Objectives

- To discuss the basic concepts of viability, vigour, dormancy, and germination in seeds
- To mention the essential structures of seedlings used to evaluate germplasm viability
- To mention general aspects of viability, vigour, germination, and essential components of plant parts for vegetative propagation

Introduction

To conserve germplasm, an essential condition is that it must be viable, that is, it must be alive and able to regenerate new plants capable of independent existence. Within this order of ideas, the verification of the biological or physiological status of germplasm targeted for conservation takes on special importance.

Once conditioning has been carried out, the material should be verified to check that it was correctly prepared to guarantee its successful conservation in terms of the bank's plans and goals. Also, following the germplasm bank's norms, the viability of conserved germplasm should be monitored at least every 5 years according to species and storage conditions; or, in their absence (as for vegetative planting materials), adjustments made as required per species. These periodic checks should be compared with each species' initial viability and conservation conditions (FAO and IPGRI 1994).

The verification of viability requires the understanding of concepts of the germplasm's physiological, physical, and health quality, as defined in *Lesson 2* on conditioning (*Module 3, Submodule B*). Moreover, as a minimum, general knowledge is needed on viability and vigour as applied to seeds and, indeed, to vegetative planting materials. Also important is being able to identify those essential structures that are evaluated to determine physiological status and interpret results. This lesson deals with these themes.

Viability and Vigour

The **viability** of germplasm refers to that property of being alive, that is, possessing the ability to regenerate new plants capable of independent existence. In other words, the germplasm is able to germinate and produce normal and vigorous seedlings that can complete again the species' life cycle. In terms of quantification, **seed viability** measures the number of seeds in a lot that are alive and could develop into plants that will reproduce under appropriate field conditions (Rao et al. 2006).

In the case of seeds, the viability of germplasm must be understood as being variable over time (i.e., longevity is variable) and, depending on the species, a function of its adaptation to the habitat where, ecologically, the plants had originally developed. The seeds of each plant species present typical germination mechanisms that respond to the effect of natural selection induced by predominant environmental conditions on the nature and physiology of seeds (Vázquez Y et al. 2004). Knowledge of this behaviour is useful for making the adjustments needed to establish viability tests to confirm physiological status.

Usually, the half-life of a seed is between 5 and 25 years. Seeds lose their viability for highly diverse reasons. One is that their food reserves are exhausted and, hence, the germinative capacity is lost. The less active a seed's metabolism is, the more long-lived it will be (García B 2004).

The **vigour** of a species' seed is its capacity for the rapid and uniform emergence and normal development of seedlings under a broad range of field conditions (AOSA 1983; OSU 2004). As ISTA defined it during its 1977 congress, vigour is the total sum of those properties of a seed or lot of seeds that determine the level of activity and capability of this during germination and seedling emergence (Perry 1981). The definition considers specific aspects of performance that had been regarded as evident variations, associated with differences in seed vigour such as:

- Biochemical processes and reactions during germination such as respiratory activity and enzymatic reactions
- Speed and uniformity of seed germination and seedling growth
- Speed and uniformity of seedling emergence and growth in the field
- The capacity of seeds to emerge under unfavourable conditions

Factors that influence seed vigour

The causes of variations in vigour are many and diverse. To clarify the concept, ISTA has established a list of the most commonly known factors that affect vigour, including:

- Genetic constitution
- Environmental conditions and nutrition of the parent plant
- State of maturity at harvest
- Seed size, weight, and density
- Physical integrity
- State of ageing and deterioration
- Pathogens

Generally, seed development involves a series of ontogenetic states such as fertilization, nutrient accumulation, seed drying, and dormancy. Any disturbance to development may alter the seed's potential performance (Delouche and Baskin 1973). The challenge for tests, then, is to determine vigour by identifying one or more quantifiable parameters commonly found in association with seed deterioration.

A hypothetical model has been developed that will eventually be used to better estimate seed vigour, even including the process of deterioration (Delouche and Baskin 1973). The model graphically visualizes the extent to which deterioration may increase and the seed's vitality diminish (Figure 1). In other words, in real terms, vigour can be seriously affected.

The beginning of cell membrane degradation precedes loss of germination. Hence, a highly sensitive test for estimating vigour would be that which monitors membrane integrity. There are sufficient experimental arguments to support this assertion. Membranes are essential for many metabolic events that occur in a seed, including respiration, which provides the seed with the energy needed for subsequent plant development (OSU 2004).

