General Comments

Plants are living things that have morphological, structural, and functional characteristics that enable them to adapt to the habitat where they are established, interacting with changing environmental conditions. Furthermore, they have internal information systems that coordinate and control all the processes pertaining to life maintenance, so that they succeed in sustaining a certain degree of permanence across space and time.

Under natural conditions, over time, and as a function of their evolution and needs for adaptation, plants have accumulated, in coded form in their genome, the results obtained. Knowledge of those coded and therefore usable plant attributes and properties converts them into valuable resources (i.e., plant genetic resources or PGRs), worthy of conservation. As described previously (*Module 1, Lesson 1, page 2*):

> *Plant genetic resources are the sum of all combinations of genes and their variants, resulting from the evolution of plant species. During evolution, a plant population is the receptacle of all past changes and of the results of selections made by the environment, which are expressed as DNA that is exactingly organized and conserved in genomes (Hoagland 1985). In other words, genes contain all the information that defines each trait or character of a living being, in this case, plants. An inheritable trait or character is meticulously reproduced in offspring. Consequently, we find in genes information on adaptation, productivity, resistance to adverse conditions such as pests, diseases, stressful climates, and poor soils, and other characteristic of a population's individuals that are usable by humans to the extent of their knowledge.*

In general and according to previous statements, important information is believed to exist in plant genomes and to express itself as morphological, structural, or functional attributes. It is contained in **germplasm**, which therefore becomes the holder of a species' entire sum of hereditary characteristics. However, it should be emphasized that, to use it, germplasm should be understood in detail, that is, the type of attributes it possesses should be determined. The process of gaining such understanding is known as **germplasm characterization.**

Information on the Module

Module 4 contains two lessons and a brief evaluation exercise for each lesson.

Objectives

When you have completed this module, you should be able to:

- Justify and conceptualize plant germplasm characterization
- Describe types of germplasm characterization

Lessons

1. General concepts of germplasm characterization
2. Ways of characterizing plant germplasm

Bibliography

Throughout this module, a bibliography is provided for each section, that is, the *General Comments* and each *Lesson*. The bibliographies follow a format of two parts:

1. *Literature cited*, which includes those references cited in the text itself. Some of these citations were used to develop the original Spanish-language course on *ex situ* conservation and may therefore appear in Spanish or Portuguese. However, where practical, references to the English versions of the original Spanish-language documents are provided.
2. *Further reading,* which is a list of suggested readings in the English language. Most cover in depth the topics included in this module.

A list of *Acronyms used in the bibliographies* is also given. The idea is to save space by not having to spell out each institution's full name each time it appears in the references.

Acronyms used in the bibliographies

AVRDC	Asian Vegetable Research and Development Center
CGN	Centre for Genetic Resources—Netherlands
CIP	Centro Internacional de la Papa
FAO	Food and Agriculture Organization of the United Nations
IBPGR	International Board for Plant Genetic Resources
IITA	International Institute of Tropical Agriculture
INIA	Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (Spain)
IPGRI	International Plant Genetic Resources Institute
RHS	Royal Horticultural Society

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Next Lesson

In the next lesson, you will study the general concepts of germplasm characterization.

Lesson 1 General Concepts of Germplasm Characterization

Objectives

- To justify the raison d'être of characterizing plant germplasm
- To conceptualize this process

Introduction

Plant genetic resources are conserved to use them. Using them is only possible if their characteristics or attributes are known in detail and their possible uses visualized (Jaramillo and Baena 2000). Such knowledge and visualization can be achieved only through the study of the morphological, structural, and functional attributes of germplasm as the carrier of all the hereditary characteristics of any given species. Heritable traits or characters of plant germplasm are studied precisely during characterization, where those aspects already mentioned and others of a population's individuals that can be used by humans are studied such as adaptation, productivity, and resistance to adverse conditions (e.g., pests, diseases, and climatic and soil stresses).

When germplasm is already physically held in a bank or collection, it must be duly accompanied by passport data (i.e., origin, geographical location, and characteristics of the habitat where it was collected and of the environment such as climate and soils). Characterization can then proceed, taking into account that it is a fundamental stage of *ex situ* conservation and that important reasons exist to justify it. That is, characterization:

- Permits estimation of the true genetic diversity that is being conserved—the principal raison d'être of a germplasm bank;
- Is valuable for providing germplasm banks with complete information on the characteristics of a given germplasm, thereby contributing to an optimal *ex situ* management of collections. Otherwise, its absence would convert such banks into simple depositories of materials of no significant usefulness.
- Facilitates the use of germplasm collections by improvement programmes and crop research.

To characterize germplasm, basic skills in botany (i.e., plant biology or phytology) are essential, particularly in the three principal divisions (taxonomy, morphology, and plant physiology). An understanding of systematics and genetics, among others, is also important.

Botanical knowledge of the germplasm of a given species conserved *ex situ* provides key information for its optimal characterization, especially to better select both materials to characterize and methodologies to use.

Taxonomy should be understood as the science that uses, as criteria for classification, properties of organisms such as morphology. The criterion that is currently accepted as the basis for taxonomy is that which reflects the phylogeny of living things and compares characters of whatever nature, whether morphological, anatomical, or cytogenetic. Consequently, taxonomy is a prime tool for germplasm characterization.

Systematics is the science of diversity. That is, it is the organization of the total set of knowledge on organisms. It includes phylogenetic, taxonomic, ecological, and palaeontological information. It permits seeing a global vision of the diversity of conserved materials, and has a predictive character, which permits the better selection of germplasm to characterize and methodologies to use, and improves analysis of results.

Genetics is the study of the nature, organization, function, expression, transmission, and evolution of the coded genetic information found in organisms. It is fundamental for a maximum evaluation of the data obtained from characterization.

Characterization

Definition

Through characterization, we can estimate the variation that exists in a germplasm collection in terms of morphological and phenological characteristics of high heritability. Such variation may also include the variability expressed by biochemical and molecular markers, that is, by characteristics whose expression is little influenced by the environment (Hidalgo 2003; Jaramillo and Baena 2000; Ligarreto 2003). In the characterization of plants, the expression of constant qualitative characters is recorded throughout a given plant's various physiological stages (phenotype). Data are taken according to specific descriptors, for example:

- For the *seedling stage*: hypocotyl colour and pubescence, length of the primary leaf, and petiole thickness;
- For the stages *before and during flowering*: plant height and growth habit, leaf position, flower colour, and days to flowering; and
- During the stage of *production*: number, size, and shape of fruits and yield.

These data are added to the passport data previously recorded during the collection or procurement of materials (Jaramillo and Baena 2000).

