Lesson 1 Harvesting

Objectives

- To describe fruit types, seed parts, and propagules
- To review the principal aspects of harvesting germplasm and the care needed to guarantee its integrity

Introduction

Once the germplasm targeted for conservation has been multiplied or regenerated (whether under field conditions or in the greenhouse, mesh house, or laboratory), it is harvested. During multiplication or regeneration, natural biological processes occur that lead to the formation of reproductive structures. For plants that reproduce primarily by seed, flowers form, pollination occurs, and the ovule develops and matures into seed while, simultaneously, the ovary becomes fruit that eventually contains harvestable seeds. Certain types of plants not only produce seeds but also reproduce vegetatively. These plants also form propagules that carry one or more growth buds that, once independent, generate roots to give rise to new plants. New individuals may also result from natural or mechanical fragmentation of any piece of the plant. These are harvestable for conservation purposes.

To develop the theme, this lesson will deal with aspects related to harvesting and, in the next lesson, to conditioning and quantification. Before describing what is harvesting, and to help understanding of the process, we will discuss fruit types, principal seed parts, and the propagules associated with plant reproduction.

Fruit Types

A fruit is the mature ovary that contains the plant's seed or seeds (Figure 1). To the extent that the ovary develops after fecundation, it changes size, consistency, colour, chemical composition, and shape. Transformations are of two types: (1) dry fruits in which the cells become enveloped in very thick walls that lignify and harden; and (2) fleshy fruits in which the walls gelate and their tissues lose their cohesion, becoming more or less aqueous on ripening. Structures other than the ovary may become part of the fruit such as parts of the floral axis or tissues of foliar origin. Thus, in tomato, for example, the fleshy part is formed by the carpels, which form the ovary; in blackberry, this tissue is formed by petals that have been conserved; and in figs (green or ripe), receptacles of inflorescences form the flesh.

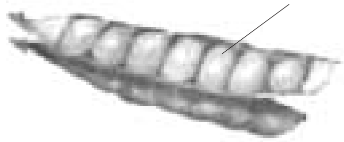
During maturation, specific physical and chemical changes occur that lead to fruit senescence and seed dissemination. One very obvious change is the drying of fruit tissues. In certain fruits, this leads to dehiscence and discharge of seeds. The colour of fruits and seed coats may change, and the fruits may soften. Immature fruit is invariably green because of the presence of chlorophyll, but as it ripens, the chlorophyll decomposes and may disappear altogether, exposing other colours, particularly those with certain pigments.

Three main criteria are used to classify fruit types: origin, composition, and description. The last is the most useful for harvesting and conditioning purposes.

Fruits

A fruit contains the seeds of a plant. True fruits develop exclusively from the ovary, whereas false fruits may also develop from nonovarian tissues such as the receptacle (e.g., strawberry). The fruit's outside wall is known as the pericarp and is sometimes divided into an outer skin or epicarp, a fleshy part or mesocarp, and an inner layer or endocarp. Main fruit types are listed below.

- **Legume** or **pod** (e.g., pea). The seeds adhere to the internal face of the fruit wall. To open, the pod breaks longitudinally.

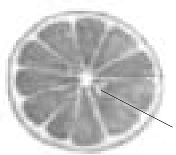


Pea pod

- **Achene.** A small dried fruit with only one seed. 'Winged' achenes (e.g., American sycamore or buttonwood) are known as **samaras**, **keys**, **helicopters**, or **whirligigs**.

Sycamore samara

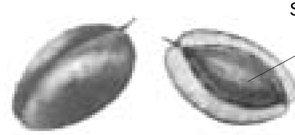
- **Berry** (orange, blackcurrant). A fleshy fruit that contains many seeds.



Orange

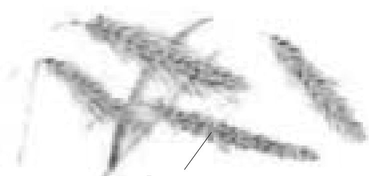
Blackcurrant

- **Drupe** (plum). Fleshy fruit, in the centre of which is a hard seed that is often called a 'stone'.



Plum

- **Grain** or **caryopsis** (wheat). The wall of this small fruit is fused with the seed sheath.

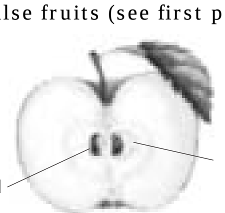


Wheat grains

- **Nut** (hazelnut, walnut). Dry fruit with a hard shell that contains only one seed.

Hazelnut

- **Pome** (apple). This type of fruit has a thick outer layer, a fleshy layer, and a core. Its seeds are enclosed within a capsule. Pomes are examples of false fruits (see first paragraph).



Apple

Figure 1. Examples of different types of fruits and their definitions (from Stockley 1991).

Essentially, a fruit can be classified as dry, fleshy, or originating from an inflorescence. The category depends on whether the ovary concerned had formed hard or fleshy structures, or the flower had one or more pistils, or the flower had been part of an inflorescence.

Dry fruits

Dry fruits are lignified structures that may or may not open spontaneously. Those that do not open are known as **indehiscent** and tend to contain a single seed. Such fruits include:

- The **achene**, which is a fruit with a single seed, for example, those of the composite family such as the pappuses of daisies and sunflowers;
- The **caryopsis** is similar to the achene, but has the pericarp welded onto the seed, as occurs in grasses;
- The **nut** is also similar to the achene but has a hard pericarp, sometimes stony, like acorns and hazelnuts, and
- The **samara**, which has winged structures that help its dissemination by wind, as occurs in elms and several other big trees;

Dry fruits that open are known as **dehiscent**. They tend to contain more than one seed. The fruit types falling into this category are:

- The **follicle**, typical of the Ranunculaceae, which opens along the line of suture of its only carpel;
- The **legume** or **pod**, typical of legumes, is similar to the follicle but opens along two sutures;
- The **silicle** or **silique**, common in the Crucifer family, has two halves separated by a partition that persists after dehiscence; and
- The **capsule**, which varies considerably in how it opens and the number of compartments it contains; it is typical of the Papaveraceae, Liliaceae, and Primulaceae families.