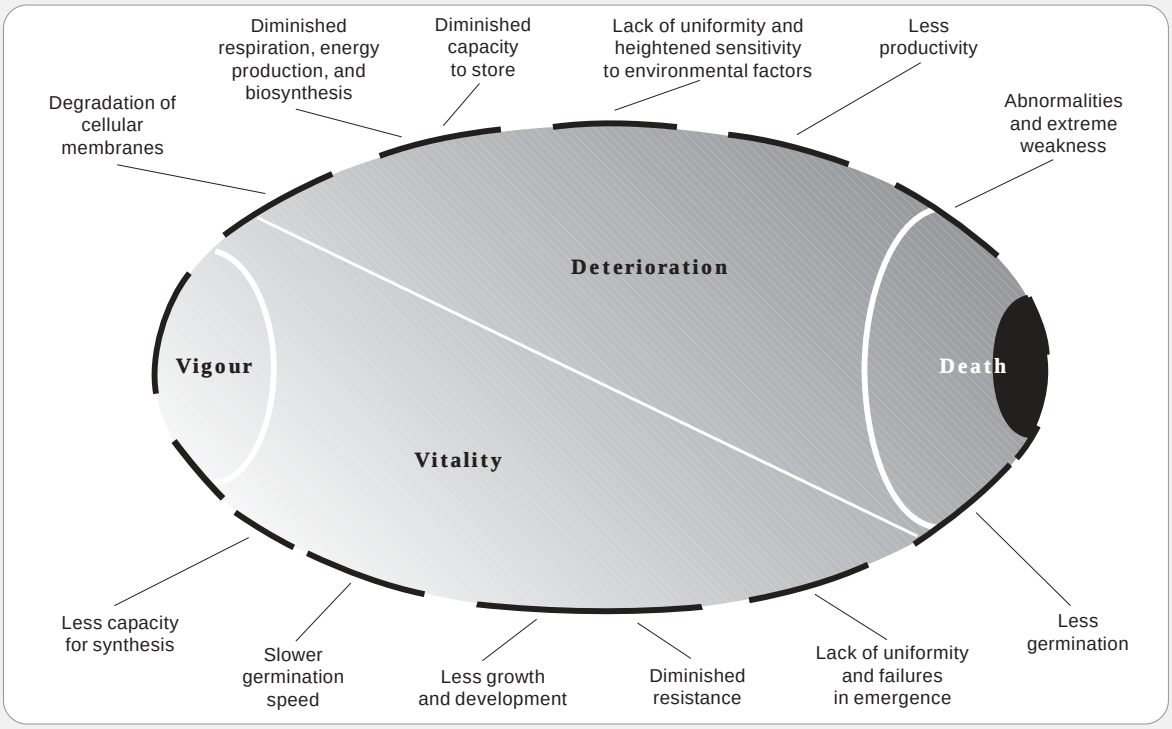

Figure 1. Hypothetical model that shows the relationship between vigour, vitality, and deterioration, and the indicators proposed (model adapted and redrawn by B Pineda from Delouche and Baskin 1973).

The endoplasmic reticulum is an organelle made up of membranes where many enzymes are produced that bring about the translation of RNA (ribonucleic acid). Thus, any disturbance in the function of such membranes can reduce the ATPs associated with the supply of energy to cells and retard the synthesis of specific enzymes essential to growth. With loss of respiration and biosynthetic capacity, the germination rate is reduced, as manifested in a lack of uniformity in seed lots. Other events associated with deterioration are loss of storability and of the capacity to resist disease. When degraded seeds are subjected to biological and environmental pressures, they show a poor rate of emergence under field conditions and poor yields. Finally, these subtle manifestations of loss in seed quality find expression in an increased incidence of abnormal seedlings—a component of germination tests (OSU 2004).

Dormancy

Higher plant forms may show dormancy or interruption of growth in meristematic tissue, for example, in growth buds of branches, as well as in seeds. In seeds, dormancy, also known as latency, resting, or quiescence, is the last phase of its ontogeny or formation and development. It is an essential stage for seed survival as it maintains them in waiting until environmental conditions are propitious for germination and plant production. In practical terms, dormancy refers to the state in which viable seeds fail to germinate, even under conditions normally favourable for germination (Rao et al. 2006).

The development of seed dormancy involves water loss, differentiation of the seed's integuments or coats, interruption of genetic transcription and protein synthesis, and reduced respiration and other activities of intermediary metabolism (Vázquez Y et al. 2004). During the final stages of ontogeny, particularly that of storing food reserves and maturation, and according to species, carbohydrates (starches and sugars), proteins, and lipids are accumulated. When this process ceases and the seed has completed its development, drying begins. The synthesis of oligosaccharides increases. These substances are involved in the tolerance of drying and may, as does raffinose, prevent the cytoplasm from crystallizing in the desiccated mature seed. In some species, during maturation, the plant hormone abscisic acid (ABA) plays an important role in controlling dormancy until conditions are such that the seed can germinate and initiate the formation of a new seedling (Bolaños 2004; Vázquez Y et al. 2004).

When the seed separates from the plant that produced it, it enters quiescence or reduced metabolism, that is, it does not show external signs of activity within it. This **resting** is called **quiescence** when the reason for the lack of germination is basically a lack of water, as when seeds are stored under artificial conditions or remain in the fruits united to the parent plant for long periods (Gooding et al. 2000; Vázquez Y et al. 2004). However, resting is called **dormancy** when the seed does not germinate, despite conditions being optimal in terms of temperature, air, and humidity for radicle emergence and seedling growth (Gooding et al. 2000; Vázquez Y et al. 2004). The lack of germination can be attributed to the existence of a chronologically regulated period of interrupted growth and reduced metabolism during the plant's life cycle. This is an **adaptive strategy** to survive unfavourable environmental conditions (Vázquez Y et al. 2004).

