in strains or closely related species of plants. The AFLP technology has been used in population genetics to determine slight differences within populations, and in linkage studies to generate maps for quantitative trait locus (QTL) analysis. AFLPs can be applied in studies involving genetic identity, parentage and identification of clones and cultivars, and phylogenetic studies of closely related species.

The disadvantages of AFLP include the need for purified, high molecular weight DNA, the dominance of alleles, and the possible non-homology of co-migrating fragments belonging to different loci.

4. Simple Sequence Repeats (SSRs)

Simple Sequence Repeats (SSRs), or **micro-satellites**, are polymorphic loci present in nuclear and organellar DNA that consist of repeating units of 1-6 base pairs in length. They are multi-allelic and co-dominant. SSRs are used in population studies, genetic diversity analysis and to look for duplications or deletions of a particular genetic region.

Micro-satellites can be amplified through PCR, using the unique sequences of flanking regions as primers. Micro-satellites developed for particular species can often be applied to closely related species, but the percentage of loci that successfully amplify may decrease with increasing genetic distance. Point mutation in the primer annealing sites in such species may lead to the occurrence of ‘null alleles’, where micro-satellites fail to amplify in PCR assays.

Importance of characterization and evaluation

Information derived from characterization and evaluation of germplasm collections can be used to:

- ✓ identify information for an accession;
- ✓ monitor identity of an accession over a number of regenerations;
- ✓ locate specific traits;
- ✓ assess genetic diversity of the collection;
- ✓ fingerprint genotypes;
- ✓ Identify duplicates;
- ✓ determine gaps in the collection;
- ✓ facilitate preliminary selection of germplasm by end-users;
- ✓ study genetic diversity and taxonomic relationships ; and
- ✓ develop core collection (a limited set of accessions of a crop species and its wild relatives which would represent with a minimum repetitiveness the genetic diversity of a crop species and its wild relatives).

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Lecture 4. Documentation & Information Management

LECTURE 4. Documentation and Information Management

Introduction

Various inter-related activities generate a large amount of data throughout the course of PGR conservation and management. The usefulness of the data generated can only be fully realized if they are transformed into meaningful information, for the benefit of both the conservationists and the users. The information can be used to make critical decisions on what germplasm needs to be collected, how much material is in store, when to regenerate accessions in the collection, which of the collection may have potential for utilization.

Correct information can be retrieved if a reliable data storage and information retrieval system, or more technically, a documentation system, is available. A documentation system must therefore be able to reliably store, retrieve and process data, and generate information from across all activities, in support of the conservation, management and utilization of PGR.

The usefulness of a germplasm database system can be influenced by the following factors:

- ✓ **information quality -** refers to accuracy, precision, completeness, timeliness and source.
- ✓ **information accessibility** - refers to the relative ease in obtaining and manipulating information.
- ✓ **information presentation** - pertains to the capability of the system to format and summarize information the way users want it.

To facilitate data gathering and allow accurate observation, the data collector must have knowledge of the descriptors being scored. Bioversity International issues a compendium of descriptive information and traits that characterize a particular accession of a certain plant group called a descriptors list. It provides a comprehensive list of characters and aims to standardize plant characterization by setting an international format. Included in a descriptors list are passport data, characterization and evaluation.

A descriptors list is a product of conscientious deliberation of a team of experts for the plant group considering the known variations present. It has a selection of expressions called states that define the descriptor and further describe the particular trait. The descriptor states cover whole range of variation for a certain trait.

The use of standardized universal descriptor lists and states simplifies description of accessions and minimizes misinterpretations. Since descriptors can be considered as recommendations, the curators can add or delete certain descriptors or states in the list to suit their needs.

In cases where there are no published descriptors, the researchers can formulate their own, following the standard format and using the published Bioversity descriptors as model.

In PGR documentation, volumes of data sets derived across the different component activities are generated. In most instances, users of the documentation system looking for information are normally interested only in a fraction and very specific information out of the entire documentation system. Therefore, the PGR documentation system must be equipped with an effective search function.

The search function should execute according to the specification supplied by the user, i.e. which tables should be searched, and how the search output should be organized. The nature of data and information that each user requires depends largely on the specific interest of the user. The search capability of the system must therefore respond to every requirement.

The report function is the output component of the documentation system or the end-result of the search execution. It can be regarded as the visualization of the search function. With every execution of the search function, the system generates the results containing a subset of the database. It is then subsequently visualized either on-screen or printed directly as a hardcopy or softcopy as a system file.

The display appearance of the report could vary greatly from user to user because information requirements are different from each other. As the interface between the user and information report, the display should be likewise visually comfortable to the user. This user-friendly feature should extend to data manipulation needs such as statistical and geographic information system (GIS) analyses; during file transfer from the database to another application; and for sharing information through the Internet.

The usefulness of germplasm databases is magnified in direct proportion to the number of users who can access it. Putting germplasm databases on the web can increase access to them many times over. There are many comprehensive germplasm databases that can be accessed via the internet. Some outstanding examples are the databases of the United States Department of Agriculture (USDA) (www.ars-grin.gov), the Center for Genetic Resources The Netherlands (CGN) (www.cgn.wur.nl), the Nordic Genebank (www.ordgen.org), the Asian Vegetable Research and Development Center (AVRDC) http://203.64.245.173/avgris), the Ministry of Agriculture, Forestry and Fisheries of Japan (www.gene.affrc.go.jp) and the CGIAR centers which can be accessed from their own websites or through the System-Wide Genetic Resources Program (SGRP) of the CGIAR.

Objectives

1. Discuss the importance of documentation systems in the management and use of plant genetic resources in genebanks; and
2. Discuss the aspects of a documentation system.

Description of topics to be covered

The lecture will discuss the importance of a germplasm documentation system. It will also outline steps that should be taken prior to the database system development and discuss the important elements of the database system design. Also it will also discuss the desirable features of a documentation system.

Lessons to learn

- ✓ Documentation system will facilitate the effective and efficient use of germplasm materials in the genebank.
- ✓ An operational documentation system will help genebank managers to identify, acquire, classify, store, manage, and disseminate information on germplasm materials in genebanks.
- ✓ An organized documentation system will facilitate access and exchanges of germplasm materials among genebanks.

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Centro Internacional de Agricultura Tropical (CIAT), Universidad Nacional de Colombia-Sede Palmira and Bioversity International, Red de Instituciones Vinculadas a la Capacitacion en Economia y Politicas Agricolas en America Latina y el Caribe (REDCAPA) and Centre technique de cooperation agricole et rurale (CTA). 2007. Multi-institutional distance learning course on the *ex situ* conservation of plant genetic resources. Cali, Colombia.