Variability as a major element in characterization

When characterizing a species, the variability existing in the genome of the population of individuals forming it is estimated. Thus, the genome of a given species of animals or plants contains all the information coded in the form of genes and their variants, which are needed both to establish the morphological identity of the members of that species and to develop all the processes and functions vital for their survival. For higher plants, any given species is estimated to have more than 400,000 genes with particular functions. As a result of evolutionary and environmental effects, many of these genes also have variants, which are accumulated among the different members composing the species. The sum of all effects of the genes and their variants is designated as the genetic variability of that species (Hidalgo 2003).

All variability produced during evolution and/or domestication is stored in the genome, that is, among the members of the populations forming the species. It may, or may not, find expression in characteristics that identify those members. Accordingly, with respect to its expression, the variability contained in the genome of a species can be separated into that which (1) finds expression as visible characteristics that form the phenotype, and (2) does not find expression as visible characteristics but generally deals with the plant's internal processes or products (Hidalgo 2003).

The first category, which refers to characterizing visually detectable variability, includes a plant's:

- Morphology and structure, used primarily for its botanical and taxonomic classification;
- Characteristics that affect its agronomic management and production and are therefore of interest to breeders and agronomists; and
- Reaction to environmental stimuli, whether biotic such as pests and diseases or abiotic such as droughts, mineral deficiencies, and temperature changes. This type of characterization is called evaluation and, for its correct quantification, usually requires experimental designs that are separate from morpho-agronomic characterization trials (Hidalgo 2003).

The second category, which characterizes variability that is not detectable by simple visual observation, is called molecular because it refers to the identification of cellular products and/or internal functions (Hidalgo 2003).

Evaluation

Evaluation consists of recording those characteristics that depend on environmental differences (e.g., disease resistance or susceptibility to drought). Hence, an accession may be evaluated in many sites, with perhaps significantly differing results for several descriptors. However, another accession characterized in many different sites may well yield similar results across sites. Once evaluated, an accession is unlikely to be characterized again, except to control its integrity and to check if it still represents the genetic composition of the original entry.

Evaluation may also describe the variation existing in a specific collection for attributes of agronomic importance that are strongly influenced by the environment such as yield. It is carried out in different sites, results varying according to environment and to genotype-by-environment interaction (Jaramillo and Baena 2000).

Characterization *per se* and evaluation are complementary activities that describe the qualitative and quantitative attributes of the accessions of a given species to differentiate them; determine their usefulness, structure, genetic variability, and relationships among them; and identify the genes that promote their use in crop production or improvement. The two activities require precision, care, and constancy, and include a significant data-recording component. These two activities have in common the use of descriptors, which are characters that are considered to be important and/or useful for describing a sample population of species. A descriptor may assume different values—it can be expressed as a numerical value, scale, code, or descriptive quality (Jaramillo and Baena 2000).

Objectives of characterization

In characterizing a collection, regardless of size, the following objectives can be established (Hidalgo 2003):

- Identify the accessions of a germplasm collection so that they can be clearly distinguished or individualized.

- Measure the genetic variability of the group under study; for which one, several, or all possible categories of variability can be included, that is, phenotypic, evaluative, and molecular, using previously defined descriptors.
- Establish the collection's representativeness and its relationship with the species' variability in a region or with its entire range of variability.
- Study the genetic structure, that is, the way the collection under study is composed in relation to variants or their combinations, forming groups or identifiable populations. The foregoing is influenced by *in situ* demographic factors such as population size, reproduction biology, and migration.
- Identify the percentages of duplication of accessions that can exist within a single collection or compared with other collections of the species.
- Identify special genes or particular alleles that may be of individual character or found in unique combinations, and may find expression in visible characters (morphological or of evaluation) in different stages or combinations of stages. These genes are usually called 'genetic stocks' and are used for research of immediate practical application, as in the case of resistance to biotic factors.

Stages of characterization

Characterization, together with its methodologies, is a comprehensive tool that can be used for both germplasm acquisition and the adequate management of the different stages of *ex situ* conservation. It includes:

- Comprehensive knowledge of one or more species.
- Presentation of questions that help improve understanding of the conserved germplasm.
- Use of improved and suitable methodology for characterization.
- Data analysis, using the best statistical techniques available, or where the data obtained are descriptive only, their presentation according to good logic.

Descriptors

For the characterization and evaluation of accessions, descriptors are used. These generally correspond to characteristics or attributes whose expression is easy to measure, record, or evaluate and which refer to an accession's form, structure, or behaviour. Descriptors help differentiate accessions by expressing their attributes precisely and uniformly, thereby simplifying the accessions' classification, storage, and recovery, and the use of their data. In other words, descriptors are the characteristics through which germplasm can be known and its potential usefulness determined. They should be specific to each species, differentiating genotypes and expressing each attribute precisely and uniformly. Many attributes can be used to describe a material but the really useful ones are those that can be detected by the naked eye; be easily recorded; have high heritability, high taxonomic and agronomic value; are readily applicable to small samples; and can differentiate one accession from another (Hidalgo 2003).

Types of descriptors

To identify germplasm entries (accessions), lists of descriptors have been established for use in accordance with the management stage in which information must be collected. These include (Hidalgo 2003; IPGRI 2004):

- **Passport data**, which provide the basic information for the accession's general management, including registration in the germplasm bank and other information for identification. They also describe the parameters that must be observed when making the original collection.
- **Management descriptors**, which provide the bases for managing the accessions in the germplasm bank and help during multiplication and regeneration.
- **Descriptors of site and environment**, which describe the specific parameters of the site and environment. They also help in the interpretation of results when characterization and evaluation trials are carried out.
- **Descriptors for characterization and evaluation**.

This lesson will emphasize descriptors for characterization and evaluation, although all are important when analyzing a germplasm collection in an integrated manner.

Descriptors for characterization

Characterization descriptors permit relatively easy discrimination between phenotypes. They are usually highly inheritable characters that are easily detected by the naked eye and find expression in all environments. Descriptors related to phenotypic characters mostly correspond to the morphological description of the plant and its architecture. These characters are called **morphological descriptors** and can be grouped into two types: botanical-taxonomic and morpho-agronomic.

Botanical-taxonomic descriptors correspond to morphological characters such as the shape of the root, stem, leaf, flowers, fruit, and seeds (Figure 1) that describe and identify the species and are common to all individuals. Most of these characters have high heritability and present little variability.

Morpho-agronomic descriptors include those morphological characters that are relevant in the use of cultivated species. They can be qualitative or quantitative, and may include some botanical-taxonomic characters and others that do not necessarily identify the species, but are important in terms of agronomic needs, genetic improvement, marketing, and consumption. Examples of these characters include leaf shape; pigmentation of roots, stems, leaves, and flowers; colour, shape, and brilliance in seeds; size, shape, and colour of fruits; and plant architecture as expressed in growth habit and branching types. Some germplasm bank curators include descriptors related to yield components to indicate the potential of the conserved germplasm for this character. Most of these descriptors have acceptable local heritability but are affected by environmental changes. These latter are also called evaluation descriptors.