Dormancy allows seeds to distinguish a good site for germinating. For example, those that require light to germinate will not do so if they are buried in soil or shaded by other plants. Thus, through dormancy, seeds perceive information on external environmental conditions, including the season in the year! For example, the seeds of some species must undergo a period of low temperatures, close to zero degrees, indicating that winter has arrived. Then, as temperatures increase and the first rains fall, thus indicating the beginning of spring, germination occurs and the seedling is established during a time of the year when survival is most likely. This limits those species to certain geographical regions where temperatures drop in winter (Moreno C 2004).

The establishment of dormancy is regulated by hereditary factors that determine a plant's endogenous physiological mechanisms, which interact with factors of the environment in which it grows. In the long run and over millions of individuals, this gives rise to evolutionary changes in the plants. Among the most important environmental conditions are (1) climatic variations in temperature and relative humidity, (2) microclimatic variations derived from physiographic and biotic aspects such as the spectral quality of light and thermoperiod, and (3) the specific characteristics of the place to which the plants are adapted for establishment and growth. Micro- and macroclimatic variations, and the hormonal and nutritional conditions of the parent plant greatly influence the establishment of dormancy in its seeds during their development. This means that variations may exist between harvests of seeds from a given species, depending on the time and place of production (Vázquez Y et al. 2004).

The permanence of dormancy depends on the plant's species and on its adaptation to the conditions of the habitat where it originally grew or evolved. The duration of dormancy may vary considerably, from very short periods to several years.

Types of dormancy

Several types of dormancy have been defined. Rao et al. (2006) group all types into either embryo dormancy or seed-coat dormancy. However, for purposes of our theme of *ex situ* conservation, we will briefly describe those most mentioned:

Innate or endogenous dormancy becomes manifest when the embryo stops growing (while the seed is still in the parent plant) and continues until the endogenous impediment ceases. From that moment, the seeds are ready to germinate when suitable environmental conditions occur. The presence of chemicals in the embryo inhibiting germination or the embryo's immaturity is probably the main cause of such dormancy. The duration of innate dormancy is highly variable according to species and, in some cases, may even differ between seeds from one individual. Some experiments indicate that, certain tropical seeds possess processes comparable with the postmaturation that is characteristic of many temperate-climate trees, whose seeds germinate only after winter has elapsed. That is, in the laboratory, when such tropical seeds are exposed or stored under low temperatures, or given applications of plant hormones such as gibberellic acid, they show dormancy (Vázquez Y et al. 2004).

Physiological dormancy involves mechanisms that physiologically inhibit the embryo's radicle from emerging. Such dormancy can be attributed to the low permeability of the integuments around the embryo to oxygen; or to the embryo's lack of sufficient growth potential to break the seed coats, as the endosperm cells restrict the radicle's growth. Physiological dormancy may range from **non-deep** through **intermediate** to **deep dormancy**, differing according to species (Baskin and Baskin 1998b).

Induced or secondary dormancy occurs when seeds are in a physiological position to germinate but are in an environment that is highly unfavourable such as having little oxygen, CO_2 concentrations that are higher than those of the atmosphere, or high temperatures. These unfavourable factors induce reversible physiological alterations in the seeds, whereby they fall into a state of secondary dormancy and are no longer able to germinate, even though they remain alive. In some cases, this type of dormancy can be broken, using hormonal stimulation. Induced dormancy can sometimes compound other types of dormancy or replace them (Vázquez Y et al. 2004).

Secondary dormancy can be induced in mature seeds once they are separated from the parent plant or, if dormancy was already present, induced into a deeper level. This process occurs when the seeds are subjected to unfavourable conditions for germination, for example, anaerobiosis, which occurs when there is excess water or low oxygen levels in the atmosphere, or the seed coats are not readily permeable. High temperatures may also have the same effect. Sometimes the lack of a single requirement for germination such as light will induce dormancy. However, it should be remembered that each species responds in particular ways (Moreno C 2004).

Imposed or exogenous dormancy. In nature, this dormancy occurs in seeds that can germinate under suitable conditions of humidity and mean temperature, that is, suitable for

the habitat that they occupy. However, they remain dormant because of one factor or another such as lack of light or particular requirements of temperature or oxygen. This dormancy is controlled by the physical conditions of the environment surrounding the seed. It occurs in seeds found in the soil and which germinate only after a disturbance that modifies, for example, the light regime or oxygen content (Vázquez Y et al. 2004).

Physical dormancy. A major reason for a seed not germinating is the impermeability of its seed coats to water. This impermeability is associated with the presence of one or more walls of impermeable palisade cells (Baskin and Bask in 1998b). Many plants produce seeds whose external coat is hard and impermeable to water or gases and even their micropyle is provided with a barrier that impedes the penetration of water to the embryo. This characteristic appears frequently in several plant families, particularly the Fabaceae or legumes, Malvaceae, and Bombacaceae (Vázquez Y et al. 2004). In forest soil, the seed coat gradually becomes permeable through weathering, microbial degradation, soil factors such as saponins, or temperature fluctuations, with germination occurring slowly. This mechanism of passive dormancy is particularly frequent in dry tropical forests, and may have originated as a mechanism for seed persistence in the soil over the season that is unfavourable to growth (Vázquez Y et al. 2004).