Fleshy fruits

Fleshy fruits are aqueous and do not open. Seed is liberated when birds or animals devour the flesh or when this decomposes after falling to the ground after ripening. Principal types of fleshy fruit are:

- **Drupe**, in which the endocarp tends to be hard and the mesocarp fleshy, as occurs in olives, walnuts, almonds, plums, peaches, or myrobalans;
- **Berry**, in which both mesocarp and endocarp are fleshy, as in grapes and tomatoes;
- **Hesperidium**, which is a berry that is fleshy between the endocarp and seeds, as in citrus fruits;
- **Pome**, which has a coriaceous endocarp and an external part that derives from the floral receptacle, as in apple, pear, or quince;
- **Infructescence**, cluster of fruits, derived from an inflorescence or group of inflorescences. Principal types are:
  - **Multiple fruits**, in which individual ovaries of many separate flowers cluster together on a common axis. Types include:

- **Syconium**, in which a large number of small drupes from an entire inflorescence are enclosed within a cavity, as in fig; and
- **Sorosis**, which is a group of berries is traversed by a fleshy axis, as in the pineapple and others of the *Ananas* genus.

– **Aggregate fruit**, in which a group of separate fruits develop from the carpels of one flower, as in strawberry or blackberry.

Seeds and Their Parts

Botanically, an angiosperm seed is a mature ovule that is enclosed within the ovary or fruit. The seeds and fruit of different species vary greatly in the aspect, size, shape, place, and structure of their embryos and presence of food storage tissues. In terms of seed management, the seed cannot always be separated from the fruit, as they sometimes form a unit. In such cases, the fruit itself is treated as 'seed', as with maize and wheat (Hartmann and Kester 1971). A seed has three basic parts: embryo, tissues for storing food, and seed coats (Figure 2).

Embryo

The embryo is a newly formed plant that results from fertilization, that is, from the union of the male and female gametes. Its basic structure consists of an axis with growing points at each extreme, one for the stem and the other for the root, and one or more seminal leaves (cotyledons) set at the embryonic axis. Plants are classified according to their number of cotyledons. Monocotyledonous plants (e.g., grasses and onion) have one cotyledon, whereas dicotyledonous plants (e.g., beans, cowpea, or peach) have two. Gymnosperms (e.g., pine and ginkgo) may have as many as 15.

Food storage tissues

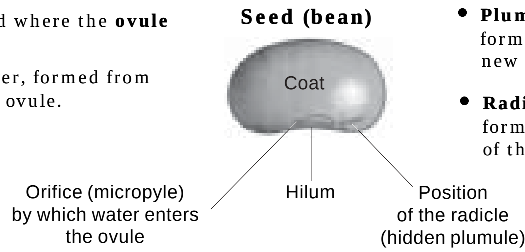
Food storage tissues in a seed may comprise **cotyledons**, **endosperm**, **perisperm**, or, as in gymnosperms, the haploid **female gametophyte**. Those seeds in which the endosperm is large and contains most of the stored food are called **albuminous seeds**. Those that either lack the endosperm or have it reduced to a thin layer surrounding the embryo are called **exalbuminous seeds**. In the latter, food reserves are found in the cotyledons and the endosperm is digested by the embryo during its development. The **perisperm**, which originates in the nucellus, occurs in several plant families such as the Chenopodiaceae and Caryophyllaceae. Normally, during seed formation, it is digested by the endosperm as the latter develops.

Seed coat or testa

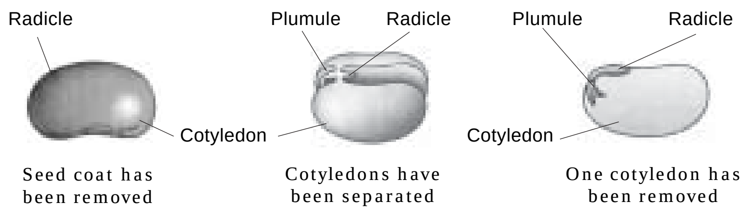
One or two seed coats (rarely three) may be formed by sheaths from the seed, from residues of the nucellus, and sometimes by part of the fruit. The coats derive from the integuments of the ovule. During development these coats are modified and at maturity present a characteristic aspect. In general, the outside seed coat dries, hardens, thickens, and takes up a colour that may be coffee coloured or other tone. The inside coat usually remains thin, transparent, and membranous. Within this layer remnants of the nucellus and endosperm may be found, sometimes forming a distinct continuous layer around the embryo.

Seed parts

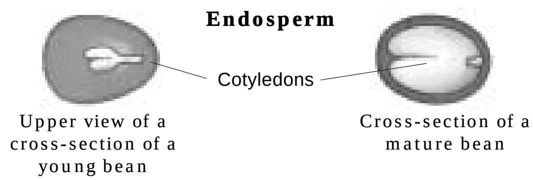
- **Hilum.** Scar on the seed where the **ovule** had joined the **ovary**.
- **Coat** or **testa.** Seed cover, formed from the **integuments** of the ovule.



- **Plumule.** First or **primary bud**, which forms within the seed and becomes the new plant's first shoot.
- **Radicle.** First or **primary root**, which forms within the seed and becomes part of the new plant.



- **Endosperm.** Layer of tissue within the seed, covering the developing plant and contributing food. In some plants such as the pea, the **cotyledons** absorb and store all the endosperm before the seed matures; in others such as grasses, the endosperm is not completely absorbed until the seed **germinates**.



- **Cotyledon** or **seed leaf.** A simple leaf that forms part of the developing plant. In certain seeds such as those of beans, this leaf absorbs and stores all the **endosperm's** food. **Monocotyledons** (e.g., grasses) are plants with only one cotyledon and **dicotyledons** (e.g., pea) are plants with two cotyledons.

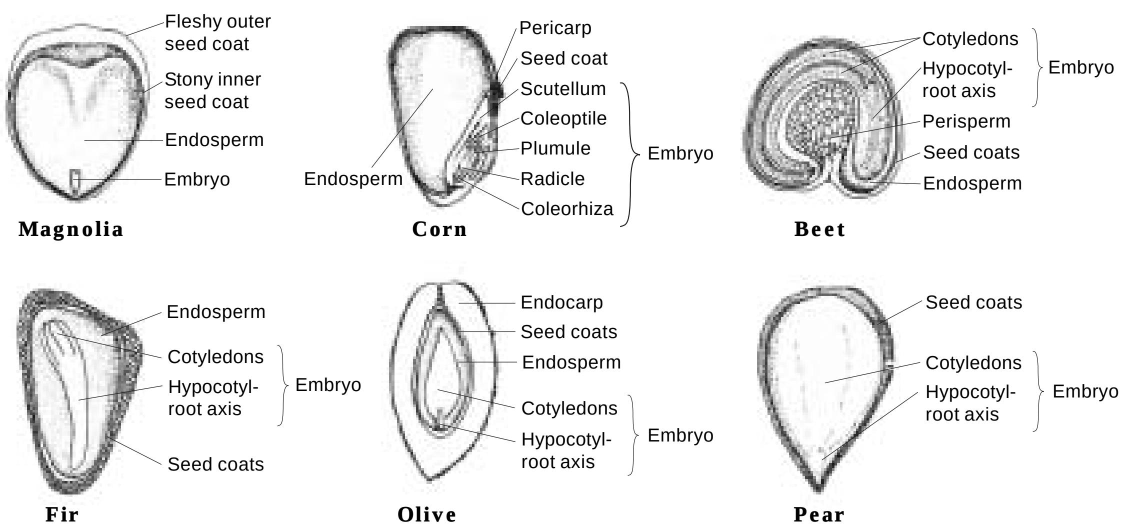


Figure 2. Seeds parts in different plant species (upper drawing from Stockley 1991; lower drawing from Hartmann and Kester 1971).