Evaluation descriptors

Characters for this type of descriptors include yield, agronomic productivity, and susceptibility to stress. They also include biochemical and cytological characters, which are usually of greater interest for crop improvement. Not all plant characteristics are expressed with the same intensity. Some, especially the quantitative, can present different degrees of expression, and are recorded in terms of scales of value (usually between 1 and 9), known as descriptor states (IPGRI 1996). Such descriptors are found for resistance or susceptibility to different types of biotic (pests and diseases) and abiotic stress (drought, salinity, acidity, or

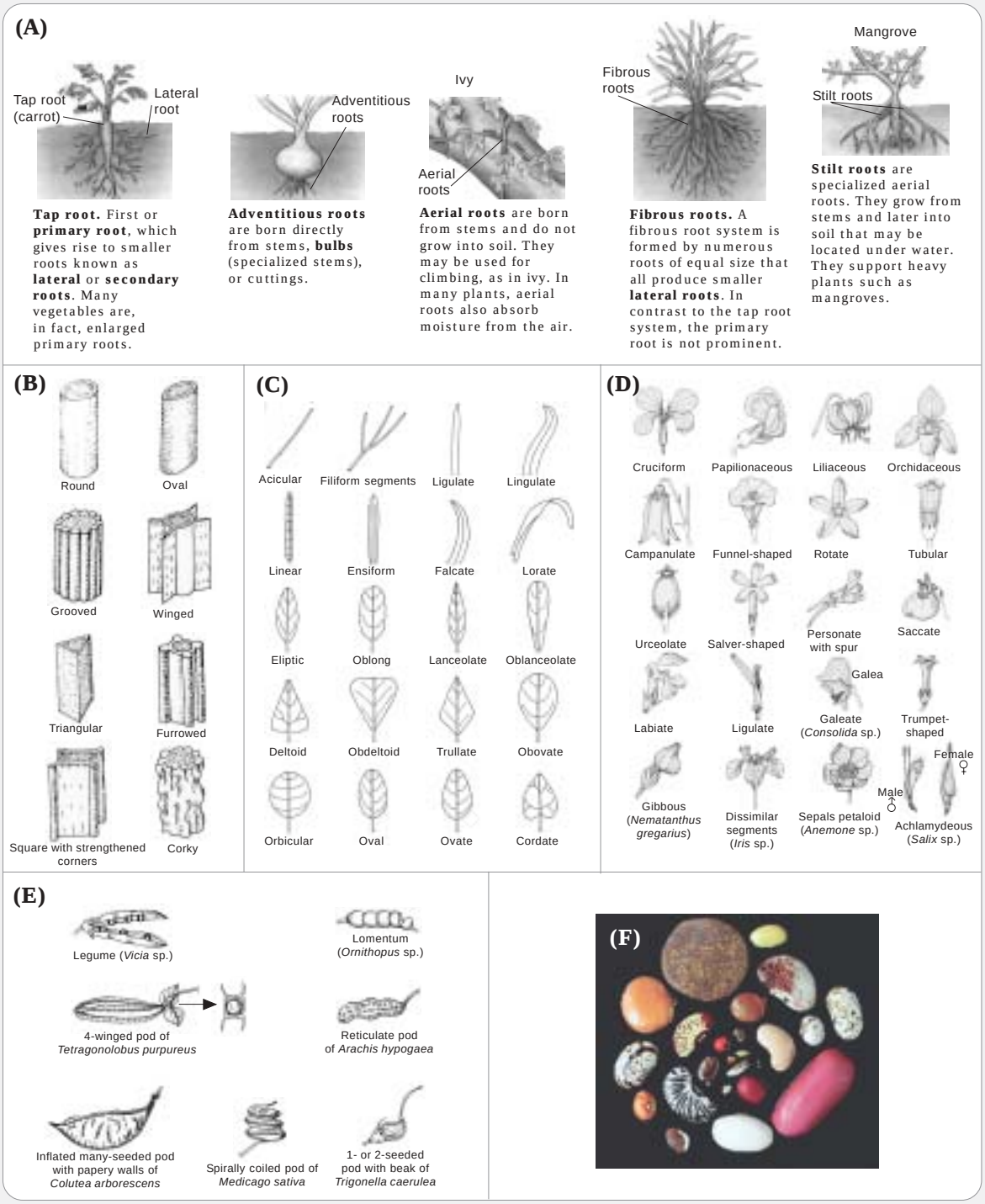

Figure 1. Examples of morphological characters that are used as descriptors to characterize germplasm accessions. **(A)** Root types; **(B)** stem forms; **(C)** leaf shapes; **(D)** flower types; **(E)** fruit types; **(F)** seed types. (From Stockley 1991 [**A**]; Hickey and King 2000 [**B**, **C**, **D**, and **E**]; D Debouck 2004, GRU, CIAT [**F**].)

low soil fertility). Most of the descriptors of this category depend on the environment for their expression and, accordingly, require special experimental designs for their evaluation. Evaluation may also involve complex methods of molecular or biochemical characterization.

Other descriptors

Sometimes, characterization data and morpho-agronomic evaluation are insufficient for establishing differences between species or between accessions. In these cases, genome characteristics may be studied such as the karyotype, chromosome number, and ploidy level. The genome itself can be studied directly, using biochemical (isoenzymes; Simpson and Withers 1986) and molecular markers (microsatellites, restriction fragment length polymorphisms or RFLPs, randomly amplified polymorphic DNA or RAPD, and quantitative trait loci or QTLs). These methodologies help locate genes of interest with greater accuracy but do not evaluate the effect of the environment on the expression of those genes (Westman and Kresovich 1997). Accordingly, they do not replace—but complement—characterization and morpho-agronomic evaluation (IPGRI 2004; IPGRI and CIP 2003).

Descriptor attributes

Depending on whether they involve characterizing or evaluating germplasm, the descriptors used may have various attributes. The principal ones are summarized in Table 1.

Practical Recommendations for Characterization

The characterization of genetic variability has several limitations that are common to almost all germplasm banks at national and international institutions. These should be taken into account when planning procedures. First is the limited quantity and poor quality of available seeds, which does not permit flexibility in multiplication and characterization tasks. Second is the poor documentation of collections, mainly because a high percentage of germplasm existing in banks was the product of opportunistic collections that were made, using criteria based on agronomic characteristics rather than on genetic resources. Finally, resources are scarce for the sustainable maintenance of germplasm banks. This is reflected

Table 1. Attributes of characterization and evaluation descriptors for plant germplasm.