Chemical dormancy occurs when inhibitors of germination are deposited in the pericarp or seed coats. These substances include compounds that are produced in the seed or are transmitted to it, thus blocking the embryo's growth (Baskin and Baskin 1998b).

Embryo dormancy occurs when an embryo that has been extracted from its seed is incapable of germinating under suitable conditions. This type of dormancy is controlled, on the one hand, by the cotyledons that inhibit growth of the embryonic axis and, on the other, by substances that inhibit germination such as abscisic acid (Moreno C 2004).

Coat dormancy. The structures responsible for this mechanism are the seed coats, but frequently include other seed parts such as the endosperm, pericarp, and, in grasses, the glume, palea, and lemma. These structures impose dormancy in several ways by interfering with the entry of water and gas exchange, containing chemical inhibitors, impeding the escape of inhibitors present in the embryo, modifying the light arriving at the embryo, and mechanical constriction.

Interference with the entry of water is a major cause of dormancy in seeds with hard coats, particularly in legumes, but also in species of Convolvulaceae, Cannabaceae, Chenopodiaceae, Gramineae, Malvaceae, and Solanaceae. Many seeds have mucilaginous coats and, in some cases, this mucilage is believed to produce impermeability as in, for example, *Sinapis arvensis* and *Blepharis persica*. Some species, mainly from the Mimosoideae (legumes) group, present a small aperture in the coat, which has a plug known as the strophiole that must be broken for water to enter. In some cases, such as for *Iris* or *Rosa* spp., the coat contains substances that inhibit germination. Sometimes these inhibitors are inside the seed and the coat's impermeability prevents their escaping. For example, in oats, dormancy is maintained by the lemma and palea. When these are removed and the naked seeds placed in a moist environment, the seeds germinate.

In the vast majority of seeds that require light to germinate, the coat imposes dormancy. It acts as a filter through which light must pass. It should be remembered that seeds

requiring light to germinate are stimulated when a certain ratio of active to inactive phytochromes is established—a product of the combined action of red and far red light. Seed coats have different thicknesses and different pigmentations, thus modifying the quantity and quality of light that reaches the embryo. For example, in *Chenopodium album*, germination rates of seeds exposed to 15 min of light are, for thick coats at 49–53 µm, only 27%; 44–99 µm thick, 47%; and 24–28 µm, 62% (Moreno C 2004).

Influence of the parent plant's environment on seed dormancy

The seed's germinative characteristics have a strong genetic component that manifests itself during the seed's development. At the same time, the environmental conditions to which the parent plant is subjected affect this process and influence the type and degree of dormancy of the seeds. Seeds of a given species (e.g., *Lactuca sativa* or *Stellaria media*) produced in a period of low temperatures presented a deeper dormancy than seeds produced in warmer periods. The daylength that the parent plant experiences (especially during the last days of seed maturation) affects the dormancy of some species. Some desert species show a correlation between daylength and coat permeability. The environmental humidity that the parent plant experiences during seed maturation can also determine the seed coat's degree of impermeability (Moreno C 2004).

Classifying seeds by their dormancy

Hartmann and Kester (1971) suggest that seeds can be classified according to their response to specific environmental conditions and management methods. The groups are:

Group I. Seeds that have a hard and impermeable coat and, accordingly, the embryo cannot absorb water. This group includes seeds with such hard coats that they resist the growing embryo.

Group II. Seeds with dormant embryos that respond to pre-germinative chilling.
- A. Seeds that need a single period of chilling.
- B. Seeds that require a warm period for the root or embryo to develop before the chilling period.
- C. Seeds that need two consecutive chilling periods separated by a warm one.

Group III. Seeds that combine an impermeable coat with a dormant embryo.

Group IV. Seeds that contain chemical inhibitors that can be removed by percolation.

Group V. Recently harvested dormant seeds that become germinable after dry storage (postharvest dormancy).
- A. Seeds in which germination is promoted by light.
- B. Seeds in which germination is inhibited by light.
- C. Seeds in which germination is inhibited by high temperatures.

The Association of Official Seed Analysts, in its *Rules for Seed Testing* (AOSA 1983), differentiates between hard and dormant seeds. 'Hard seeds' are those that cannot absorb moisture as they possess an impermeable coat (groups I and III above). 'Dormant seeds' are those that can absorb moisture, but do not germinate because of restrictive influences within the seed that block some physiological reaction in the embryo, thus impeding the initiation of germination (groups II, IV, and V above) (Hartmann and Kester 1971).