In some plants, parts of the fruit adhere to the seed, so that both are regarded as 'seed'. In certain classes of fruits such as achenes, caryopses, samaras, and schizocarps, the fruit and seed layers are contiguous. In other fruits such as acorns, the fruit and seed coats are separate but the fruit coat is indehiscent. In still others such as the 'stone' in many fruit trees (e.g., peach and almond) or the 'peel' of the common walnut, the coat is a hardened part of the pericarp but is dehiscent and can be removed without much difficulty. The seed coats provide the embryo with mechanical protection. Hence, the seed can be handled without damage and therefore be transported long distances and stored over long periods. Seed coats significantly influence germination.

Vegetative Reproduction: Propagules and Plant Fragments Used for Reproduction

Many plants can reproduce vegetatively, that is, through plant parts. Such reproduction is possible because those plants have organs with regeneration capacity. Stem parts can form new roots and root parts can regenerate new stems. Leaves can regenerate new stems and roots. A stem and a root (or two stems), when suitably combined, such as in grafting, form continuous vascular connection to produce a new plant (Hartmann and Kester 1971; Vázquez Y et al. 2004).

Vegetative reproduction is one type of asexual reproduction, which typically involves only one progenitor with no fusion of gametes (sexual cells). Plants use diverse mechanisms to reproduce vegetatively. These include:

- Specialized storage organs, known as **propagules,** including:
  - **Rhizomes**—horizontal underground stems
  - **Bulbs**—bases of swollen leaves
  - **Stem tubers**—thickened underground stems
  - **Root tubers**—swollen adventitious roots
  - **Corms**—solid stem structures, with well-defined nodes and internodes
  - **Stolons**—creeping horizontal stems (or runners), which throw out roots that give rise to new plants
  - **Bulbils**—small bulbs that grow on the stem or instead of flowers, fall, and grow as new plants
  - **Propagule or adventitious shoots**—minute plants that become aligned along leaf margins before these fall to the ground, where they grow into adult plants.
- Natural or mechanical fragmentation where new individuals originate from any piece or fragment of the plant such as **cuttings** or **stakes**. At least one node of the stem or branch is needed to provide a growing point with potential to produce a new plant. Such fragments are almost always vegetative parts of the plant such as stems, modified stems (rhizomes, tubers, corms, and bulbs), leaves, or roots.
- Use of **shoots** that are induced naturally or artificially to form roots from a stem that is still joined to the mother plant. Such stems, once rooted, separate to become new plants that grow with their own roots.
- Today, new plants can be obtained from single cells, tissues, or organs. Any plant part is isolated and cultured in an aseptic, artificial, nutritive environment (***in vitro*** **tissue culture**). One well-known application is the use of **apical meristems** or apices, based on the principle that these structures perpetuate themselves and are responsible for the continuous formation of primary tissues and stem appendages (e.g., leaves and stipules).

Harvesting the Germplasm

After the plants have grown and borne fruit (in the sense of containing seeds or propagules capable of generating new individuals), harvesting is carried out. The procedures for each case are inherent to the type of germplasm being handled and its predominant reproductive system.

Harvesting germplasm that reproduces by seed

Harvesting germplasm that reproduces by seed consists of collecting the plant's fruits, once they are physiologically mature, that is, they are carrying seeds capable of germinating and initiating the development of new plants. When harvesting, the following should be taken into account:

- The species being harvested and its type of seed (determines conditioning—drying is critical for species with recalcitrant or short-lived seeds, as they are sensitive to drying, whereas orthodox ones tolerate it better)
- Stage of maturity of the fruit (physiological maturity according to fruit type is preferred)
- Procedures for collection (manual or use of special equipment)
- Selection of fruits during collection (i.e., harvesting only ripe fruits that are not damaged by insects or showing symptoms of pathogen attack)
- Type of packaging to use (preferably clean cloth or paper bags) and germplasm identification system
- System of bulk collection and transport to sites for temporary storage
- Conditions for temporary storage and pre-drying of fruits before final conditioning

In general, harvesting should be selective and timely. Fruits that are green, damaged, or diseased should not be harvested. In no way should overripe fruits or those decomposing through saprophytic micro-organisms be included. During harvest, utmost care should be taken to prevent damage or injury likely to degrade the fruits' physical integrity and their contents.

Mechanical injuries produced during harvest may reduce seed viability and lead to the production of abnormal seedlings. Some injuries are internal and cannot be seen at the time but, after storage, manifest themselves as reduced viability. Damage to the seeds is a potential factor in any operation that implies hitting the seeds, especially when machinery is not duly adjusted. Usually, seed suffers less damage if its moisture content is 12%–15% during harvest.

As the objective of conservation is to maintain the germplasm's genetic identity as closely as possible to the lots originally entered, only the offspring of the materials planted originally should be harvested while avoiding atypical materials or other entries or plants that do not correspond to the planted germplasm. However, cross-pollinated species may have natural segregations that may have to be confirmed later. Hence, the reproduction system of the species should be taken into account before 'atypical' materials are discarded.

A seed reaches maturity when it can be separated from the fruit or plant without endangering its germination. Usually, harvest is facilitated if the fruit is ripe, that is, has acquired the characteristics that lead to natural dissemination. The maturation stages of fruit and seed may not coincide. If the seed is harvested too early or if the embryo has not developed sufficiently when the fruit matures, then the seed may be thin, light, shrivelled, and of poor quality. If the harvest is delayed, then the fruits may open, fall, or be eaten by birds or animals. The tendency for fruit drop, that is, a premature fall of fruits and therefore seeds, varies considerably according to plant class. Losses can be reduced by careful management. Usually, harvesting should take place before the fruits dry up too much. Cutting early in the morning when dew is still present may, in some cases, reduce drop (Hartmann and Kester 1971), but the risk that the seeds will be severely affected by fungi is higher. As a result, harvesting should, preferably, take place after the dew has evaporated.