Activity	Attributes of characters or descriptors	Examples
Characterization	Qualitative Environmentally stable Mendelian heredity Mono- and oligogenic Easily manipulable in genetic improvement	Flower and seed colours Proteins Isoenzymes Marker-based PCR
Evaluation	Quantitative Influenced by the environment Additive heredity Oligo- and polygenic Difficult to handle in genetic improvement	Yield Plant height Protein contents Flowering Maturation time

by low numbers of accessions and reduced quantities of seeds per accession (Hidalgo 2003). Accordingly, when characterizing a germplasm collection, the following recommendations should be considered:

- A complete knowledge of the species' biology is necessary, especially on reproduction—sexual, asexual, autogamous, and allogamous—as well as on the centres of origin and domestication.
- Adequate documentation provides useful elements for establishing a preliminary idea of the reference collection. With that idea, the variability to be found in the materials can be inferred, even before initiating characterization. Also, by clearly defining the objectives for characterization, unnecessary steps can be saved.
- Objectives should be clearly established, taking into account the goals being sought, whether these be ascertaining variability in the group or representativeness of the collection, studying the structure, identifying duplicates, or detecting special genes.
- Regardless of established objectives, prior experimental planting should be carried out to discover, in general, the overall variability of the collection, the facility in recording descriptors, and the usefulness of descriptors for seed characterization and multiplication.
- Before attempting the definitive characterization, accessions should be homogenized according to their morphotypes. This is especially important for wild forms and native landraces, which, in their original state, are frequently mixtures of morphotypes in terms of, for example, seed types, growth habits, flower colours, and fruit types. Even if the germplasm bank conserves a complete sample of the original, the characterization of an accession that has a mixture of morphotypes enormously hinders data analysis. If standardization cannot be made when preparing seeds, then, where possible, prior experimental planting should be attempted to achieve this purpose.
- To obtain better and more information for the statistical analysis and reliability of differences among materials and variables, 3 to 5 plants per accession and a minimum of two replications should be established.
- When the availability of seeds or planting materials is low, thereby making the establishment of replicated plots of each accession impossible, then the most homogeneous plot possible should be selected to prevent the effects of variable soil conditions. In these cases, the correct acquisition of data will facilitate comparative analysis between accessions and even between variables.
- If the principal objective is to measure group variability, then descriptors should be selected that are as discriminatory as possible. This will help save time by avoiding repetitive data collection and will simplify analysis. Accordingly, the descriptor lists published by IPGRI for the species under study should be consulted.
- When designing characterization tasks, a statistician or related professional should be consulted on field design, suitable ways of recording and analysing data, and interpretation of results (IPGRI 2001).
- The use of currently available automated programs helps in understanding the procedures related to advanced statistical methods for data analysis for characterization, especially multivariate ones. The key is to know how to interpret results at the point where biological knowledge of the species is important in explaining the results of data analysis (IPGRI 2001).

Evaluating this Lesson

After this lesson, you should be familiar with the basic concepts of plant germplasm characterization and with the types of descriptors used in this process.

Before going on to the next lesson, answer the following questions:

1. If you have had personal experience in germplasm characterization, comment briefly on the type of descriptors you used and why?
2. If you do not have personal experience in germplasm characterization, give your opinion on the process and its importance for *ex situ* conservation, based on the contents of the lesson and the *Recommended Reading* list.

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Next Lesson

In the next lesson, you will study ways of characterizing plant germplasm.

Lesson 2 Ways of Characterizing Plant Germplasm

Objective

To describe types of plant germplasm characterization

Introduction

Under natural conditions, or during domestication, any population of individuals that is part of a plant species is found in permanent dynamic interaction with its environment. It constantly adapts to the biotic and abiotic factors of that environment by adapting the information contained in its genome to the needs for survival in the environment (Hidalgo 2003). The result of such adaptive interaction is an accumulation of genetic information that is stored in the genome, leading to variability among the members of the population. Such variability may or may not find expression in characters, which should be appropriately identified through an activity known as 'characterization'.

During characterization, the existing variability in the genome of the population of individuals is estimated, and methodologies designed for this purpose are used to encompass all aspects related to biodiversity. However, this lesson will focus mainly on **morphological, biochemical, and molecular characterization,** which have the most applicability to plant germplasm.

What Is Involved in Characterization?

During characterization, each entry or accession is systematically described. Descriptors are selected and used according to the category of activity (see *Module 4, Lesson 1*). The best characterization is that where all the characteristics of a germplasm can be observed and recorded. Hence, different methodologies should encompass the categories of diversity that is characteristic of plant germplasm, including those that are biological (i.e., morphological, physiological, and anatomical), taxonomic, ecological, geographical, biochemical, molecular, genetic, and cytogenetic in nature.

Each category of characterization offers a series of opportunities for acquiring information that could be very useful for understanding the germplasm. Nevertheless, the categories most used by germplasm banks are those that deal with **morphological, biochemical**, and **molecular characterization**. For each of these categories, different methodologies or techniques have been developed, which can be applied individually or complementarily to characterize germplasm. None of the available techniques is superior to the others, even over a wide range of applications, as each permits the observation and recording of different parts of the total diversity available for characterization.

The three categories of characterization should be used in a complementary way to estimate the genetic diversity of collections and, accordingly, help establish criteria for improving their representativeness. None of the three is replaced or excluded by another, as each has a different history and can show different facets of the diversity being examined. When a technique must be selected, the following activities should be carried out:

- Define the type of information needed in terms of the desired results.
- Define the level of discrimination, that is, the taxonomic level (e.g., within and/or between populations, between species, or between genera) at which genetic variation will be estimated.
- Estimate reproducibility, as this parameter will genetically identify a collection's accessions and estimate its genetic variation.
- Define the genomic coverage or number of loci that the technique is likely to include.
- Define the number of alleles required in individual loci—this is needed when hypervariability is required.
- Know the mode of inheritance.
- Verify the availability of samples for each technique considered. Thus, germplasm will not be sacrificed to develop a process. Normally, recording morphological descriptors will not consume samples, as the multiplication carried out in the greenhouse and field can be used at the same time to characterize germplasm.
- Estimate the costs of the respective techniques.
- Estimate the speed each technique would take.
- Trained personnel must be available.

Morphological characterization

Characterization is carried out on a representative population of an accession, using a list of descriptors for the species. The representative population of the species is that which represents the accession's total genetic variability so that all the characteristics that it possesses can be observed and recorded. With regard to variability, a representative population is expected to contain at least 95% of the accession's alleles. Population size will be determined by the species' type of reproduction. For example, if it is allogamous (i.e., highly variable), the population should be larger than if it were autogamous (i.e., variability is low).

Descriptors are those characteristics by which germplasm can be known and its potential usefulness determined (see *Module 4, Lesson 1*). For characterization, lists of very useful descriptors have been prepared by IPGRI for more than 110 plants species (IPGRI 1996, 2004), including crops of African importance such as sweet potato (*Ipomoea batata*; Huamán 1991), taro (*Colocasia esculenta*; IPGRI 1999), yam (*Dioscorea* spp.; IPGRI and IITA 1997), and shea tree (*Vitellaria paradoxa*; IPGRI and INIA 2006). However, if little studied crops are being characterized and a list of descriptors is unavailable, then the most relevant characters for the case must be identified.