Germination

Germination is known as the resumption of active growth of the embryo, which results in the rupture of the seed coats and the emergence of a new seedling capable of independent existence. When the seed separates from the plant that produced it, it is quiescent, that is, it does not show external signs of activity. For germination to take place, three conditions should be met:

- The seed should be viable, that is, the embryo should be alive and able to germinate.
- The seed's internal conditions should be favourable for germination, that is, any physical or chemical barrier to germination should have disappeared.
- The seed must be exposed to favourable *environmental* conditions. The essential factors are availability of water, appropriate temperature, provision of oxygen, and, sometimes, light. Although each of these conditions may have different effects on any given seed, more frequently, the interaction among them determines the beginning of germination (Hartmann and Kester 1971).

Phases of germination

Germination encompasses a complex sequence of processes (Figure 2) that imply complex changes of a biochemical, morphological, and physiological nature. These processes are described by authors such as García B (2004), Grierson and Covey (1984), Hartmann and Kester (1971), and Moreno C (2004). In general, the processes are seen as occurring in five stages, as described on the next page.

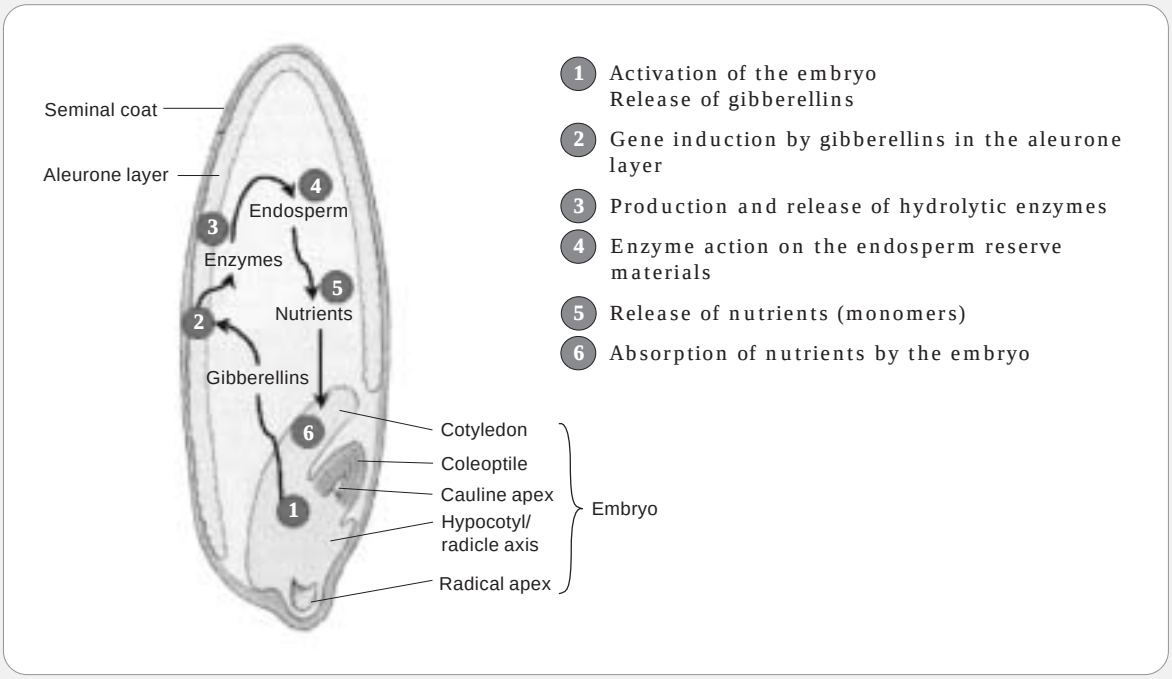

Figure 2. Sequence of germination processes in cereals such as wheat and barley (from García B 2004).

The first stage starts with the dry seed imbibing water, its coats softening, and the protoplasm hydrating. This process is largely physical and occurs even in nonviable seeds. As a result of absorbing water the seed swells and its coats may break.

In the second stage, cellular activity in the seed begins. It is characterized by the appearance of specific enzymes and the elevation of the respiration rate, particularly in cereal grains such as barley and wheat. The process is controlled by the embryo, which uses gibberellic acid, previously synthesized by the embryonic axis and/or scutellum (cotyledon in grasses) to send signals to the aleurone layer. At the same time, the coleoptile of the growing embryo begins to synthesize indoleacetic acid, which initiates the differentiation of vascular tissue through which gibberellic acid is transported towards the aleurone layer. In response to the gibberellic acid, the aleurone synthesizes the enzymes required for germination, except β-1,3-glucanase, the synthesis of which is still not completely understood.

The third stage involves the enzymatic digestion of the insoluble complex reserve materials (mostly carbohydrates and fats, with some proteins) into soluble forms that are translocated to the areas of active growth. This whole process is carried out by enzymes secreted by the aleurone layer that surrounds the endosperm. The enzymes are α-amylase, protease, ribonuclease, and β-1,3-glucanase, the last attacking the hemicellulose in the walls. All are secreted towards the endosperm and, in only 3 or 4 days, they totally liquefy it. The endosperm begins dissolving; the cell walls degrade; the protein reserves are hydrolyzed to form amino acids; and the starch is also hydrolyzed to form reduced sugars that then become sucrose to be transported to the embryo. Other enzymes appear in the aleurone, weakening the seed coats and permitting the root end to pass through them. Cell elongation and root emergence are events that are associated with the beginning of germination. Cellular division can also occur in early stages but is, apparently, independent of cell elongation.