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LECTURE 5. Germplasm Exploration and Collection

Introduction

The assembly of plant genetic resources entails systematic exploration and collecting of germplasm materials. Field collecting, requests and exchanges from institutions or genebanks, or donations from individuals or institutions can accomplish this. Germplasm materials can be acquired in the form of whole plants, seeds, stocks or scions or tissue cultures.

Germplasm may be acquired for conservation, research and study, crop improvement, direct utilization, distribution, and gap filling. The aim of germplasm acquisition is to collect materials with maximum amount of useful genetic variability within a strictly limited number in the least amount of time. Priorities in collecting in terms of species, populations and geographic location among others should be established to insure maximum use of time and resources.

Objectives

1. Describe the methods of characterization and evaluation of germplasm; and
2. Discuss the importance of germplasm characterization and evaluation.

Description of lessons covered

The lecture will discuss the setting of priorities, planning for collecting and the methodologies employed in collection.

Lessons to learn

- ✓ Exploration and collecting requires methodical planning to optimize the use of time and resources.
- ✓ Collecting requires knowledge of the crop, the location and the proper timing for collection.
- ✓ Collecting requires knowledge of the ethical and cultural concerns, legal requirements, cooperation with local authorities and quarantine requirements. It will be useful for the collecting team to be familiar with the International Code of Conduct for Plant Germplasm Collecting and Transfer.

Criteria for Acquisition of Germplasm

Genebanks may consider the following criteria in setting priorities for germplasm acquisition.

The species' state of conservation - based on sufficiency of its representation in collections so that conservation activities do not duplicate already existing ones.

Urgency for conservation - depends on threat to the species or population within a crop species. For wild related species, the *IUCN Red List of Threatened Species* maybe consulted.

Contributions in terms of genetic variability - selected species and populations may provide additional diversity.

Potential usefulness of the species - species that contribute to the satisfaction of basic needs (e.g., food, medicines, and housing).

Relative cost of conservation - depends on the capacity of the conservation unit.

Cultural importance to the community - contribution to the aesthetic, symbolic, or cultural value of a species for a community (i.e., the role that it fulfills in cultural or religious activities may determine whether it should be conserved.)

Phases in Germplasm Collection

1. Planning
   - ✓Technical
   - ✓Logistical
2. Field work
3. Processing and Documentation

Phase 1. Planning

Technical planning

Technical planning addresses the scientific issues involved in launching a collecting mission. In particular, it addresses the following questions:

- ✓ What should be collected and in what form?
- ✓ Where it should be collected?
- ✓ When it should be collected?
- ✓ How it should be collected?

Planning for the collecting mission should start well in advance. The collecting team must acquire all available information such as:

- ✓ environmental heterogeneity;
- ✓ history distribution, and phenology of crop;
- ✓ reproductive biology, seed storage behavior;
- ✓ cultural diversity;
- ✓ history of movement of people; and
- ✓ political climate of the country or region to be explored.

Logistical Planning

Logistical requirements of a collecting mission:

- ✓ Composition of the team;
- ✓ Collecting permits (local and national);
- ✓ Itinerary;
- ✓ Transport required; and
- ✓ Equipment and supplies.

Phase 2. Field Work

- ✓ Field sampling (distribution of sites, number of sites, delineation of sites, distribution of plants sampled in site, number of plants sampled);
- ✓ Population identification; and
- ✓ Material for collection (passport data, voucher specimens, germplasm, live plants).

Sampling strategy

The general principle in sampling is to collect a bulk sample from the site by randomly harvesting panicles or fruits of a number of plants from several spots in the site. The frequency of sampling (number of samples per site) and the size of each sample would be governed much by the extent of genetic diversity and geneflow within a taxon and the agro-ecology of the site. A practical approach and on-the-spot observations to devise the best sampling technique should be used.

Sampling frequency
Sampling intervals can vary depending on ecological/edaphic variation in environment or site. The intervals can be large when the environment is uniform and proportionately small when the collecting sites include much faster changes in topography/altitude, soil types, farming practices or other features as observed in an agro-ecologically diverse environment. The collector should use his own judgment especially when the collecting trip covers a large area and is planned for a relatively longer period or when erratically distributed crops like fruit tree species, and root and tubers are collected.

Sampling methods
Sampling methods must ensure the collection of representative within-population variation as well as that associated with geographical patterns of variation. The most appropriate sampling method should be used.

Sample size
Sufficient quantity of seeds should be collected, keeping in mind the seed requirement for base and active collections as well as for duplicate conservation.

Phase 3. Processing and Documentation

Collected materials should be processed on site and immediately upon arrival to the genebank. Passport data and associated information should be collated and documented.

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Lecture 6. Use of Molecular Markers

LECTURE 6. Use of Molecular Markers for Diversity Analysis

Introduction

Molecular data, usually in the form of qualitative or discrete data such as presence or absence of alleles, markers or bands, or the number of alleles in a given primer, are used primarily to study the genetic relationships between individuals or populations, and to calculate population genetics parameters, specifically diversity at different levels. They are also used to determine quantitative traits loci and to map the genes of individuals.

The analysis of genetic relationships among samples is done by constructing a matrix specifying the character state of each marker for each sample, which is then used to construct a sample by sample matrix of pair-wise genetic similarities. The genetic distance between two samples based on the differences between them can be calculated using Nei's genetic distance.

The resulting distance matrix can be analyzed and displayed by using the Principal Coordinate Analysis (PCoA) to produce a 2- or 3-dimensional scatterplot of the samples where the geometrical distances among the samples in the plot reflect the genetic distances among them; or by using Cluster Analysis to produce a dendrogram which links together in clusters those samples that are more genetically similar to each other compared to the other samples. The clustering methods most frequently employed are the Unweighted Pair Group Method with Arithmetic Averages (UPGMA) and Ward's Method.

Genetic diversity can be measured using Wright's F-statistics and Nei's G-statistics. In both cases, estimate of genetic diversity is based on allele frequencies, making them useful in analyzing RFLP and SSR data.

There are several software that can be used to analyze molecular data. Examples are NTSYS pc (Numerical Taxonomy and Multivariate Analysis System), Statistical Analysis Software (SAS) and GENEPOP.

Objectives

1. Describe the methods of analyzing molecular characterization data for diversity analysis; and
2. Discuss the interpretation of molecular data for genetic relationships and diversity analysis.

Description of lessons covered

This lecture will discuss the different measures of genetic diversity using molecular data.

Lessons to learn

- ✓ Knowledge of the extent of genetic diversity of the collection is essential in the germplasm management. It is particularly important in filling the gaps in collection and in the identification of duplicates and establishing the core collection.

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LECTURE 7. Utilization of Plant Genetic Resources in Pre-breeding/Genetic Enhancement

Introduction

The low utilization of conserved plant genetic resources in most genebanks is due to lack of documentation and inadequate description of collections, lack of the information desired by breeders, and lack of evaluation of collections. In order to fully utilize available genetic diversity in genebanks, pre-breeding or genetic enhancement of exotic/unadapted materials should be undertaken.