Pre-drying fruits

Great care should be taken when pre-drying fruits and their contents, as any neglect or error may lead to reduced seed viability and, in extreme cases, to the loss of germplasm. The reason for drying fruits and their seeds is to reduce moisture content to levels that will increase longevity during storage and, therefore, the intervals between regenerations. Several drying methods exist, the most common being the use of a drying chamber or de-humidifier (FAO and IPGRI 1994; Hong and Ellis 1996). When drying fruit, the species seed type must be taken into account.

Seed moisture content will determine storage time. Species with short-lived seeds or seeds sensitive to drying (recalcitrant) should be dried out with more care than long-lived ones whose moisture content can be more severely reduced (orthodox seeds). Other seeds can be highly sensitive to moisture loss, being able to tolerate storage for only some days, such as those species with fleshy fruits belonging to the Myrtaceae family (e.g., myrtles, *Luma* spp., *Myrceugenia* spp., and Chilean guava). In these cases, seeds should be planted immediately after being extracted from the fruit (Hartmann and Kester 1971; Sandoval 2000).

The methods used will depend on available equipment, number and size of samples to be dried, local climatic conditions, and economic cost (Grabe 1989). Preferable ranges for drying are temperatures between 10° and 25°C and relative humidity (RH) between 10% and 35%, whether using a dryer or drying chamber. A suitable drying product is silica gel, which can reduce moisture content to the extremely low levels that characterize ultra-dry seeds. Harvested materials should be dried out as soon as possible after collection to prevent any significant deterioration. The drying period will depend on the size of fruits and seeds, the quantity to be dried, the fruits' initial moisture content, and the level of relative humidity maintained in the drying chamber.

Personnel of germplasm banks must keep in mind that dried seeds, particularly those that are very dry, are often fragile, and therefore susceptible to mechanical injury. Hence, they must always be handled with utmost care (FAO and IPGRI 1994; Hong and Ellis 1996).

Some management procedures are described below, according to whether seeds are from woody or herbaceous plants, or from trees and shrubs.

Woody plants

To harvest the fruits of woody plants, we need to know the characteristics that indicate optimal conditions for harvesting a given class of seed. These include moisture content (dryness), general appearance, and the state of the more-or-less milky colour of the seed. In some pine species, the specific weight of recently harvested cones is valuable for judging their state of maturity. Some seeds, if they are harvested before the fruit has ripened completely and have not been allowed to dry, germinate better in spring or the season immediately following harvest. Once those seeds dry up and their coats harden, they may not germinate until the second spring or season after they were produced, except by using special handling methods. Examples of those plants for which this practice of early harvest has been found desirable include *Cornus*, *Cotoneaster*, *Carpinus*, *Cercis*, *Hamamelis*, *Rhodotypos*, *Viburnum*, *Juniperus*, and *Magnolia kobus* (Hartmann and Kester 1971).

Herbaceous plants

Dry fruit seeds. The dehiscent (follicles, capsules, pods, and siliques) and indehiscent fruits (caryopses and achenes) of some materials can be harvested, using special combine harvesters. However, for most plants, fruits or mature *i*nfructescences are collected, then cut, and allowed to dry for 1 to 3 weeks before being threshed. Plants may be placed in rows, stacks, or piles to dry. Those plants whose fruits open easily on drying, as for many ornamental species, are cut (frequently by hand) and placed on a canvas or tray. When many plants are dealt with, they may be cut and put out to dry by placing them inverted in a bag and hanging them (Hartmann and Kester 1971).

Fleshy fruit seeds. Fleshy fruits (e.g., tomato, pepper, chilli, eggplant, and cucumber) may be harvested ripe or, in exceptional cases, overripe (e.g., cucumber and eggplant). If lots are small, the fruits may be broken and separated, and the seeds cleaned and dried out by hand. Otherwise, seed is separated from the flesh through fermentation, mechanical means, or washing in screens (Hartmann and Kester 1971).

Trees and shrubs

Both dry and fleshy fruits from trees and shrubs can be harvested by shaking them over a canvas, felling them with poles, using conical hooks fixed on long poles (as for conifers), or picking them by hand. The seeds of some street trees such as elms can be collected with brooms. Seeds of small trees and low shrubs may be harvested by hand, cutting or striking seed-bearing branches.

The viability of seed from trees and shrubs varies considerably from year to year, from place to place, and from plant to plant. Before collecting seeds from a specific source, several fruits should be opened and the seeds examined to determine the percentage of well-developed embryos. Such an examination is known as the *cutting test*. Although it is not a reliable test of viability, it helps prevent harvesting seeds from a source that is producing only empty seed. Another test is to examine fruits by X-ray (Hartmann and Kester 1971).

Dry dehiscent fruits. Seeds of plants such as certain ligneous legumes (e.g., *Acacia triacantha*), *Caragana* spp., *Ceanothus* spp., poplar, and willow are extracted from capsules and pods. The fruit of these plants are dried by spreading them out in thin layers on canvases; cloths; on the floor; or on shelving in open sheds, using trays with wire mesh bottoms. Air-drying takes 1 to 3 weeks.

Fleshy fruits. Fleshy fruits include berries (grape), drupes (peach, plum), pomes (apple, pear), aggregate fruits (raspberry, strawberry), and multiple fruits (blackberry). With these kinds of fruits, the flesh should be removed as soon as possible to prevent decomposition and before the seed is damaged. Methods that are suitable for small lots of seeds are cleaning by hand, trampling in vats, and scraping through screens. Relatively large fruits can be conveniently cleaned by placing them in a wire basket and washing them with water at high pressure. For larger lots, a hammer mill or macerator can be used. The macerator is constructed with a hermetic feeder, the water is passed through it, together with the fleshy fruits, and the crumbled mass passes to a tank where both flesh and seed are separated by flotation.

Germplasm that reproduces vegetatively

Harvesting vegetative planting material depends on the type of propagule of the species (Figure 3), and the procedures to apply depend on the management established for the case (Vázquez Y et al. 2004). The procedures established for managing parts of stems, roots, leaves, or specialized structures (e.g., tubers, bulbs, corms, stolons, rhizomes, tuberous roots, and buds) should be revised so that identical, whole, and healthy plants are regenerated. With respect to health, acquisition should necessarily guarantee, where possible, during harvest and conditioning, planting materials that are free of pathogens. One way of guaranteeing this is to pay attention to three basic aspects: isolation of the production site, adopting health and inspection measures, and periodic testing (Hartmann and Kester 1971).