Fourth stage. Those substances in meristematic regions are assimilated, providing energy for cellular activities and growth, and the formation of new cellular components.

Fifth stage. The seedling grows by the usual processes of division, growth, and division of new cells in the growing points. The seedling depends on the seed reserves until such time as the leaves can adequately handle photosynthesis.

To summarize, germination occurs in the following stages: imbibition or hydration; germination as such (including enzymatic and respiratory activity, digestion, translocation, and assimilation) when water absorption is reduced; growth, characterized by a renewed increase in water absorption and respiratory activity; and the associated emergence of the radicle.

Essential Structures of the Seed and Seedling

To evaluate their physiological status and interpret the viability indicators in the respective tests, the essential structures of seed and seedlings must be identified. The following description also complements what was already discussed in *Module 3, Submodule B, Lessons 1* and *2*.

A seed constitutes three basic parts: the **embryo**, **food storage tissues**, and **seed coats**. The embryo is a new plant that results from fertilization, that is, from the union of a male and female gamete. Its basic structure consists of an axis with growing points at each extreme—one for the stem and one for the root—and one or more seminal leaves (cotyledons) fixed at the embryonic axis.

In **dicotyledonous plants**, the embryo consists of three components: **radicle**, which will form the root; **plumule**, which will give rise to the stem; and two **cotyledons**, which are part of the seed's reserve tissues and which will ultimately be used as photosynthetic organs (Figure 3A).

In **monocotyledonous plants**, only one **cotyledon** is found. It does not have photosynthetic characteristics during germination and constitutes a protective tissue called the **scutellum**. It separates the embryo from the endosperm, which is the principal reserve tissue in all monocotyledons (Figure 3B).

Development of seedlings and essential structures

The seed's absorption of water, increased respiration, and the biochemical changes that it undergoes (as described above) awakens the embryo into growing and subsequently developing into a growing seedling. Normally, 'seedlings' refers to tender young plants that emerge from the soil, having developed directly from seed embryos.

Usually, the first sign of germination is the appearance of the **radicle**, from which originates the primary root. In monocotyledons, this part of the seedling lasts only a very short period, as it promptly develops secondary roots. In gymnosperms and dicotyledons, the radicle develops into the primary or principal root that lasts the plant's entire life.

In other species, **hypocotyl** growth is the first visible manifestation of germination. This pattern is found in families such as the bromeliads, palms, Chenopodiaceae, Onagraceae, Saxifragaceae, and Typhaceae. The growth of this structure is important in **epigeal germination**, as it lifts the cotyledons out of the soil (Figure 4A). As the episperm tears, the cotyledons become exposed to sunlight, converting them into photosynthesizing organs during the seedlings' first stages of growth. The **epicotyl** develops late.

Epigeal germination is common in seeds with endosperm, for example, in castor bean (*Ricinus communis*), onion (*Allium cepa*), and *Rumex* spp. It is also found in seeds without endosperm such as those of bean (*Phaseolus vulgaris*), squash (*Cucurbita pepo*), cucumber (*Cucumis sativus*), mustard (*Sinapis alba*), groundnut (*Arachis hypogaea*), and lettuce (*Lactuca sativa*).

In **hypogeal germination**, the hypocotyl grows very little and the cotyledon usually remains within the soil. The **epicotyl** starts elongating early so that the first two leaves are in the air and receiving sunlight (Figure 4B).

Hypogeal germination appears in seeds with endosperm such as those of wheat *(Triticum aestivum*), maize (*Zea mays*), barley (*Hordeum vulgare*), date palm (*Phoenix dactylifera*), and rubber (*Hevea* spp.). It also appears in seeds without endosperm, for example, pea (*Pisum sativum*) and broad bean (*Vicia faba*).