Pre-breeding refers to all activities designed to identify desirable characteristics and/or genes from unadapted (exotic or semi-exotic) materials, including those that, although adapted have been subjected to any kind of selection for improvement. Exotic materials include any germplasm that do not have immediate usefulness without selection for adaptation for a given area.

Pre-breeding is a vital step to link conservation and use of plant genetic resources especially in breeding programs. It aims to reduce genetic uniformity in crops through the introduction of a wider base of diversity, as well as to increase yields, resistance to pests and diseases, and other quality traits.

Pre-breeding programs can generate new base populations for breeding programs and also assist in identifying heterotic patterns for hybrid programs. Pre-breeding aims to provide breeders with enhanced germplasm materials which have specific traits of interest as well as a means to broaden the diversity of improved germplasm.

Objectives

1. Discuss the value of pre-breeding or genetic enhancement in the utilization of conserved germplasm in genebanks; and
2. Discuss methods of genetic enhancement/pre-breeding.

Lessons to learn

- ✓ Characterization and evaluation are key to identification of useful genes; and
- ✓ Knowledge of the genepool concept is important in pre-breeding/ genetic enhancement in the germplasm collection.

Methods of pre-breeding/genetic enhancement

- ✓ Synthesis of new base populations; and
- ✓ Introgression.

Considerations in pre-breeding/genetic enhancement

- ✓ Reproductive system of crop;
- ✓ Economic importance of crop, users' interest in the trait concerned;
- ✓ Genetic distance (breeding line, landrace, wild) of material with variant of interest from modern breeding lines;
- ✓ Ease of phenotyping ;
- ✓ Genetic control of trait;
- ✓ Generation time;
- ✓ Effective population size; and
- ✓ NARS capacity.

Genepool Concept - classification of crop species based on genetic relationships.

Plants are divided into different genepools as follows:

Primary Genepool: GP1

- ✓ Primary breeding material;
- ✓ Gene transfer is usually simple; and
- ✓ Hybridization is easy and hybrids are fertile.

Secondary Genepool: GP2

- ✓ Can be used for breeding with some effort;
- ✓ Can be crossed with members of GP1; hybrids are usually sterile, but some maybe fertile; and
- ✓ Gene transfer is possible using conventional methods, but may be difficult .

Tertiary Genepool: GP3

- ✓ Extreme outer limit of potential genetic reach;
- ✓ Can be crossed with members of GP1, but hybrids are sterile; and
- ✓ Gene transfer is only possible with radical measures.

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LECTURE 8. Genebank Development and Management

Introduction

Virtually all countries in the world maintain germplasm collections, with varying facilities, budget, capabilities including staff, physical environment and policy framework. Maintaining plant genetic resources under optimum conditions of management is of prime importance if only to insure that the objectives of germplasm conservation and sustainable use will be met.

Genebank management begins with institutional legal and policy framework. In addition, the relationship of the genebank with national and global policy regimes should be clearly understood.

The effect of infrastructure and budgetary considerations on the management of germplasm collections, in addition to the constraints posed by the physical environment in which the genebank facilities are located, should also be considered.

The germplasm managers and curators should always keep abreast of and take into account in the management of genebanks and germplasm collections the good practices from acquisition to registration to regeneration to conservation to characterization to distribution to documentation.

Objectives

1. Discuss the options and considerations in developing effective genebank management strategies;
2. Discuss the important elements of management of the genebank and the collections; and
3. Discuss options for efficient and cost effective management of seed collections in genebanks.

Description of topics to be covered

This lecture will discuss the biological factors that influence breeding system, seed storage characteristics, conservation and utilization concepts and maintenance of seed quality; and the routine operations of genebank operations and practices and their implications for maintaining genetic integrity.

Lessons to learn

✓ The lessons learned from the previous modules will be integrated into an effective genebank strategy that takes into account policy, infrastructure, personnel, physical facilities and routine operations.

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PART 2.
Practicum

Practicum 1. Seed Processing & Drying

PRACTICUM 1. Seed Processing and Drying

Introduction

The seed samples for storage should meet the minimum standards for seed quality and quantity. The operational sequence to integrate an accession into the genebank involves cleaning, moisture content (mc) determination, drying, viability testing and packaging. The management of seed collections requires that germplasm accessions be maintained with a high proportion of viable seeds and this involves storage under appropriate conditions, periodic monitoring of seeds for viability and quantity and regenerating them when the situation warrants.

Objectives

1. To expose the participants on the best practices in seed processing and drying; and
2. To gain practical experience on processing, seed drying and packaging.

Description of topics to be covered

The practicum will demonstrate the good practices on seed processing and drying used in genebanks. It will also allow the participants to do actual seed processing, drying and packaging for storage.

Lessons to learn

- ✓ Seed processing and drying should be done within the shortest possible time after harvest and upon arrival of collected materials in the genebanks.
- ✓ The proper precautions in seed cleaning, drying and packaging should be observed to insure that seeds of good quality will be stored.

Seed cleaning

The different steps used in manual and/or mechanical threshing and cleaning of the seeds will be performed. The practices necessary to maintain accession integrity and identity during the cleaning process will be done.

Seed drying and processing

In conjunction with threshing and cleaning, the good practices in drying seeds will be performed. The exercise will include initial drying and final drying, and the use of different drying methods and facilities.

Seed moisture content (mc) determination

Different methods of seed mc determination will be conducted. The appropriate oven drying methods and the use of moisture meters will be done.

ISTA (1996) has prescribed two kinds of oven-drying methods for determining mc:

- Low-constant temperature oven method for oily seeds; and
- High-constant temperature oven method for non-oily seeds.

Seed mc is usually expressed as the weight of water contained in the seed as a percentage of the total weight of the seed before drying (i.e. wet or fresh weight basis), as follows:

Equation 2.

$$MC\ (\%\ wb) = \frac{\text{fresh weight} - \text{dry weight}}{\text{fresh weight}(g)} \times 100$$

Moisture content can also be expressed on a dry weight basis. It is expressed as the ratio between weight of water and dry weight of tissues.

Equation 3.

$$MC\ (\%\ dw) = \frac{\text{fresh weight} - \text{dry weight}}{\text{dry weight}} \times 100$$

Packaging

1. Different types of containers could be used depending on the size, shape of the seeds and the purpose of conservation (i.e. whether for long-term or short-term). Decide on the most suitable container you will use for storing the seeds in your genebank.
2. Prepare and label your containers. Also prepare a label for inclusion within the seeds inside the container. The labels should contain at least the following minimum information:
    a. Accession number;
    b. Genus and species;
    c. Container number;
    d. Weight of seeds; and
    e. Date of storage.
3. Weigh out each labeled container empty.
4. Fill the containers with the seeds and weigh again. Calculate actual weight of seeds.
5. Add the label prepared for the inside and seal the container immediately to prevent moisture from entering the seeds.
6. Check quality of the container after sealing by making visual examination of each container to make sure that there is no leakage.
7. Any container found below standard should be replaced immediately.