Depending on species and type, propagules are usually short-lived and should be planted within a very short period after harvest. In general, when harvesting vegetative planting materials, the following should be taken into account:

- The species being harvested
- The propagules' stage of maturity
- Procedures for collection (manual or use of special equipment)
- Selection of the propagules during collection (i.e., harvesting only mature propagules that are not damaged by insects or nematodes, or showing symptoms of pathogen attack)
- Type of packaging to use (e.g., baskets or pita-fibre sacks) and germplasm identification system
- System of bulk collection and transport to sites for temporary storage
- Conditions for storing and conserving the materials before new plantings begin

Evaluating the Lesson

After this lesson, you should be familiar with the most important aspects of harvesting germplasm to help guarantee its integrity such as fruit types, seed parts, and propagules.

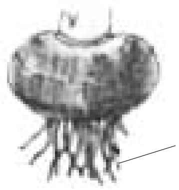
Before going on to the next lesson, comment on your experiences with harvesting and managing fruits or propagules to obtain seeds or planting materials for germplasm conservation. Emphasize the procedures and care needed to be successful.

If you are not directly familiar with these processes, list and discuss the criteria that, in your opinion, should be taken into account when harvesting and managing fruits and propagules destined for conservation.

Vegetative reproduction

Besides producing seeds, some plants possess a special type of asexual reproduction known as vegetative reproduction or propagation. That is, a part of the plant can give rise to a new plant by itself.

- **Corm** (e.g., saffron). A short thick stem, similar to the bulb, except that it stores food within the stem itself.

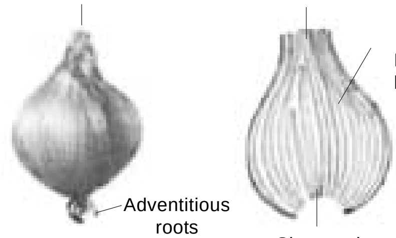


Saffron corm

- **Rhizome** (grasses, ferns, lilies). Thick stem with layered leaves and growing horizontally underground. It produces roots along its length and buds that give rise to new shoots.

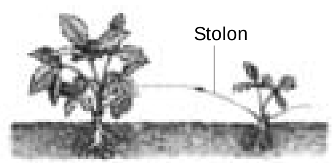
Rhizome of mint

- **Bulb** (daffodil). Short thick stem surrounded by layered leaves and containing food reserves. It forms in the soil from an old and dying plant, and represents the first latent stage of a new plant that will emerge as a shoot at the beginning of the following season.



Daffodil bulb

- **Stolon** or **runner** (strawberry). A stem grows horizontally from a point close to the plant base. It then produces roots at intervals along the stem and new plants grow from these points.



Old strawberry plant **New plant**

- **Tuber** (potato). Short, thickened, subterranean stem that stores food and produces buds that give rise to new plants.

Potato tubers **Potato plant**

Microphotograph of a longitudinal section of a stem apex from *Coleus* sp.

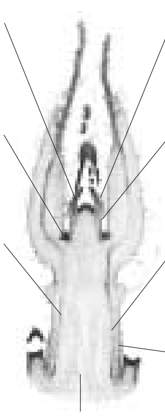


Figure 3. Different types of propagules (corm, rhizome, bulb, stolon, and tuber) for vegetative reproduction (from Stockely 1991). The microphotograph shows a longitudinal section of a stem apex from *Coleus* sp. (from Kindersely 1994).

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Next Lesson

In the next lesson, you will study the principal aspects of conditioning and quantifying germplasm after its multiplication and regeneration.

Lesson 2 Conditioning and Quantification

Objectives

- To discuss the concept of quality for germplasm
- To describe the process of conditioning germplasm
- To describe the generalities of quantifying the harvest

Introduction

If the multiplication and harvesting tasks have been successful and the germplasm's identity is successfully maintained, then the tasks of conditioning and preparing for storage for conservation become essential. Conditioning is perhaps the most delicate process, requiring special attention because the long-term viability of materials depends on it. An error in drying, for example, may lead to an inexorable reduction of viability and thus to loss of germplasm in the short term.

Once the germplasm is harvested, then obtaining the seed or propagules becomes essential. Acquisition is based on fructifications or on collected plant parts. A series of processes and controls is applied to ensure that germplasm with the requisite quality for conservation is acquired. Given that conditioning is a critical stage in managing germplasm for conservation and that its successful conservation is a function of its **quality**, this theme will first be discussed.

The Concept of Quality

The total quality of a given germplasm refers to the degree of adequacy that its genetic, physiological, physical, and health attributes have for that material's conservation.

Genetic quality

This attribute refers to the degree to which the germplasm conserves its original genotypical characteristics, that is, the degree to which it carries the genes that are to be conserved and were present in the material when it was first introduced into the germplasm bank or collection. Genetic quality can be ensured by planting authentic and pure seeds, and maintaining this authenticity and purity during multiplication through preventive methodologies such as isolation, selection of appropriate fields, verification inspections, and rigorous management to prevent undesirable mixtures.

Physiological quality

The tangible result of physiological quality lies in the seed's faculty to germinate, emerge, and give rise to uniform and vigorous plants. Good physiological quality implies integrity of structures and physiological processes that permit the seeds to be kept not only alive, but also with high vitality index.

Physical quality

For seeds, this refers to such attributes as size, shape, brilliance, colour, and weight that were characteristic of the accession or entry. It also includes the seed's own integrity, that is, it is not fractured, damaged by insects, or stained by the action of micro-organisms, and is free of any contaminant.

For vegetative planting materials, physical quality refers to the organs or plant fragments containing functional generative parts (e.g., buds, meristems, apices, roots, and primordia) showing no physical or mechanical deterioration.

Seed health quality

This quality includes that set of characteristics that the germplasm must possess to ensure absence of pathogens transmittable by plant parts and/or micro-organisms that cause deterioration during conservation.

Conditioning

Conditioning consists of appropriately preparing the germplasm after harvest to achieve conservation goals by treating seeds or propagules accordingly. Those procedures most used for treating seeds and vegetative planting materials are reviewed below.

Dry fruit seeds

Seeds are conditioned by applying procedures that take into account the type of fruit from which they come. For dry fruit seeds, procedures include threshing or shelling fruits, cleaning by blowing or sieving, drying (20°C; 22% relative humidity or RH), temporary storage in cold rooms (5°C and 22% RH), final selection of seeds, final drying with cool air (20°C; 22% RH), and final packaging in hermetic containers or vacuum-packing in aluminium bags for conservation in cold rooms (-20°C), according to goals. When managing and packaging seeds during different stages of the process, they should be placed in cloth bags (muslin), especially during drying, and then in hermetic containers to prevent the seeds from rehydrating.