Material to be characterized is planted in the field or greenhouse, in duly identified plots and under uniform management conditions. Once the targeted populations are established, the characteristics of the species are observed throughout various developmental stages and their expression recorded in terms of a selected set of descriptors. A case of characterization can be seen in Box 1, for ulluco (*Ullucus tuberosus*). Data are systematically and consistently taken and recorded in an orderly way to facilitate their later statistical analysis and to ensure that the information, based on the same descriptors but obtained from different regions, is comparable and compatible.

Box 1

Characterizing ulluco (*Ullucus tuberosus*)

7. Plant descriptors

The entries to be characterized should be maintained in the same environment, receive the same agronomic and conservation management, and be planted at the same density and in the period most appropriate for their growth and development. Plant characters should be recorded during full flowering (130–150 days after planting), whereas tuber characters should be recorded immediately after harvest. The characters of both plant and tubers should be recorded for a representative number of the population for each entry. The recording of data on plant colour and especially tubers is complex and difficult because of the variation existing among most of them. Hence, attempts have been made to simplify the variation of each colour and indicate the most representative. These should be recorded, using the *Colour Chart* of the Royal Society of Horticulture (*RHS Colour Chart*).

The characters indicated below are stable and appropriate for identifying morphotypes and/or duplicates. The numbers and letters in parentheses correspond to the colour or colours listed in the *RHS Colour Chart*.

7.1. Plant data

7.1.1. Plant growth habits

1 Erect
2 Creeping

7.1.2. Elongated stems

Where elongated stems are present during full flowering, three to seven stems per plant stand out from the foliage, with a tendency to be decumbent to creeping, and covering more than 50% of the furrow towards the end of the plant's vegetative cycle.

0 Absence of elongated stalks
1 Erect elongated stems
2 Decumbent elongated stems
3 Creeping elongated stems

7.1.3. Stem colour

1 Pale yellowish green (145A–D)
2 Pale yellowish green (145A–D) predominant, with pale red (pink) (51C, D) irregularly distributed along the length of the stem
3 Greyish red (178B) predominant, with yellowish green (146C, D) irregularly distributed along the length of the stem
4 Greyish red (178A, B; 182A, B)

7.1.4. Pigmentation of aristas/stem angles

0 Absent
1 Present

7.1.5. Leaf blade shape

See Figure 3

1 Ovate
2 Cordate
3 Deltoid
4 Semi-reniform

(Continued)

Box 1. (Continued.)

7.1.6. Foliage colour
1 Pale yellowish green (145A, 146D)
2 Yellowish green (146A)
3 Dark yellowish green (147A)

7.1.7. Colour of lower leaf surface
1 Pale yellowish green (146B–D)
2 Pale yellowish green (146B–D), with reddish purple (59A–D)
3 Reddish purple* (59A-D)

7.1.8. Petiole colour
1 Pale yellowish green (144A–D)
2 Yellowish green (144A, B; 146A–C), with arista/angle pigmented
3 Greyish red (178A–D) predominant, with yellowish green (146B)
4 Greyish purple (183D) predominant, with yellowish green (146B)

7.1.9. Flowering habit
0 Absent
3 Scarce
5 Moderate
7 Abundant

7.1.10. Shape of inflorescence axis (rachis)
1 Predominantly straight
2 Predominantly zigzag

7.1.11. Colour of inflorescence axis (rachis)
1 Pale yellowish green (144A–D)
2 Yellowish green (144A, B; 146B–D), with reddish purple (58A, B) irregularly distributed
3 Reddish purple (58A; 59A, B) predominant, with green

7.1.12. Sepal colour
1 Yellowish green (150D; 154C, D)
2 Pale reddish purple (58C, D)
3 Reddish purple (58A; 59A, B)

7.1.13. Petal colour
1 Yellowish green (151C, D)
2 Yellowish green (151C, D), with reddish purple (59B–D) apex
3 Yellowish green (151C, D) with reddish purple (59A–C) apex and margins
4 Reddish purple (59A, B), with yellow orange (14C; 15C, D) base

7.1.14. Tendency to form flowers with more than five petals
0 Absent
1 Present

7.2. Data on tubers

7.2.1. Predominant colour of tuber surfaces
1 Yellowish green (145B–D, 147D, 148D)
2 Yellowish white (8D)

* The dish purple becomes progressively more intense towards the end of the cropping cycle.

(Continued)

Box 1. (Continued.)

3 Yellow (10A)
4 Dark yellow (13B)
5 Greyish yellow (162C)
6 Yellow orange (19A)
7 Pale orange (22A; 24B)
8 Orange (26A, B)
9 Reddish orange (33A)
10 Pale red (pink) (51C, D)
11 Red (46D)
12 Reddish purple (61A)

7.2.2. Secondary colour of tuber surfaces
0 Absent
1 Yellowish white (4D)
2 Pale red (pink) (54C)
3 Reddish purple (61A)

7.2.3. Distribution of secondary colour on tuber surfaces
0 Absent
1 Eyes
2 Irregularly distributed
3 Eyes and irregularly distributed

7.2.4. Tendency to produce chimeras
0 Absent
1 Present

7.2.5. General tuber shape
See Figure 4
1 Round
2 Cylindrical
3 Semi-falcate
4 Twisted

7.2.6. Colour of cortical area
1 Yellowish green (145B, C)
2 Yellowish white (4D)
3 Yellow (12C; 13A)
4 Orange (26A)
5 Reddish orange (33A–D)
6 Pale red (pink) (50C; 51C, D)
7 Red (46D, 53B)
8 Reddish purple (61A)

7.2.7. Colour of central cylinder
1 Yellowish green (145B, C)
2 White (155A–D)
3 Yellowish white (4D)
4 Yellow (12C; 13A)
5 Yellowish orange (20B)

7.3. Notes
Add any further information here

SOURCE: IPGRI and CIP (2003).

Information is sometimes taken from observations that record the presence or absence of a characteristic (e.g., thorns or trichomes) or sometimes taken as quantities, for example, number of fruits, plant height, or number of stamens. Structures should then be counted and/or measured, using tape measures, rules of several sizes, and graduators. Highly precise recording of data will need tools such as:

- Colour charts, for example, the *RHS Colour Chart* (1982), the *Methuen Handbook of Colour* (Kornerup and Wanscher 1984), or the *Munsell Plant Tissue Charts* (Munsell Color 1975, 1977)
- Vernier calibrators
- Microscopes or stereomicroscopes
- Balances
- pH meters
- Durometers (to measure the resistance or hardness of peel and pulp)
- Stoves (to calculate quantities of water and dry matter)
- Chemical reagents
- Laboratory instruments (for enzymatic and molecular characterization and evaluation)

Biochemical characterization

In this type of characterization biochemical markers are used that are principally isoenzymes (metabolism enzymes) and total proteins (e.g., seed storage proteins). These markers have two very useful characteristics as tools for characterizing plant germplasm: they occur naturally, and their expression is not influenced by epistatic effects (Simpson and Withers 1986).