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PRACTICUM 2. Purity and Health Testing

Introduction

Seeds to be stored should be of high quality (high purity and free from quarantine objects) to ensure maximum potential seed longevity. To insure a healthy seed harvest, collaboration with pathologists and entomologists is necessary for them to periodically monitor occurrence of pests and diseases during the growing season.

Maintenance of seed purity is essential to insure that the accession stored is true to type, maximize the use of storage space, and prevent contamination by seeds of weeds and other species. Seed health refers to the presence or absence of disease-causing organisms, pests and deleterious physiological conditions. Seed health tests determine the health status of a seed sample and by inference that of the seed lot, thus gaining information that can be used to compare quality of different seed lots.

Objective

To gain practical experience on seed purity and seed health testing of representative accessions of cereals, legumes and vegetables.

Description of topics to be covered

The practicum will cover methods of seed purity and seed health testing and procedures in plant quarantine

Lessons to learn

- ✓ Seed cleaning is a vital step to maintain seed purity and health, which in turn are key to the maintenance of the identity of the accessions.

Seed Purity

Seeds of an accession are said to be pure if all seeds are genetically identical, whole, undamaged and free from pests, diseases and debris. This is the case in genetically homogeneous accessions or pure lines. In heterogeneous accessions the seeds of which are not genetically identical, the other factors are still relevant.

Seed purity can be improved by cleaning, i.e. separating pure seed, other seeds, and inert matter or debris. Debris can consist of a wide range of plant and soil-derived matter which were not separated from the seeds during harvesting and threshing. They can include soil, sand, stones, chaff, plant parts, pests etc. It is easy to determine purity in seeds of genebank accessions due to the small number of seeds involved, which makes possible the removal of all seeds of weeds and other crop species and debris by hand cleaning.

Analytical purity indicates how much of the seed in the stock is of the correct botanical species. It is determined by examining a small sample and removal of impurities. The remaining pure seed is weighed and expressed as percentage of the weight of the whole sample.

Species purity is determined by examining a sample for contamination by seeds of another crop species of similar type and expressing purity as the total number seeds less the number of foreign seeds in the sample.

Seed Health

Seed-borne inoculums reduce storage longevity and cause poor germination or field establishment. Seed-borne inoculums also bring about disease development in the field, reducing the value of the crop. Exchange of diseased and infested seeds leads to introduction of diseases and pests into new regions. Therefore, genebank curators should ensure that seeds prepared for conservation are healthy and free from the seedborne diseases and pests.

Eliminating pests and diseases from germplasm

Seed materials received at the quarantine station are subjected to visual and microscopic examination. Once a pest, pathogen or weed is detected, appropriate eradication treatments such as fumigation, heat treatment or chemical dressing are given before release of the material for sowing.

Fumigation

Fumigation of material under atmospheric pressure or under reduced pressure is an acceptable quarantine treatment against insects and mites. Fumigants like methyl bromide, HCN, phosphine and EDTA are commonly used. However, seed treatments must be conducted carefully as the safety margin between the dose that is toxic to insects and a dose that will cause no loss of germination or vigour of seeds is very small.

Important points to consider are:

- ✓ strict adherence to recommended dosages and exposure periods;
- ✓ avoidance of excessive temperatures; and
- ✓ thorough aeration of the seeds after fumigation.

The moisture content of the seeds is a critical factor in many instances, especially when methyl bromide is used. Great care must be taken to ensure that the seed is dry (usually below 12 percent moisture content for prolonged storage).

Heat treatment

Hot water treatment or hot air treatment are used for eradication of insects, mites, nematodes, fungi, bacteria and viruses. The basic principle involved is that treatment temperature should be sufficiently high to kill the pest/pathogen but not the seeds. However, the margin of safety is very narrow and temperature should be very accurately controlled.

To eradicate fungi, seeds are first soaked in cold water at 20-30^{o}C for 5 hrs to induce dormant mycelium to grow and then immersed in hot water at 50-54^{o}C for 10 minutes to kill the mycelium.

Chemical treatments

Dressing seeds with fungicides is one of the most common methods of chemical control of diseases. Chemicals commonly used for treatment are: Thiram, Captan, Carboxin, Tricyclazole and applied as dust, spray, slurry or as dip. The dosage of the chemical should be enough to eradicate the inoculum but should not kill the seed. The treatment has to be done before sowing as treated seeds cannot be stored.

Soil treatments

Soils harbor a large number of pathogens. In addition to seed treatments, treatment of soil affords protection against seedling diseases. Soil treatments are largely curative and mainly aim at killing pathogens making it safe for the growth of the plant. Most common the soil treatments are:

Solarization: Soil solarization is generally used to control soil-borne pathogens like *Pythium*, *Rhizoctonia*, *Fusarium* etc. and nematodes. Irrigate the nursery bed to moisten the soil to a depth of 10 cm. Cover the bed after 2 days with thin transparent polythene sheet for 4-6 weeks. Irrigate the beds once in a week to increase thermal sensitivity of resting fungal structures.

Chemical treatment: Involves the use of chemicals to control soil-borne fungi. Requisite quantity of fungicide suspension is applied per unit area so that fungicide reaches a depth of 15 cm. Commonly used chemicals are: Emisan, PCNB, Carbendazim, Copper fungicides, etc.

Plant Quarantine (PQ) is a strategy of control to prevent the spread of pests and diseases. PQ covers all regulatory actions taken to exclude animal or plant pests or pathogens from a site, area, country or group of countries.

Plant collectors often import germplasm from areas where pests, pathogens and host species have co-evolved. The exchange of plant propagation material from genebanks also carries the concurrent risk of spreading crop associated pests and diseases to new areas. Thus, pests of cultivated plants can move and establish in new areas, where they are previously unknown or organisms that are not recognized as damaging in their native areas can become damaging pests when move into new areas. PQ helps in safe introduction of new propagating material from other areas/countries.

Quarantine is usually a government responsibility. However, germplasm curators also have the responsibility to reduce the potential for harboring pathogens in the collections they manage. Compliance to phytosanitary regulations is vital to protect the country's agriculture from potential damage or destruction from introduction of new pests and diseases.
Quarantine regulations are generally similar but execution may differ among nations.

Plant quarantine practices have two main functions:

- ✓ Exclusion or regulatory actions to prevent or reduce the risk of entry of exotic pathogens, pests or parasites.
- ✓ Assisting exporters to meet quarantine requirements of importing countries by granting Phytosanitary Certificates.