Threshing or shelling. Shelling or threshing can be carried out manually or be mechanized. The procedures used depend on the species and fruit type. Any threshing operation basically implies a process whereby the harvested fruits are beaten or passed through rollers to separate the seeds from the rest of the plant. A heavily used machine is the combine thresher, the central part of which is a revolving cylinder that works as a beater. It also has couplings with other devices that separate the threshed seed from husks and straw. This type of machine is used to harvest large seed lots. With small lots, seeds can be separated by threshing and cleaning them by hand in a screen (Hartmann and Kester 1971).

For legumes, seeds are extracted by striking or trampling the pods and sieving them through a screen, shelling them by hand, or rubbing them with a special implement (Figure 1). However, care must be taken to verify seed performance as according to species, because striking them is sometimes counterproductive, cracking and therefore spoiling them.

The extraction of conifer seeds requires special procedures. The cones of some species will open if they are dried in the open air for 2 to 12 weeks. Others must be forcibly dried at higher temperatures in special ovens. On drying, the cone scales open, exposing the seeds.

Figure 1. Conditioning seeds. Procedures and equipment used (photographs by B Pineda, GRU, CIAT; diagrams from CIAT 1989).

They must then be shaken or raked to separate the seeds, which should then be immediately removed, as the cones may close again (Hartmann and Kester 1971).

The seeds of some grasses (Poaceae) and cereals have aristas, beards, or glumes, which cannot be completely separated during threshing, thus impeding their effective classification. To remove them, the seeds must be either rubbed manually or placed into a specialized machine that rubs the seeds against revolving hammer arms that remove the coats, thresh the spikes, and, generally, polish the seeds (CIAT 1989).

Conifer seeds have appendages or wings, which are removed, except in species where the seed coats damage easily, as in incense cedar (*Libocedrus* sp.). Fir (*Abies* spp.) seeds also damage easily, but they can be separated from the wings if care is taken. Seeds from *Sequoia* spp. have wings that cannot be separated from the seed. In small lots, the wings can be removed by rubbing the seeds between wet hands, or else trampling or striking seeds in partly filled sacks. For larger lots, special dewinging machines are used. After dewinging, the seeds are cleaned to remove residues of wings and other light materials. The final step is to separate the filled and heavier seeds from the lighter ones, using pneumatic separators or gravity (Hartmann and Kester 1971).

Cleaning and selection. Once threshed, the seeds must be cleaned to remove rubbish, twigs and other unwanted plant parts, parts of foreign plants, and seeds of other crop or weed species. Small lots can be cleaned, using a screen or passing them through a container to another and allowing air to drag away the lightest materials. Removing the rubbish is a pre-cleaning operation by which materials that are larger or smaller than the seeds are separated from them. The operation is manual, using sieves or screens (Figure 1) and a seed blower, or with cleaners that combine hoppers, sieves, and ventilators to eliminate the light materials (CIAT 1989; Hartmann and Kester 1971).

Seeds can be separated mechanically from undesirable materials during cleaning only if they differ from them in one or more physical properties. The properties most used correspond to weight or density, colour, texture, size, width or thickness, length, and electrostatic properties. These permit the design of specialized devices that take advantage of the differences between seeds and contaminants to clean. The devices usually combine air currents (Figure 1), different-sized screens, gravity (Figure 1) or texture separators, slopes, vibrators, magnetic cylinders, and photoelectric cells (CIAT 1989; Hartmann and Kester 1971).

When cleaning equipment is used, care must be taken to remove harvest residues or seeds remaining in its interior before processing another accession to prevent contamination and undesirable mixing. Also, the equipment should be cleaned of dust and other residues to prevent contaminating the seed with the reproductive structures of micro-organisms such as fungi, bacteria, and nematodes that usually associate with plant materials during the plants' growth and fructification in the field.

After carrying out the basic cleaning processes and having obtained the seeds, the procedures for finishing or final selection are conducted (Figure 1). Seeds are examined under low-powered magnifying glasses to discard those with otherwise invisible spots, fissures, wounds, or deformities. Special equipment such as pneumatic or gravity separators is also used to eliminate empty or low-density seeds.

Drying. Drying consists of reducing the moisture content of seeds to a minimum level for metabolic activity, without their losing viability. To dry or reduce the seed's moisture content, its original moisture content must first be determined by quantifying, either directly or indirectly, the water they contain.

Direct determinations are made through gravimetric methods, chromatography, or spectrophotometry. Indirect methods include hygrometric methods, infrared spectroscopy, nuclear magnetic resonance, and chemical reactions of seeds (Grabe 1989). Currently on the market are electronic analysers (moisture meters) or special balances with infrared heating chambers that permit rapid and precise quantification of moisture content of small samples of seeds (Figure 2). If such technology is not available, then the other methods mentioned

Figure 2. Drying seeds. Left, balances to determine seed moisture content and, right, cool-air drying room (20°C; 22% RH) (photographs by B Pineda, GRU, CIAT).

above can be used. All these methodologies are described in the following publications: *Seed Technology for Genebanks* (Ellis et al. 1985), *A Protocol to Determine Seed Storage Behavior* (Hong and Ellis 1996), and *Manual of Seed Handling in Genebanks* (Rao et al. 2006).

To precisely determine moisture content, the gravimetric method (ISTA 1999) is recommended with some modifications to sample size, given that this method is destructive. Many pre-postharvesting operations require rapid determinations (which are less precise) that can be carried out with portable equipment. Such determinations are based on the electrical properties of water in the seeds such as conductivity and capacitance (ability of an electric conductor to carry a charge at a given potential; ability to store electrical charge).

The physical relationships between the moisture content (MC) of a seed, temperature, and relative humidity form the basis for drying. The maximum quantity of water that air can contain depends on the temperature. An indirect measure of air humidity is relative humidity (RH). The concept of RH can be expressed as follows: if air at 10°C contains 5 g of water/kg of dry air, but its capacity for saturation is 20 g of water/kg, then its RH is $5/20 \times 100 = 25\%$. For the same water vapour content of the air, if the air's temperature rises, then its RH drops and vice versa.

Water in the seed (i.e., MC) tends to balance (moisture content balance or MCB) out with the humidity of the surrounding air. Hence, dry air, that is, with a low RH (20%–25%), can rapidly dry the seed to reach an MCB. The time taken to reach the MCB depends on the species (anatomy and food reserve tissues) and temperature. Likewise, humid air increases a seed's MC. Thus, the air used for drying should be recycled and dried out. Certain chemicals are able to absorb moisture from the air; perhaps the most common is silica gel.

A relationship exists between seed longevity, storage temperature, and seed MC, thus demanding that a suitable combination of temperature and moisture be taken into account. The first requisite to consider is the low MC of seeds, which can be as low as 5%. A calculation made with sesame (*Sesamum indicum* L.) found that reducing seed MC from 5% to 2% will increase seed longevity by 40 times. However, a lower limit of tolerable MC exists, depending on the species. Hence, a limit of 5% is usually established (FAO and IPGRI 1994).