Isoenzymes. These molecular forms of a single enzyme have affinity for a given substrate found in the tissue of an organism and are coded by different loci. These differentiate among themselves according to size (weight), shape, and electrical charge. When they are coded by different alleles from a simple locus they are called **alloenzymes**. Isoenzymes can be found in the same subcellular compartment, in different compartments of the cell, or in different cells or tissues of an organism; and can be produced in any developmental stage of the plant.

The principal characteristics of isoenzymes include simplicity, a minimum quantity of material for study, low cost, a genome coverage of 10 to 20 loci per species, and the absence of epistatic and environmental influences. Allelic expression is codominant, permitting comparisons between species or between populations of a single species, and the detection of hybrids and gene introgression.

Generally, applications of isoenzymes in the characterization of plant germplasm conserved *ex situ* can be summarized as follows: germplasm identification (fingerprinting), detection of redundant germplasm (genetic duplicates), analysis of the genetic structure of plant populations, plant systematics, evolution of domesticated plants, and molecular studies (Simpson and Withers 1986).

Total proteins are components of plants and, as genetic markers, they are characterized by:

- A high level of polymorphism;
- Limited environmental influence on their electrophoretic patterns;
- Simple genetic control;
- A complex molecular base for genetic diversity; and
- Homologues among protein types of different taxons, which enables them to be identified (taxonomy) or have their relationships established (phylogeny).

Seed proteins have been heavily used in the analysis of genetic diversity within and between populations, and in the study of relationships between genomes or species, especially serial polyploidy. Table 1 provides a comparison of attributes of isoenzymes and seed proteins as biochemical markers.

Table 1. Attributes of total seed proteins and metabolic enzymes (isoenzymes or alloenzymes) that can be used in studies on characterization, evolution, genetics, and germplasm improvement.

Attributes	Seed proteins	Enzymes (isoenzymes or alloenzymes)
Polymorphism	High	Low
Environmental stability	High	Moderate to high
Stability when domesticated	Unaffected by selection pressures	Unaffected by selection pressures
Genome coverage	Moderate (<10 loci)	Low (<50 loci)
Inheritance	Biparental and codominant	Biparental, maternal, and codominant
Molecular base	Complex	Simple
Comparability of studies	High	Good
Sample types for analysis	Dry seeds (conservation time is not important)	Multiple conserved tissues
Practicability	Quick, simple, inexpensive, and easy to transfer	Quick, simple, inexpensive, and easy to transfer

Molecular characterization

In the last 2 decades, molecular characterization of plant germplasm has gained great importance for both the quantity and quality of results obtained (Mendoza-Herrera and Simpson 1997; Westman and Kresovich 1997). Previously, these results could not be obtained, as they were based on characterizing the phenotype, especially morphologically, and, to a lesser extent, biochemically. Now, a large variety of molecular methodologies, based on DNA, is now available, making the direct characterization of the genotype possible (Westman and Kresovich 1997). Hence, these modern methodologies provide the means of

knowing the genetic diversity of a germplasm collection, in terms of its measurement and distribution. Furthermore, conclusions can now be drawn on the phylogenetic relationships among the accessions of a collection and even among taxons.

However, the use of this type of germplasm characterization carries with it the same philosophy that is applied to morphological and biochemical characterization: that it be used to complement the other types. Hence, together, they provide the best technique for characterizing the genetic diversity of a germplasm collection, meaning that phenotypic characterization can never be ignored in the study of the targeted biological material.

Currently, thanks to advances in molecular biology, markers are now being used. These markers have a DNA molecular nature and are highly sensitive to changes in the genotype of individuals. This situation has permitted major advances in studies on the genetic characterization of plant germplasm. The selection of the marker to use depends on the study objectives, the availability of the germplasm to characterize, cost, and the marker's inherent characteristics.

Research on DNA-based technologies has been favoured with the availability of numerous markers such as those based on restriction fragment length polymorphisms (RFLPs) and the polymerase chain reaction (PCR). From these two techniques multiple techniques have derived, for example, random amplified polymorphic DNA (RAPD); amplified fragment length polymorphism (AFLP); and variable number tandem repeats (VNTR), that is, both minisatellites and microsatellites (or simple sequence repeats or SSR). A monomorphic molecular marker is invariable in all organisms studied, but when a marker presents differences in molecular weight, enzymatic activity, structure, or restriction sites, it is polymorphic. The degree of variation is sometimes such that these markers are called hypervariable. To characterize PGRs, the following markers are used:

RFLPs (restriction fragment length polymorphisms). The technique that uses these markers was developed at the end of the 1970s. It is based on detecting, through digestion with the same restriction enzyme, DNA fragments of different molecular weights in different organisms. The use of RFLPs in plants represents a good alternative for conducting various studies related to the three genomes that exist in plants: nuclear (nDNA), mitochondrial (mtDNA), and chloroplast (cpDNA). This technique has proved very useful in studies on plant phylogeny and genetic diversity, and for identifying cultivars for varietal protection.

RAPDs (random amplified polymorphic DNA). This technique has proved to be one of the most versatile since its development in 1990. It is very convenient and quick, requiring little DNA that, moreover, does not need to be very pure. It does not presuppose previous knowledge of the sequence, and can quickly and simultaneously distinguish many organisms. However, one drawback is that the amplified fragments tend to correspond to redundant DNA rather than to DNA that is linked to some trait. Nor does the technique give information on the number of copies of the amplified sequence in the genomic DNA.

This technology has been used to catalogue fruit, select varieties, and differentiate among clonal lines. It is also used to analyse varieties of celery, grape, lemon, and olive, and to study the genetic diversity of crops and their relationships with wild ancestors.

AFLPs (amplified fragment length polymorphisms). The technique was developed in 1995. It combines the use of restriction enzymes and oligonucleotides for PCR, so that very specific molecular markers are obtained without needing to know the sequence beforehand. One special advantage of this technique is its capacity to generate many molecular markers in a single reaction.

Minisatellites (VNTRs). These are repeated sequences that occur in eukaryotes. They are found repeated in tandem and are scattered throughout the genome, representing many loci. Each locus has a distinct number of variable repeats, thus associating itself with specific alleles of high variability. These sequences are used as probes of SSRs. Minisatellites have been used to study genetic diversity and to identify ('fingerprint') individuals in various species, both for accession description and detection of genetically duplicated accessions (i.e., redundant germplasm).