The objectives of plant health quality control include:

- ✓ Reduction of the risks involved in transferring germplasm from one country or region into another
- ✓ Maintenance of material free of pathogens of quarantine interest
- ✓ Facilitating the availability of germplasm without plant health restrictions for the users

Quarantine regulations

Any importation, exportation, and movement of plant propagating materials or plant products is subject to quarantine action which generally includes:

- ✓ **Prohibition of importation** – When the pest risk is very high and safeguards available in the country are not adequate
- ✓ **Restriction** – Pest risk is not high and import regulated through permits stipulating conditions of entry, inspection and treatment.

- ✓ **Import inspection** – for compliance to regulations regarding documentation and general health status of the imported material upon arrival
- ✓ **Detention** – when accompanying documents are not in order
- ✓ **Treatment** – material found to be infected or infested upon inspection will be treated
- ✓ **Isolation and observation (post-entry quarantine)** – for plants with high risk and known to be hosts or from endemics suspected to be infected/ infested with quarantine pests
- ✓ **Refusal of entry** - if material cannot be disinfected or documents are not in order
- ✓ **Destruction/ Re-shipment** - Plants, plant products and other regulated items imported in contravention of the PQ regulations are destroyed, disposed or refused entry.
- ✓ **Release** - when PQ requirements are fully met.

Third country/Intermediate quarantine

Material is grown, and tested/indexed for hazardous plant pests in a temperate country without much risk because either the possible hosts are not present there or the environment is unfavorable for disease establishment.

Post-entry quarantine

PQ regulations in some countries require that germplasm must be planted and periodically inspected before it can be safely released (because many pathogenic fungi and viruses borne deep inside the seeds escape chemical treatments).

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Practicum 3. Storage Conditions, Viability Testing and Monitoring

PRACTICUM 3. Storage Conditions, Viability Testing and Monitoring

Introduction

Storage life of seeds will depend on initial viability, seed moisture content, relative humidity and temperature of storage facility. We have to ensure that the seeds should maintain its mc. It is also important that storage conditions specially temperature and relative humidity should be maintained at optimum levels.

The number of seeds to be kept in storage depends on the breeding system of the crop, heterogeneity of the accession and type of storage (short-, medium- or long-term). In active collections, an additional consideration is the frequency of distribution (frequently requested seeds).

Viability testing and monitoring are done to determine initial viability, to determine the trend of loss of viability and to provide data to predict when regeneration needs to be done.

Objectives

1. To provide the participants with the good practices on seed storage, viability testing and monitoring in genebanks; and
2. To gain practical experience on viability testing and monitoring.

Description of topics to be covered

The practicum will demonstrate the methods of testing and monitoring seed viability, applications of viability equations for predicting viability of seeds in storage. A tour to the different storage facilities of RDA will be done.

Lessons to learn

- ✓ The viability equation can predict the storage life of seeds and useful in determining the economical ways to monitor seed viability.
- ✓ Seeds deteriorate in storage, hence monitoring seed viability is an important consideration in genebank management.
- ✓ Regular monitoring of seed viability ensures the quality of seeds conserved and distributed by genebanks.

Monitoring viability

Viability is monitored by conducting germination test on a fixed sample size or by sequential germination.

Fixed sample size germination test
It is recommended to use a minimum of 200 seeds as two replicates of 100 seeds for the fixed size germination test.

1. Identify and make a list of the accessions that require testing
2. Remove the containers from storage and leave them overnight at room temperature to warm up.
3. Open the container and draw a sample of seeds needed for the test and close the containers.
4. Conduct the germination tests
5. Calculate the mean percentage germination of the accession form the results of the two replicates. *Repeat the germination test if the difference between the two replicates exceeds the maximum tolerance limits at 2.5% probability (Table 1)*
6. If the mean germination decreases to less than 85% of the initial germination percentage (i.e. germination at the beginning of storage), schedule the accession for regeneration
7. If the percentage germination is above 85% of the initial germination percentage (at the beginning of storage), continue to store the accession.

Sequential germination tests

The sequential germination tests use less number of seeds per replicate than the standard size germination test. Otherwise, the methods and conditions for germination are same as described for standard size germination test.

The number of seeds required for each replicate can vary. However, it is recommended to use at least 40 seeds per replicate.

- ✓ Conduct the germination test using 40 seeds.
- ✓ Count the number of seeds germinated after the prescribed period of testing.
- ✓ Compare the results of the test with the number germinated in Table 2 looking at the line with the value of 40 in the first column (number of seeds tested).
    - If the number of seeds germinated is 29 or less, the accession will require regeneration.
    - If the number of seeds germinated is more than 29, then the test will have to be repeated with another sample of 40 seeds exactly as described above.

Use the same number of samples when repeating the test, so that the different samples can be treated as replicates.

- ✓ Count the number of seeds germinated in the second test and add this to the results of the number of seeds germinated in the first test.

- ✓ Compare the results of the test for number germinated in Table 2 as before looking the line with the value equal to the total number of seeds used for all tests (80) in the first column (number of seeds tested).
  - If the number of seeds germinated is 64 or less, then the accessions will need to be regenerated.
  - If the number germinated is above 75, the accession can be continued in storage.
  - If the number germinated is between 65 and 75, the accession will require testing again with another of sample of 40 seeds and compare the results with the value equal to the total number of seeds used in all tests (i.e. 120 seeds) in Table 2.
- ✓ Continue the test in this way until a decision - whether to regenerate or to continue storing the accession - is reached or the test is repeated 10 times.

Predicting viability of seeds in storage

There is a need to predict seed longevity in storage seeds to provide a rational basis for an economical regime of monitoring tests in storage. Variation in seed longevity (stored at a constant MC and temperature) results to a normal distribution of deaths in time. Survival curves conform to negative cumulative normal distribution. Under constant storage conditions, probit viability, *v*, is related to storage period, *p* (in days) as follows:

Equation 4.

$$v = K_i - p/\delta$$

where
v = probit percentage viability
K_i = initial probit viability
p = days of storage
ð = standard deviation of the normal distribution of seed deaths in time

For any given storage experiment (constant storage environment) the parameters K_i and ð can be estimated by fitting the linear relationship within a probit analysis.

The improved equation for predicting viability of seeds in storage is as follows:

Equation 5.

$$v = Ki - p/10\ (KE - CW\log_{10}(m) - CHt - CQt2)$$

where:
V = probit percentage viability
P = days of storage
t = temperature
m = seed moisture content
Ki, KE, CW, CH, CQ = species constants in time

Monitoring seed quantity

Seed quantity can be monitored by checking the inventory data file. This can be best done through a computer based genebank documentation system.