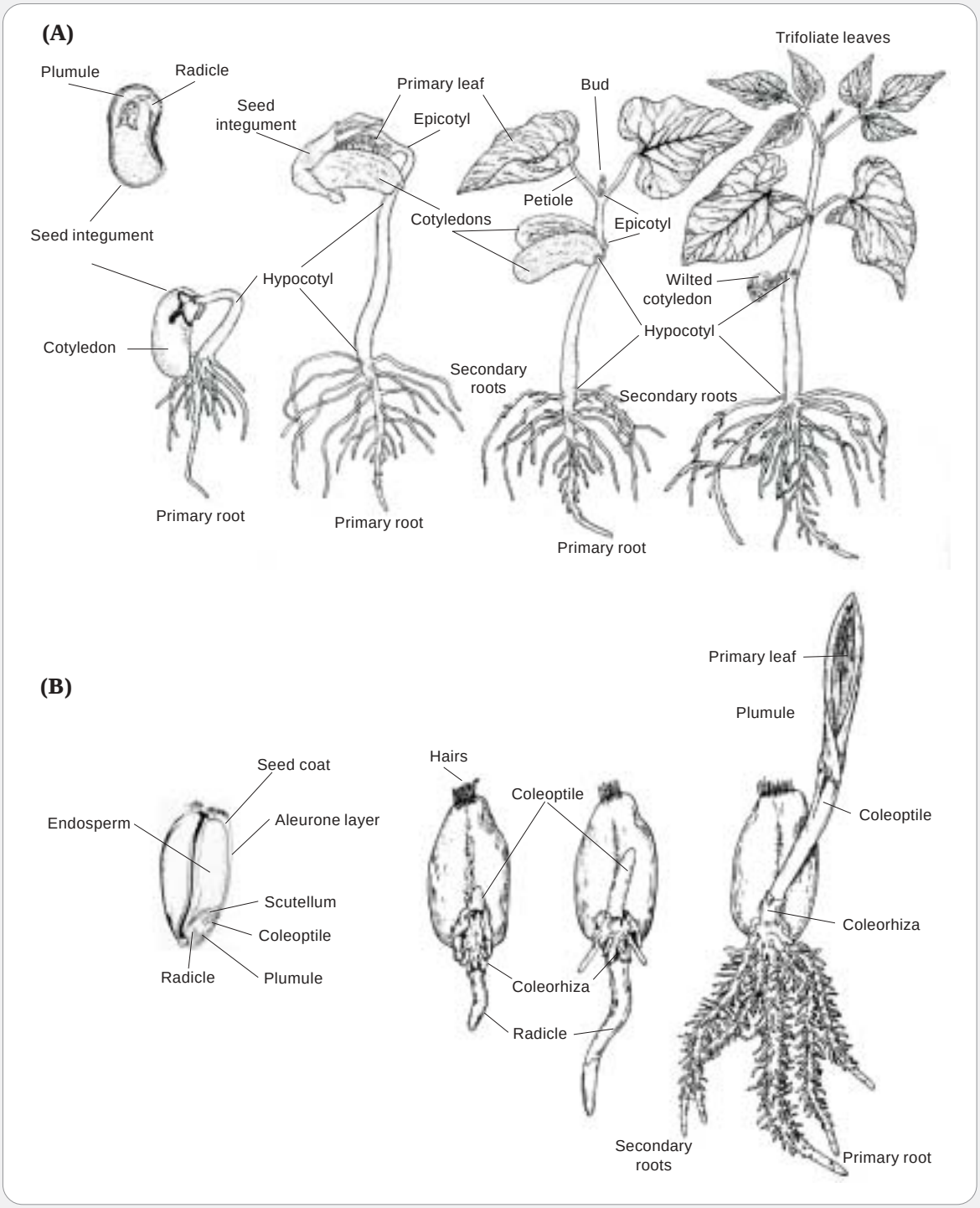

Figure 3. Essential structures of seeds and seedlings. **(A)** Dicotyledons (Magnoliopsida), for example, beans (*Phaseolus vulgaris* L.). **(B)** Monocotyledons (Liliopsida), for example, wheat (*Triticum aestivum* L.) (from Robbins et al. 1966).