Before proceeding with drying, the procedures and degrees of desiccation that the materials require should be well understood. In terms of maintaining the viability of the germplasm, deficient or excessive drying without sufficient basis is a very high risk, to which seeds should not be exposed. Hence, experiments should be carried out to determine the type of drying that can be applied with minimal risk.

Most seeds should be dried after harvest. Seeds with more than 20% MC heat up if they are piled up for several hours, thus reducing their viability. Drying must begin in the field, immediately after collection and/or extraction of seeds (see *Module 3, Submodule B, Lesson 1*).

Drying can occur naturally in the open air or artificially by heat or other methods. Drying temperatures should not be higher than 43°C (110°F) and, if seeds have high moisture content, the ideal temperature is 32°C (90°F). Too rapid a drying can cause shrivelling and fracture of the seeds and sometimes harden the coats. The MC at which seeds can be conserved without risk is between 8% and 15%, although some seeds should be conserved moist. Nevertheless, drying at temperatures at more than 40°C can be disastrous for germplasm conservation. In Latin America, there have been cases where seed longevity has been no longer than 5 years and the percentage of germination no more than 25% (Daniel Debouck 2004, personal communication).

Methods of natural drying (e.g., drying in the open air under shade) do not reduce MC below 8%–10%, which is suitable for short-term conservation, that is, 2 to 3 years. Drying in the direct sun is not recommended because, in many cases, the germplasm can be exposed to high temperatures for too much time, thus causing irreversible damage to seed viability.

Artificial drying can, with the help of equipment that permits air circulation at different temperatures or silica gel, be an easy and effective method (Hong and Ellis 1996). Electronic driers permit programming of drying cycles, temperatures, flows, and speeds of the drying air. Drying should be carried out in rooms especially designed for the purpose (Figure 2). In such rooms, combinations of dry (20%–22% RH) and cool (temp. 15°–25°C) air can be managed to reduce the percentage of MC in the seeds to 4%–6%, suitable for long-term conservation. However, the species should be taken into account because, in some cases, they would be overdried.

The type of substances in the seed's reserves also influence the MCB in the drying room. Sugars have the most affinity for water, followed by proteins, starches, and oils. This means that, for a given RH, oleaginous seeds may contain less moisture than proteinous or starchy seeds.

Once the drying is finished, the MC is measured again to confirm that the required level (5%–12%) has been reached and to determine if the samples need to be submitted to a new cycle of drying or rehydration. The temperatures and times of drying must be established accurately so not to endanger the samples, as repetitive procedures can reduce viability. Fluctuations in MC reduce the seeds' longevity, as they increase the seeds' respiratory rate. The increase causes the seeds' reserves, which are designed to feed the embryo during germination, to be consumed through respiration as metabolism is increased, thereby reducing the seeds' quality (Hartmann and Kester 1971).

Most long-lived or intermediate seeds can tolerate drying (orthodox) to 4%–6% for storage over prolonged periods at low temperatures. Moisture content can be increased, but only if the temperature is reduced. The seed's MC will determine the duration of storage. In general, short-lived seeds are sensitive to drying (recalcitrant). These seeds have a high MC and lose their viability when this is reduced (Hartmann and Kester 1971). Seeds of this type are found in species such as oaks (*Quercus* spp.), walnuts (*Juglans* spp.), araucaria pines (*Araucaria* spp.), Chilean hazel (*Gevuina avellano*), and *Beilschmiedia* spp. (Hartmann and Kester 1971; Sandoval S 2000).

Fleshy fruit seeds

These seeds must be separated from the flesh that surrounds them. For tomato, macerated fruits are placed in barrels or large vats and left to ferment for about 4 days at about 21°C (70°F), stirring occasionally. If the fermentation is left for too long, the seeds may germinate. At higher temperatures, fermentation time will be shorter. As seeds separate from the pulp, the healthier and heavier ones sink to the bottom of the vat. The pulp remains at the surface, together with the empty seeds and other foreign materials. After extraction, the seeds are washed and dried, either in the sun or in a drier. Additional cleaning is sometimes needed to remove dried pulp and other materials. For cucumbers and similar fruits, special machines are used to extract and clean the seed from the pulp. After separation, the seeds are washed and dried as is done for fermentation (Hartmann and Kester 1971).

The small berries of some species of *Juniperus* and *Viburnum* are difficult to process because of their size and the difficulty of separating the seeds from pulp. One way of managing such seeds is to pound them with a kitchen roller, soak them in water for several days, and remove the pulp by flotation. A better method for extracting seeds from small fleshy fruits is to use an electric mixer of the type used in soda fountains or a blender. To prevent damage to the seeds, the blender's metal blades can be replaced with a piece of rubber that is cut from a tyre and fixed horizontally to the machine's revolving axis. A fruit and water mixture is then placed into the blender's glass and agitated for 2 min. When the flesh has separated from the seed, it can then be removed by flotation. Some fruits such as those of juniper (*Juniperus* spp.) must be pounded before extracting the seed (Hartmann and Kester 1971).

Plant parts

Plant parts are conditioned according to the type of propagule of the species, and to the management established for its case. Where no information is available, then the requisite research must be conducted. The procedures for conditioning stem parts, root parts, leaves, or specialized structures (e.g., tubers, bulbs, corms, stolons, rhizomes, tuberous roots, buds, meristems, and apices) targeted for conservation are specific to each species. For example, for cassava (*Manihot esculenta*), the factors to take into account when conditioning stakes include the plant's age, its health status, stem parts to use, stem diameter and length, number of buds, type of cut to make, and the treatment, if any, before temporary storage (Lozano et al. 1977).

Generally, because they concern specialized organs, parts, or fragments of live plants, plant parts cannot have their MC reduced. Nor can they be exposed to long-term storage. As a result, they must be handled with great care.

If materials are to be conditioned for *in vitro* conservation, explants are extracted, preferably from the youngest plants, for micropropagation. This procedure consists of (a) disinfecting the explants in a solution of sodium or calcium hypochlorite, or ethanol; (b) planting them in an *in vitro* culture medium until new shoots develop; and (c) rooting the shoots to obtain whole plants (Frison 1994; George 1996; George and Sherrington 1984; Roca and Mroginski 1991).

Packaging

Once conditioning is finished and the verifications of MC are carried out, the material, in the case of the seeds, is ready for packing and transporting to the storage site. Both container and storage site should respond to the requirements of the species and guarantee survival of the samples.