- ✓ Record the weight of the seeds initially transferred to genebank.
- ✓ Record all subsequent seed withdrawals for distribution, regeneration and germination testing.
- ✓ Update seed stock immediately adjusting the total after all seed withdrawals.
- ✓ Query and prepare a list of accessions where the number of seeds in storage falls below critical level (usually the number required for at least three regenerations).

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Table 1. Maximum tolerable difference between the number of seeds which is rarely exceeded by chance alone (probabilities of 5.0%; 2.5%; 1.0%, or 0.1%) for two 100-seed replicates of a germination test (Adopted from Ellis et al. 1985).

Average germination (%) of all replicates		Probability (%)			
		5	**25**	**1**	**1**
99	2	3	4	4	*5*
98	3	4	5	5	7
97	4	5	6	6	8
96	5	5	6	7	9
95	6	6	7	8	10
94	7	7	8	9	11
93	8	7	8	9	12
92	9	7	9	10	13
91	10	8	9	10	13
90	11	8	9	11	14
89	12	9	10	11	15
88	13	9	10	12	15
87	14	9	11	12	16
86	15	9	11	13	16
85	16	10	11	13	17
84	17	10	11	13	17
83	18	10	12	14	17
82	19	10	12	14	18
81	20	11	12	14	18
80	21	11	13	14	19
79	22	11	13	*15*	19
78	23	11	13	15	19
77	24	11	13	15	19
76	25	12	13	15	20
75	26	12	14	16	20
74	27	12	14	16	20
73	28	12	14	16	21
72	29	12	14	16	21

Table 1. (continuation)....

Average germination (%) of all replicates		Probability (%)			
		5	**25**	**1**	**1**
71	30	12	14	16	21
70	31	12	14	17	21
69	32	13	14	17	21
68	33	13	15	17	22
67	34	13	15	17	22
66	*35*	13	15	17	22
65	36	13	15	17	22
64	37	13	15	17	22
63	38	13	15	17	22
62	39	13	15	17	22
61	40	13	15	18	23
60	41	13	15	18	23
59	42	13	15	18	23
58	43	13	15	18	23
57	44	13	15	18	23
56	45	14	16	18	23
55	46	14	16	18	23
54	47	14	16	18	23
53	48	14	16	18	23
52	49	14	16	18	23
51	50	14	16	18	23

Table 2. Modified sequential germination test plan for 85% regeneration standard for groups of 40 seeds.

Number of seeds	Regenerate if number germinated is less than or equal to	Repeat test if number germinated is between	Store if number germinated is more than or equal to
40	29	30-40	-
80	64	65-5	76
120	100	101-110	111
160	135	136-145	146
200	170	171-180	181
240	205	206-215	216
280	240	241-250	251
320	275	276-285	286
360	310	311-320	321
400	340		341

**When 400 seeds have been tested, the test can be terminated because enough tests have been done for practical decision to be made.*

Practicum 4. Documentation & Information Management

PRACTICUM 4. Documentation and Information Management

Introduction

The development and introduction of a PGR documentation system involves careful planning, analysis and design. Thus, understanding the PGR system is the basic requisite in the analysis of documentation needs and requirements to properly develop a documentation system design. In database computing this aspect is commonly referred to as *system analysis*. It attempts to give an idea of the documentation system in relation to current and projected information needs, hardware and software sophistication level, and design and implementation of the PGR documentation system.

Another important step in the creation of a database management system is the development of the logical design. A logical design is a detailed definition of how to implement the solution to the documentation problem. It gives the step-by-step procedure on how to accomplish the documentation task. The logical design is developed from the results of analysis of the PGR program. It is presented as a flow diagram indicating decision points. The logical system design serves as the guide structure in developing the physical system design.

With the system analysis and the logical design as basis, a PGR documentation system can be designed using any of the appropriate software and platforms.

Accessing the available PGR databases in the internet will give the curator and documentation staff an idea of the good features of a PGR documentation system.

Objectives

1. Understand the basic concepts of system analysis in the context of PGR conservation and management;
2. Learn the basic concepts in logical design development;
3. Learn the basic operation of selected PGR documentation systems; and
4. Develop a basic PGR documentation and information management system.

Lessons to learn

- ✓ The development of a PGR documentation system requires knowledge of all aspects of PGR conservation and management.
- ✓ A PGR documentation staff requires basic knowledge on available database software.

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Practicum 5. Regeneration Plan, Distribution Standards and Collection Security

PRACTICUM 5. Regeneration Plan, Distribution Standards and Collection Security

Introduction

The regeneration of germplasm is done to ensure that there are enough good quality seeds of an accession for conservation. The frequency of regeneration is a function of initial sample size, demand for the accession, and the length of seed viability in storage.

An important consideration when regenerating germplasm materials is the maintenance of the genetic constitution of the original sample after the regeneration process.

The decision guide for regeneration by Hamilton and Chorlton (1997) is useful for genebanks to determine the quantity of seeds required for a regeneration cycle. It considers the seed requirement for the base or active and duplicate collections which is dependent on the minimum number of distribution units (number of seeds given out per seed request), regeneration units (seeds planted every regeneration cycle), base unit (minimum number of seeds to be put in the base collection), and test units (seeds to be used for periodic viability testing). The suggested frequency of viability testing is given in Table 1.

The time when regeneration of the seeds in storage should be done will also depend on the threshold viability for regeneration. FAO/IPGRI recommends 85% of initial viability *(see Table 2)*. However, in some species or races, the threshold can be lowered especially if the initial viability is appreciably lower than that obtained in other species. Some examples are the eco-geographic races of rice specifically japonica rices and some wild species.

The distribution standards should take into account breeding system of the crop whether inbreeding or outcrossing, and this is related to whether the accession is from a homogeneous heterogeneous population, size of seed, the regeneration sample if possible should be representative of the original accession.

For the security of the collection in genebanks, the physical features of the genebank storage facilities (fire alarm, earthquake proofing if possible) and monitoring of the storage conditions should be considered.

Objectives

1. Gain practical experience on the good practices for germplasm regeneration of cereals, vegetable and legumes;
2. Prepare a plan for regeneration taking into account the decision guide; and
3. Discuss standards for distribution and security of the collection.

Description of the topics covered

The practicum will demonstrate the field set-up in regeneration, the number of seeds/plants required and the pollination control in insect pollinated and wind-pollinated crops. It will also discuss the decision guide for regeneration. It will also cover standards for distribution and security of the collection.

Lessons to learn

- ✓ The size of the population for regeneration is dependent on the mode of pollination, the frequency/amount distributed and the fecundity of the species.
- ✓ Contamination/ adulteration should be avoided to maintain the genetic identity of the population.
- ✓ The quantity of seeds for distribution and duplicate collection should represent the genetic structure of the original population.

Table 1. Suggested interval for monitoring germination of active or base collections in oily and non-oily seeds.