Microsatellites or SSRs (simple sequence repeats). This technique was described in 1989. Genomes carry a ubiquitous and abundant DNA known as 'microsatellites' that comprises mono-, di-, tri-, and tetra-nucleotides repeated in tandem. This DNA, which is highly polymorphic, has been used as molecular markers when the sequence of the repeated motif is cloned and sequenced for use in population analysis. In this way, numerous trees have been successfully studied, even though some trees showed a narrower variability of microsatellites than expected. Other variations of this technique are inter-simple sequence repeats (ISSR), inter-sequence microsatellite amplified (IMA), inter-sequence amplified (ISA), inter-sequence repeat amplified (IRA), and random amplified microsatellite polymorphisms (RAMP).

Microsatellites have also been used to measure the genetic diversity of various annual crops such as soybean (*Glycine max)*, rice (*Oryza sativa)*, maize (*Zea mays)*, barley (*Hordeum vulgare)*, wheat (*Triticum aestivum*), and rape (*Brassica* sp.). Although microsatellites have been developed for perennial species, their numbers have been few. For *Eucalyptus* spp., microsatellites have been transferred between populations and species of the same genus. Additionally, these markers help in estimating heterozygosity, thus presenting a great advantage in mapping quantitative characteristics, comparing maps that include QTLs, and studying genetic flow in trees.

Germplasm Evaluation

Once the morphological and anatomical characteristics of the germplasm are known through characterization, the information for determining its potential for use is broadened through evaluation. This process describes the agronomic characteristics of accessions in the maximum number of environments possible. These characteristics are usually quantitative variables, which are influenced by the environment and low heritability as, for example, yield or resistance to biotic or abiotic stress. The goal is to identify adaptable materials that have useful genes for food production and/or crop improvement. Most cases of evaluation are carried out by breeders (Jaramillo and Baena 2000).

Evaluation complements characterization and is also carried out on a representative population of the species, using descriptors (Box 2). It can be carried out in the field, greenhouse, or laboratory, depending on the characteristic being evaluated, and following the same procedures. Unlike characterization, where plants are planted only once, the

Box 2

Evaluating ulluco (*Ullucus tuberosus*)

8. Plant descriptors

8.1. Plant emergence in the field (sprouting) (days)
Determine from the day of planting until at least 50% of plants for each entry has emerged or sprouts
1 Early (<40 days)
2 Intermediate (40–60 days)
3 Late (>60 days)

8.2. Days to flowering
Count from the day of planting until at least 50% of plants for each entry has flowered
0 No flowering
1 Early (<130 days)
2 Intermediate (130–150 days)
3 Late (>150 days)

8.3. Duration of flowering
Record from the appearance of the first flowers in at least 50% of plants for each entry until senescence appears in more than 50% of plants
0 No flowering
1 Short (<30 days)
2 Intermediate (30–60 days)
3 Long (>60 days)

8.4. Days to harvest
Record from the day of planting until more than 50% of plants for each entry has become senescent
1 Early (<7 months)
2 Intermediate (7–8 months)
3 Late (>8 months)

8.5. Plant height (cm)
Measure during full flowering from the base of the main stem to the apical buds (it should be understood that the main stem or stems are measured, not the elongated ones)
1 Short (<25 cm)
2 Intermediate (25–35 cm)
3 Tall (>35 cm)

8.6. Leaf length (cm)

8.7. Leaf width (cm)

8.8. Length of petiole (cm)
Measure from the base of the petiole to the base of the central nervure
1 Short (<3 cm)
2 Intermediate (3–6 cm)
3 Long (>6 cm)

(Continued)

Box 2. (Continued.)

8.9. Weight of tubers per plant (kg)
1 Low (<0.7 kg)
2 Intermediate (0.7–2.0 kg)
3 High (>2.0 kg)

8.10. Notes
Add any further information here

9. Susceptibility to abiotic stress
Record under clearly specified artificial and/or natural conditions; code the observations on a numerical susceptibility scale from 1 to 9, where:
1 Very low or no visible signs of susceptibility
3 Low
5 Intermediate
7 High
9 Very high

9.1. Low temperatures
Record under natural conditions during days of frosts

9.2. High temperatures
Record under natural conditions during the hot season

9.3. Drought
Record daily under natural conditions for at least 4 weeks

9.4. Days of hail
Record during days of hail

9.5. High soil moisture
Record under flood conditions for more than 4 weeks

9.6. Soil salinity

9.7. High soil acidity

9.8. Alkalinity

9.9. Shade

9.10. Notes
Add any further information here

10. Susceptibility to biotic stress
For each case, specify the origin of infestation or infection, whether natural or inoculation in the field or laboratory. Record such information under descriptor *10.4. Notes*.
Code plant susceptibility according to a numerical scale of 1 to 9, where:
1 Very low or no visible signs of susceptibility (0%)
3 Low (1%–25%)
5 Intermediate (26%–50%)
7 High (51%–75%)
9 Very high (76%–100%)

(Continued)

Box 2. (Continued.)

10.1.	**Pests**		
	Causal organism	***Common names in English, Spanish, and Quechua***	***Symptom in:***
10.1.1.	*Cylydrorhinus* sp.	Ulluco weevil; gorgojo del ulluco; ulluku kuru	Tuber (Tu)
10.1.2.	*Copitarsia turbata*	White grub; gusano de tierra; silwi kuru	Stem
10.1.3.	*Agrotis* sp.	Cutworms	
10.1.4.	*Ludius* sp.	Wireworm; gusano alambre; k'aspi kuru	Tu
10.1.5.	*Epitrix* sp.	Flea beetle; pulguilla saltadora; piki k'uti	Foliage (Fo)
10.1.6.	*Frankliniella tuberosi*	Black thrips; trips negro; yawa; k'ello kuru	Fo
10.1.7.	*Bothynus* sp.	White grub; gusano blanco; gusano arador; lakato	Tu
10.1.8.	Scarabaeidae	White grubs; gusanos blancos; gusanos aradores; wali kuru	Tu
10.1.9.	*Nacobbus aberrans*	Potato rosary nematode; nematodo rosario de la papa	Roots
10.2.	**Diseases**		
10.2.1.	*Alternaria* sp.	Leaf spot; mancha anillada	Fo
10.2.2.	*Alternaria alternata*	Alternaria blotch; mancha anillada	Fo
10.2.3.	*Alternaria solani*	Early blight; mancha anillada	Fo
10.2.4.	*Cladosporium* sp.	Black mould; mancha	Fo
10.2.5.	*Ascochyta* sp.	Ascochyta blight; mancha oval	Fo
10.2.6.	*Pleospora* sp.	Foliar blight; mancha zonada	Fo
10.2.7.	*Aecidium ulluci*	Ulluco rust; roya de la papalisa	Fo
10.2.8.	*Botrytis cinerea*	Grey rot; pudrición gris	Fo/Tu
10.2.9.	*Pythium ultimum*	Black rot; leak; gotero	Fo/Tu
10.2.10.	*Rhizoctonia solani*	Rhizoctonia blight; rizoctoniasis	Fo/Tu
10.2.11.	*Phoma exigua*	Stem rot; gangrena	Fo/Tu
10.2.12.	*Hypochnus* sp.	Rot; pudrición	Tu
10.2.13.	*Rhizopus oryzae*	Soft rot; pudrición	Tu
10.2.14.	*Fusarium oxysporum*	Wilt; pudrición	Tu
10.2.15.	*Dematophora* sp.	White root rot; lanosa	Tu
10.2.16.	*Thielaviopsis basicola*	Black tuber rot; manchado del tubérculo	Tu
10.2.17.	*Verticillium dahliae*	Verticillium wilt; marchitez	Fo
10.2.18.	*Erwinia carotovora* subsp. *carotovora*	Bacterial soft rot; pudrición suave	Tu