To pack seeds, diverse types of containers exist, with varied shapes and materials and ranging from paper and aluminium envelopes to plastic or glass bottles (Figure 3) and tins of different metals. More than its shape or material, the container must be **airtight**, that is, it

Figure 3. Types of containers and holders for seeds and plant parts. Upper part—plastic bottles and aluminium bags for seeds; lower part—plastic crates for transporting planting materials (photographs by B Pineda, GRU, CIAT).

isolates the germplasm sufficiently to prevent it from absorbing moisture and/or becoming contaminated. Selection of the container depends on seed characteristics and on the period for which they are expected to be conserved. In practice, it is also determined by the bank's resources, as containers not only vary in shape and materials, but also in costs and availability on the market. Aluminium bags are the most recommended as they can be hermetically closed, using a heated stamp (Figure 3). Airtight containers, for example, are optimal but expensive. The investment involved depends on what the material is destined for. Jaramillo and Baena (2000) describe a series of containers commonly used in germplasm banks.

For plant parts, given their relative perishability and short storage periods, when required or when the material permits it, the containers or packaging used should maintain the germplasm fresh. It is also essential that the containers protect the material from damage to its buds or other generative areas of the material, whether from mechanical injury or deterioration caused by other agents during transport to the planting site. Currently, the market provides numerous options of plastic crates (Figure 3) that are especially designed to transport and manage perishable products that can be useful for germplasm management.

Quantifying Germplasm

Seeds or propagules are usually counted by hand. For seeds, however, automated solutions are available such as counters, counter heads connected to a vacuum system (Figure 4) and other commercial equipment. Indirect estimates can also be made such as by weight, which are less precise. The technique consists of determining the unitary weight of the seed by taking at random four replications of 100 seeds (g/100 seeds) (ISTA 1999) and making the respective calculations based on the weight of materials ready for storage.

Although the count is indeed based on apparently simple operations, it is highly significant because it forms the basis by which the germplasm bank knows what it has and for what ends. If the bank assumes the responsibility to conserve, it has the obligation to test viability on a periodic basis, check samples for plant health quality, to conserve and distribute. For these activities, it must have a record of how many propagation units will be needed to fulfil pre-established plans.

Evaluating the Lesson

After this lesson you should be familiar with the most important aspects of conditioning and quantifying germplasm, as well as with the concept of total quality.

Before going on to the next lesson, describe your experiences in conditioning seeds or propagules for storage for conservation, emphasizing the procedures and care needed to be successful.

If you are not directly familiar with these processes, list and discuss the criteria that, in your opinion, should be taken into account to condition seeds and propagules destined for conservation.

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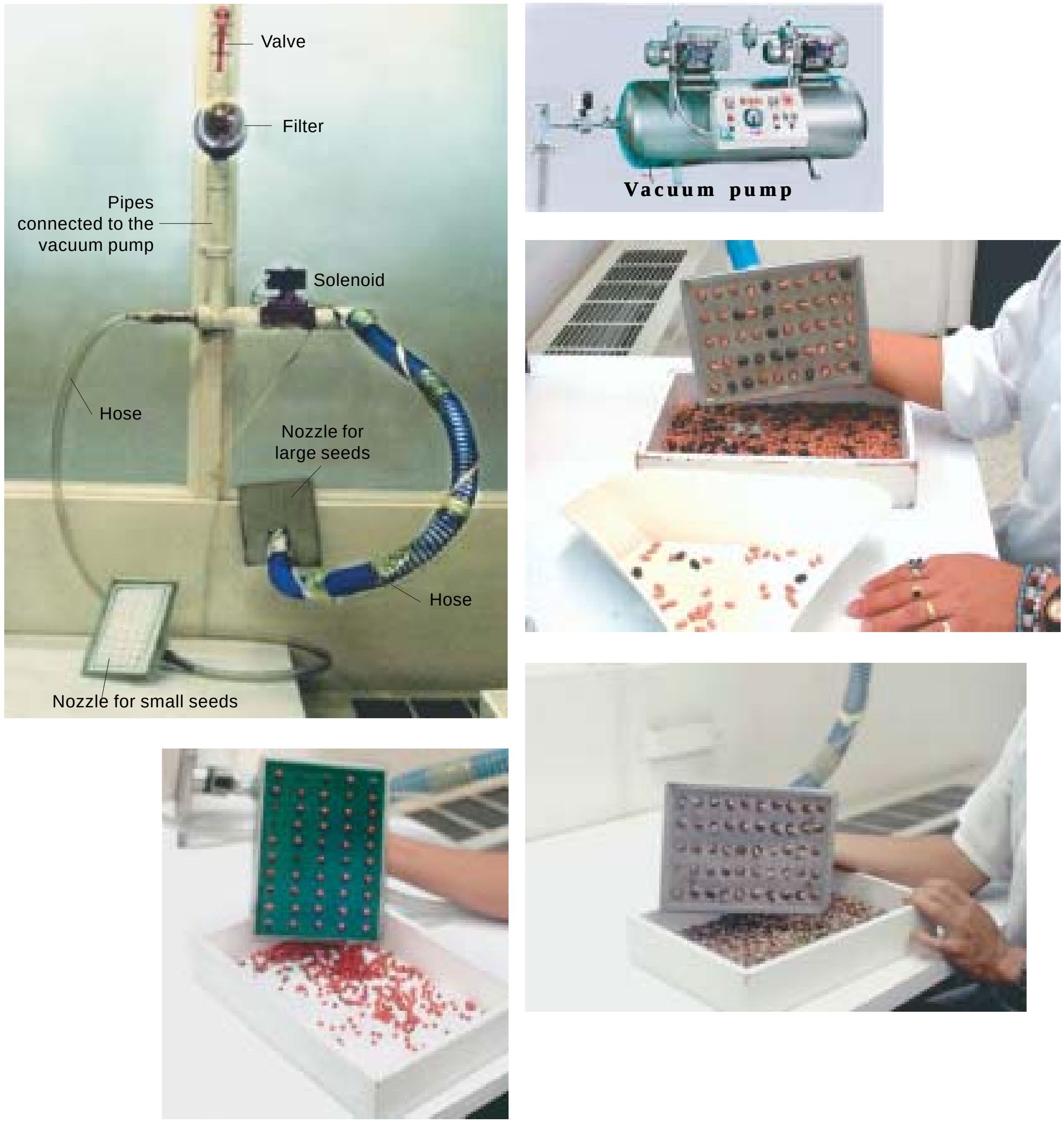

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Next Lesson

In *Lesson 1* of the next *Submodule C*, you will study the principal aspects of monitoring the biological status (physiological quality) of germplasm.