Present level of Germination (%)	Monitoring interval (years)			
	Active collection (4-5ºC)		Base collection (–20ºC)	
	Non-oily seeds	Oily seeds	Non-oily seeds	Oily seeds
<80	3	1	5	2
80-85	5	3	10	5
85-95	8	5	15	8
>95	12	8	20	12

Table 2. Threshold germination percentages for regeneration of accessions.

Initial germination	Regenerate if percentage germination after monitoring is below
100	85
99	84
98	83
97	82
96	82
95	81
94	80
93	79
92	78
91	77
90	77
89	76
88	75
87	74
86	73
85	72

Practicum 6. Germplasm Exploration and Collection

PRACTICUM 6. Germplasm Exploration and Collection

Introduction

Germplasm can be acquired through donations, requests, exchange with another institution, and through field exploration and collecting in farmers' fields and natural habitats. The types of germplasm materials that can be acquired include farmers' varieties, landraces and primitive cultivars, wild and weedy related species, and products of plant breeding, e.g. improved cultivars, obsolete varieties, mutants, genetic stocks, breeding lines etc.

Careful planning is necessary in germplasm exploration and collecting in order to collect the maximum amount of genetic diversity in the target taxon. In addition to germplasm, information including indigenous and traditional knowledge associated with the germplasm should also be collected whenever practicable. When collecting germplasm, social and ethical considerations should also be given importance.

Changes in planned itinerary can occur. Flexibility therefore should be practiced to maximize the use of time and resources in collection.

Objectives

1. Learn the good practices in collecting germplasm of cereals, vegetables and legumes;
2. Learn how to gather and document passport data and associated knowledge; and
3. Collect and prepare germplasm materials for herbarium collection.

Description of the topics covered

The practicum will discuss the pre-planning necessary for germplasm collecting including the appropriate legal instruments, demonstrate the sampling strategy (how to collect considering the number of plants/amount of seeds, number of sites); demonstrate the use of GIS and the good practices for initial processing of the collected materials, and demonstrate the procedure for gathering and documenting data during collection.

Lessons to learn

- ✓ Knowledge of the taxonomy of the target taxon is useful to the identification of related species.
- ✓ Passport data are valuable information gathered during collecting which are useful for recanvass, preliminary characterization and evaluation and use of the germplasm.
- ✓ Over-collection may contribute to the loss of the species in the target sites.
- ✓ Indigenous knowledge, if available, should be documented.

What to collect

- ✓ **Plant Germplasm-** Genetic material of plants which are of value as a resource for present and future generations of people, a set of different genotypes or varieties that may be conserved and used
- ✓ **Seeds**- cereals, vegetables
- ✓ **Pods/fruits-** legumes, fruits
- ✓ **Seedlings-** fruits, plantation crops
- ✓ **Cuttings-** rootcrops, ornamentals, medicinal plants
- ✓ **Budsticks/scions**- fruits, plantation crops
- ✓ **Specialized organs**- corms, runners,bulb, tuber, sucker, slip, crown
- ✓ ***In vitro* plants/plantlet**s- banana, orchids

Types of plant germplasm

- ✓ Farmers' varieties – crop varieties that have been selected by farmers and have evolved in farmers' fields
- ✓ Land races – primitive varieties of crops that are adapted to local environmental conditions
- ✓ Wild species
- ✓ Weedy forms
- ✓ Commercial varieties - High Yielding Varieties (HYVs), varieties from seed companies
- ✓ From plant breeders, scientists, government breeding institutions

What to bring during collecting? (Minimum list)

- ✓ Transport
  - Preferably a four-wheel drive vehicle
- ✓ Equipment
  - GPS, camera
- ✓ Supplies
  - Vehicle supplies (flashlight, spare tire etc)
  - Collecting supplies(collecting forms, net/paper bags, knife, herbarium press, etc)
  - Medical and personal supplies

Where to collect plant germplasm?

Valuable germplasm materials can be collected in the following sites:
- ✓ Farmland;
- ✓ Farm seed storage;
- ✓ Backyard/home garden or kitchen garden;
- ✓ Home seed storage;
- ✓ Village markets;
- ✓ Seed shops;
- ✓ Roadsides; and
- ✓ Natural vegetation.

How to collect plant germplasm?

The Collecting Team

Size: 3 to 5 people
- ✓ to interview/talk to farmer;
- ✓ to look around for different plants in the farm or garden;
- ✓ to record observations and information; and
- ✓ to collect seeds or plants.

Involvement of local people – as guide or source of information
Permission and assistance to collect germplasm – from national authorities, local government agencies or local leaders

Planning the Itinerary

By reading references or through interview of appropriate people, gather information on:
- ✓ eco-geographic condition of the target area (climate, topography, vegetation);
- ✓ target vegetable species (distribution, reproductive biology, fruiting time, habitat;
- ✓ the most appropriate route and roads to use (safety, availability of lodging facilities. gasoline, food); and
- ✓ cropping pattern and season of planting of the indigenous vegetables in the target area.

Time of Collecting: When is the best time to collect?

- ✓ Consult people familiar with the area (extension workers, residents, farmers)
- ✓ Consult records of previous collecting expeditions in the area

Single visit or multiple visits?

- ✓ **For single visit:** during peak seed maturity or harvesting for majority of crops.
- ✓ **For Multiple visits:** when there are:
  - differences in timing of planting
  - different vegetable species planted throughout the year
  - different varieties planted throughout the year

How long should the collecting trip be?
Duration depends on:
- ✓ Size of the area to be visited;
- ✓ The terrain of the area;
- ✓ Availability and condition of roads;
- ✓ Length of travel time; and
- ✓ Number of vegetable species and varieties to be collected.

Sampling Strategy

Sampling methods:

Coarse grid sampling
If the site is large and contains distinct differences in eco-edaphic conditions, coarse grid sampling allows sampling the maximum variation in the population. Walk across a site or a field twice - in the form of a cross or zigzag manner avoiding sampling from the borders.

Clustered sampling
Clustered sampling allows the inclusion of large variability related to both geographic and microgeographic differences in environment and better suited for wild or weedy forms. Collect several samples within a small area and repeat the sampling over several areas within the broad-based site.

Stratified sampling
Additional non-random (or selective) samples may be added as sub-samples if the collector sees any particularly interesting variant present in small numbers, which were not included in the sample by strictly random sampling. Non-random samples should not be mixed with random ones but should be kept separate and given another collecting number.

How many seeds to collect: if possible, 2,500 to 5,000 seeds.
- ✓ Amount should be enough for storage.
- ✓ Some seeds will be used for planting to produce more seeds, and to characterize the variety.

Sampling Strategy: How to collect

One variety in a big field:
- ✓ Mentally divide the area into a series of transects vertically and horizontally to form a +; or diagonally to form an x.
- ✓ Collect every interval (e.g. every 5 steps).
- ✓ Bulk the samples. Collect enough for minimum of 1,000 seeds.
- ✓ If there are variants (plants with different appearance from the others): collect seeds from the plants and separate the seeds from the others.
- ✓ Do not collect from plants with diseases or pests.