10.3. Viruses
Viruses are best coded according to the following scale:
1 Susceptible
2 Resistant
3 Immune

(Continued)

Box 2. (Continued.)

	Causal organism	***Common names in English, Spanish, and Quechua***	***Symptom in:***
10.3.1.	Ullucus virus C (UVC)	Virus C del ulluco	Fo
10.3.2.	Potato leafroll virus (PLRV)	Virus del enrollamiento de la papa	Fo
10.3.3.	Arracacha virus A (AVA)	Virus A de la arracacha	Fo
10.3.4.	Papaya mosaic virus (PapMV)	Virus del mosaico de la papaya	Fo
10.3.5.	Ullucus mosaic virus (UMV)	Virus del mosaico del ulluco	Fo
10.3.6.	Tobacco mosaic virus (TMV)	Virus del mosaico del tabaco	Fo
10.3.7.	Potato virus T (PVT)	Virus T de la papa	Fo
10.3.8.	Andean-potato latent virus (APLV)	Virus latente de la papa andina	Fo

10.4. Notes
Add any further information here

11. Biochemical markers

11.1. Isoenzymes
Indicate, for each enzyme, the tissue analysed and zymogram type. Each enzyme can be specifically recorded as 11.1.1; 11.1.2, etc. Examples: phosphatase acid (ACPH); esterases α and β (EST A and B); isocitrate dehydrogenase (IDH); malate dehydrogenase (MDH); phosphogluconate dehydrogenase (PGD); phosphoglucose isomerase (PGI); phosphoglucose mutase (PGM); and peroxidases.

11.2. Other biochemical markers (e.g., polyphenol profiles)

12. Molecular markers
Describe any specific, useful, or distinctive feature for this accession, and indicate the probe-enzyme combination analysed. Some of the more commonly used basic methods are described below.

12.1. Restriction fragment length polymorphism (RFLP)
Indicate the probe-enzyme combination (this criterion can be used for nuclear, chloroplast, or mitochondrial genomes).

12.2. Amplified fragment length polymorphism (AFLP)
Indicate the combinations of initiating pairs and the exact molecular size of the products (used for nuclear, mitochondrial, or chloroplast genomes).

12.3. DNA amplification fingerprinting (DAF); random amplified polymorphic DNA (RAPD); arbitrarily primed polymerase chain reaction (AP-PCR)
Indicate accurately the experimental conditions (initiators, etc.) and the molecular size of the products (used for nuclear, mitochondrial, or chloroplast genomes).

(Continued)

Box 2. (Continued.)

12.4. Sequence-tagged microsatellite site (STMS)
Indicate initiating sequences and the exact size of the products (can be used for nuclear or chloroplast genomes).

12.5. Other molecular markers

13. Cytological characteristics

13.1. Number of mitotic chromosomes

13.2. Ploidy level
(2x, 3x, 4x, etc.)

13.3. Chromosome pairing during cellular division
Make observations of chromosome pairing during meiosis and mitosis. Descriptions should be based on the mean of observations across several cells.

13.4. Other cytological characteristics

14. Identified genes

SOURCE: IPGRI and CIP (2003).

germplasm must be planted at the same time in different environments and over several years. Hence, evaluating all accessions is not economically feasible. Instead, a preliminary evaluation must be conducted to observe how accessions adapt to the new environment. Those that perform well against a check are further evaluated in terms of a specific objective.

The planning of evaluation tests should take into account the species, evaluation objective, sites, and follow an experimental design with several sites and replications. Germplasm evaluation also requires a uniform management of plots and a systematic collection and recording of the data observed to facilitate statistical analysis and allow conclusions to be made on the material's usefulness (IPGRI 2001).

Statistical Tools for Characterization

During characterization, visible characteristics of a species may well be *more or less* uniform, with not all expressed at the same level of intensity. Some members of the population may present sufficiently different degrees of expression that they translate into different types of data or categories of variables. Different categories of data therefore exist according to the expression of the descriptor, being either qualitative or quantitative.

If expression is **qualitative**, then binary data (i.e., double state data), sequential data (ordinal), and nonsequential data (nominal) can be generated. If expression is **quantitative**, then the generated data may be discontinuous (or discreet) and continuous. These are usually organized into a basic data matrix (Hidalgo 2003).

Germplasm characterization generates a considerable quantity of data that must be analysed. One analytical tool comprises statistics, through which scientific methods compile, organize, summarize, present, and analyse the data to obtain valid conclusions and make decisions.

From a practical viewpoint and considering the impossibility of studying an entire population or universe, **representative samples** of the population under study are examined. Because deductions at a given time may not be absolutely certain, the language of **probability** is used to formulate conclusions. Moreover, statistics may be **inferential** when referring to conditions under which a deduction is valid, or **descriptive** or deductive, when statistics are used only to describe and analyse a given group without drawing conclusions or inferences to a greater group (Spiegel and Lindstrom 2000).

Data can be analysed by using **simple** or **complex methods**, which range from the use of figures and statistics for main tendencies and dispersions to multivariates. Analysis aims to reduce the volume of information typical in studies of this nature. By applying these methods on the basic matrix of data, conclusions can be made on the variability and usefulness of germplasm. Hence, data should faithfully represent the characteristics and behaviour of the accessions studied (Hidalgo 2003).

Evaluating this Lesson

After this lesson, you should be familiar with the ways used to characterize plant germplasm. You have now finished *Module 4: Germplasm characterization*.

Before going on to the next *Module 5*, comment briefly on the type of germplasm characterization you have used in your work. If you do not have experience with these processes, consider the importance of characterization for the *ex situ* conservation of plant germplasm.

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Further reading

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Next Lesson

In the next *Module 5*, you will study aspects of germplasm bank management.