Many species and varieties in a home garden:

- ✓ Collect every variety and species.
- ✓ Bulk the seeds of the same variety in the same bag or envelope. Do not mix together seeds of different varieties.
- ✓ Collect as many seeds as you can. However, leave enough for the farmers to plant the following season

Fill in collecting labels. Use permanent ink.

Collecting Number _____
Collecting Date _____
Collecting Site _____
Crop _____

Always put two labels per variety collected. Put one label inside the bag or envelope; put the other label outside the bag or envelope.

Give unique collecting number for every sample collected. Use a standard system of numbering: e.g. ABC 09-005.

Filling in the Collection Record Sheet

Provide as much information as possible in the collection record sheet and indigenous knowledge record sheet. The IK consists of information including characteristics and uses, household and farm profile and farming activities such as the practices employed in the cultivation and harvesting of the material.

COLLECTING INSTITUTE_____
COLLECTORS_____
COLLECTION NUMBER_____
COLLECTION DATE __YR__MO__DD
COUNTRY _____
DISTRICT/PROVINCE _____
LOCATION OF COLLECTION SITE:
village _____
town _____
VARIETY NAME LANGUAGE _____
USAGE (specify) _____
GROWER NAME _____

LATITUDE OF SITE _____
LONGITUDE OF SITE _____
ALTITUDE OF SITE _____
CROP COLLECTED _____
GENUS _____ SPECIES _____
VARIETY/LOCAL NAME _____
MEANING _____

Photographs, Field Notes, Herbarium specimen

- ✓ Take photographs of plants, leaves, flowers and fruits if possible. Use an indicator of scale (ruler is ideal).
- ✓ Keep an account of the day's events and observations in a field notebook.
- ✓ Prepare herbarium specimens of lesser known wild species.

Processing of Collected Material

- ✓ Initially process the collected material to maintain high viability of seeds.
- ✓ Extract seeds and air dry them whenever possible.
- ✓ Keep the samples in separate containers, properly labeled.
- ✓ Provide the basic information used for the general management of the accession (including registration at the genebank and other identification information).
- ✓ Describe the parameters that should be observed when the accession is originally collected.

References

Centro Internacional de Agricultura Tropical (CIAT), Universidad Nacional de Colombia-Sede Palmira and Bioversity International, Red de Instituciones Vinculadas a la Capacitacion en Economia y Politicas Agricolas en America Latina y el Caribe (REDCAPA) and Centre technique de cooperation agricole et rurale (CTA). 2007. Multi-institutional distance learning course on the *ex situ* conservation of plant genetic resources. Cali, Colombia.

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PRACTICUM 7. Characterization and Evaluation

Introduction

Germplasm characterization and evaluation are linked to utilization. They are done to determine identity of the collection and for efficient management of the genebank. Gemplasm accessions in a collection are characterized and evaluated using morphological, cytological, biochemical, and molecular markers.

Characterization and evaluation start with the adoption of a descriptor list. The descriptor list is prepared following prescribed global standard, and therefore becomes a universally understood vehicle of genetic information storage and retrieval.

For morphological characterization, knowledge of the morphology of the crop species is essential. It is important to define the exact time (growth stage) to make the observation and the method of recording

For molecular characterization, the choice of the methods to be used depends on the ease of analysis, reproducibility, level of polymorphism, number and genome distribution of loci.

Objectives

1. To familiarize the participants with use of descriptor list in morphological characterization;
2. To provide the participants with practical experience in the use of morphological and molecular markers in characterization and evaluation of germplasm using the descriptor list;
3. To introduce to participants the practical application of molecular markers in assessment of diversity of a germplasm collection; and
4. To learn good practices in characterization and evaluation of crop germplasm.

Description of the topics covered

The practicum will review the morphology of the crop species to be characterized and discuss the minimum descriptors to be used and the types of measurement data. Actual morphological characterization will be conducted. The steps in molecular characterization from DNA extraction, PCR, electrophoresis, staining and scoring of bands will be demonstrated.

Lessons to learn

- ✓ The choice of the method of characterization and evaluation depends on the capacity (physical and financial) and capability (availability of trained staff) of the institution.
- ✓ The easiest and quickest gauge of the genetic diversity of the collection is through morphological characterization.
- ✓ Characterization of heterogeneous populations should provide information valuable for the utilization of germplasm.
- ✓ Molecular characterization can differentiate accessions at the DNA level.

Morphological characterization and evaluation

For crops with no descriptor list yet, a descriptor list can be developed by following these steps:

1. Assemble all published taxonomic descriptions used for the species. When gathering the information consider all the taxonomic names to which the species has been referred to in literature (e.g. synonyms). Consider also related taxa.
2. Analyze the descriptions for any pattern of variation that is recognizable. Take note if differences are obvious. Note also if the character is heritable or environmentally influenced.
3. List the morphological characters that should be included in the descriptor list.
4. Assemble a collection of accessions or make field collections from where you can directly observe the different states for each morphological trait.

When conducting morphological characterization, consider the following:

1. Measurements are made in metric units.
2. Many descriptors which are continuously variable are recorded on a 1-9 scale. Modifications may be made such as 3, 5, 7 for describing only a selection of the states.
3. Presence or absence of characters are scored as 1=present and 0=absent. For descriptors which generally are heterogeneous or not uniform throughout the accession (e.g. genetic segregation, mixtures), mean and standard deviation could be reported where the descriptor is continuous or mean and "X" where the descriptor is discontinuous.
4. Where the information does not exist (descriptor is not applicable), "0" is used.

5. Blanks are used for information not yet available.
6. Standard color charts (e.g. Royal Horticultural Society Color Chart, Methuen Handbook of Color, Munsell Color Chart for Plant Tissues) are strongly recommended for all ungraded color characters. The precise chart used should be specified in the "NOTES" Descriptor.

Molecular characterization

Details of the following steps involved in molecular characterization will be provided in the practicum.

1. DNA extraction/isolation;
2. Selection of informative primers;
3. PCR;
4. Visualization of PCR products using electrophoresis;
5. Staining using appropriate dye;
6. Scoring of bands; and
7. Data analysis.

References

Centro Internacional de Agricultura Tropical (CIAT), Universidad Nacional de Colombia-Sede Palmira and Bioversity International, Red de Instituciones Vinculadas a la Capacitacion en Economia y Politicas Agricolas en America Latina y el Caribe (REDCAPA) and Centre technique de cooperation agricole et rurale (CTA). 2007. Multi-institutional distance learning course on the *ex situ* conservation of plant genetic resources. Cali, Colombia.

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Notes



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