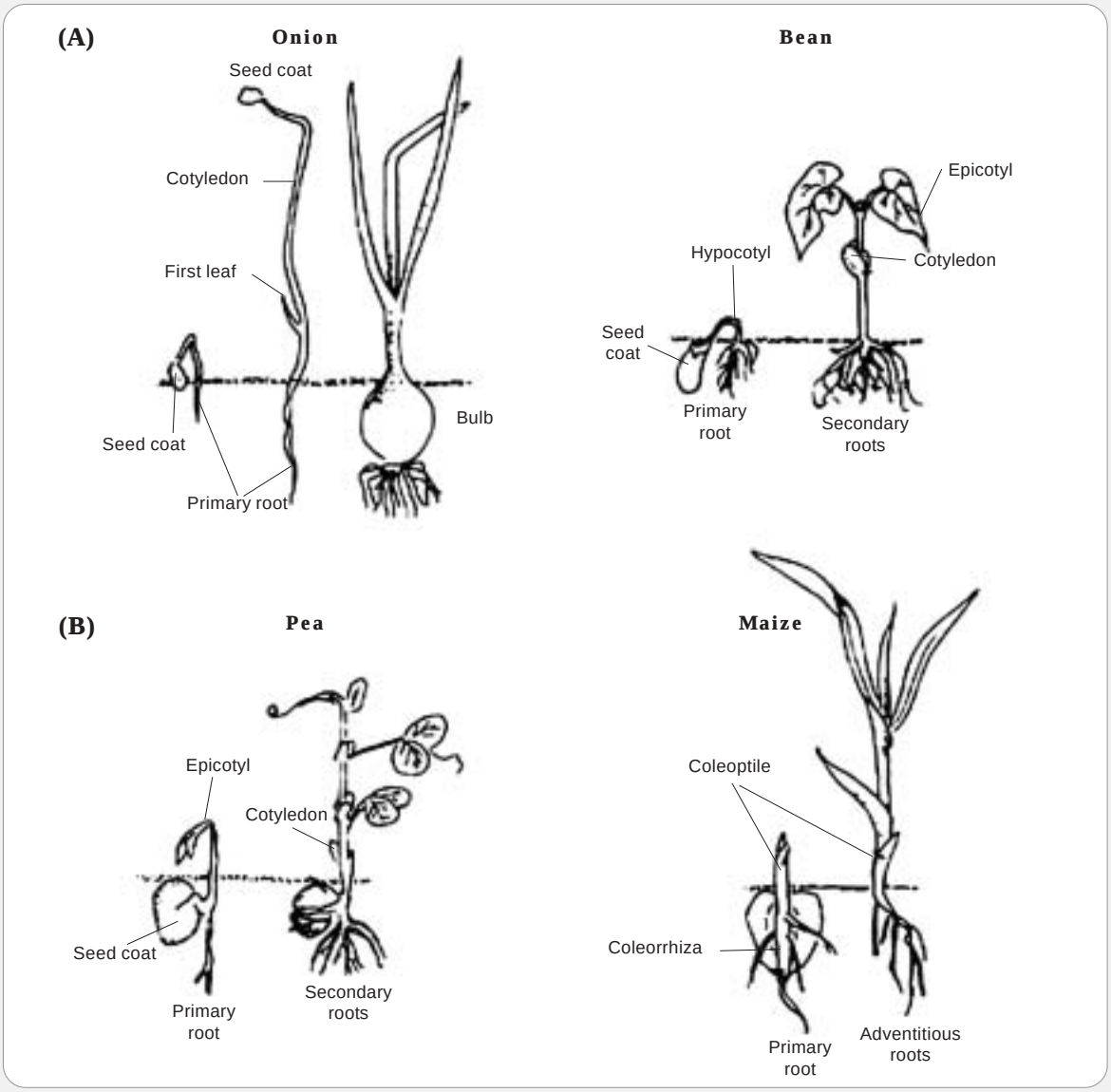

Figure 4. Types of germination. **(A)** Examples of seedlings with epigeal germination: *Allium cepa* (onion) and *Phaseolus vulgaris* (bean). **(B)** Examples of seedlings with hypogeal germination: *Pisum sativum* (pea) and *Zea mays* (maize) (from Moreno C 2004).

To evaluate the parameters of viability such as percentage and speed of germination, and seedling morphology, commercial-seed experts, as represented by the International Seed Testing Association (ISTA) define certain terms and apply criteria that should be taken into account (ISTA 1999). Nevertheless, because germplasm of cultivated species is per se diverse, these rules are a guide only. More worthwhile is to make careful observations of the performance of each species being tested for the parameters being evaluated. During laboratory tests, seedling performance should also be correlated with field performance.

'Essential structures of seedlings' refers to those structures that are essential for continuous and satisfactory plant development such as the radicular system, embryonic axis, cotyledons, terminal buds, and, for Poaceae, coleoptile (Figure 3). Depending on the species, ISTA experts (1999) define a seedling as consisting of a specific combination of the following structures, essential for subsequent development:

- Radicular system, made up of the primary root and, in certain cases, secondary or seminal roots
- Embryonic axis, made up of the hypocotyl, epicotyl, and terminal bud; and, for Poaceae, the mesocotyl
- One or more cotyledons
- In all Poaceae, the coleoptile

Viability, Vigour, Dormancy, Germination, and Essential Structures of Vegetative Planting Materials

The availability of information on viability, vigour, dormancy, and germination of vegetative planting materials (plant fragments or specialized organs) is not as abundant as for seeds. However, except for the logical differences that exist, understanding and applying these concepts to this type of material in an analogous manner as for seeds are essential.

The concepts of viability and vigour, which refer to the properties of being alive and able to generate new plants that emerge quickly and uniformly, are applied generally.

Dormancy or the interruption of growth in meristematic tissues can appear in higher plant forms. Deciduous and subdeciduous trees are best known for exhibiting this type of behaviour. They are characterized by a phenological phase of leaf fall and meristematic dormancy at the end of the growing season. Where stakes are used for propagation, serious difficulties in rooting can be caused by the presence of chemical inhibitors, as in grape vine (Hartmann and Kester 1971).

When speaking of the germination of vegetative planting materials, we cannot talk about resuming active growth in the embryo, because this type of structure does not exist in such materials. Instead, we must refer to meristematic buds or regions that can be activated to generate shoots that will develop into new plants.

With respect to the essential structures for vegetative reproduction, propagules (see *Module 3, Submodule B, Lesson 1*) or plant fragments should have buds or meristematic tissues able to generate shoots, and have accumulated nutritive reserves to feed them until they develop photosynthesizing organs able to sustain an independent plant (Figure 5).

Evaluating the Lesson

After this lesson, you should be familiar with the most important concepts of germplasm viability and vigour, and how it relates to deterioration, and the phenomena of dormancy and germination.

Before going on to the next lesson, answer the following question: When planning and executing methods to verify a germplasm's viability, which of the concepts revised in this lesson do you consider as the most important? Briefly explain why.

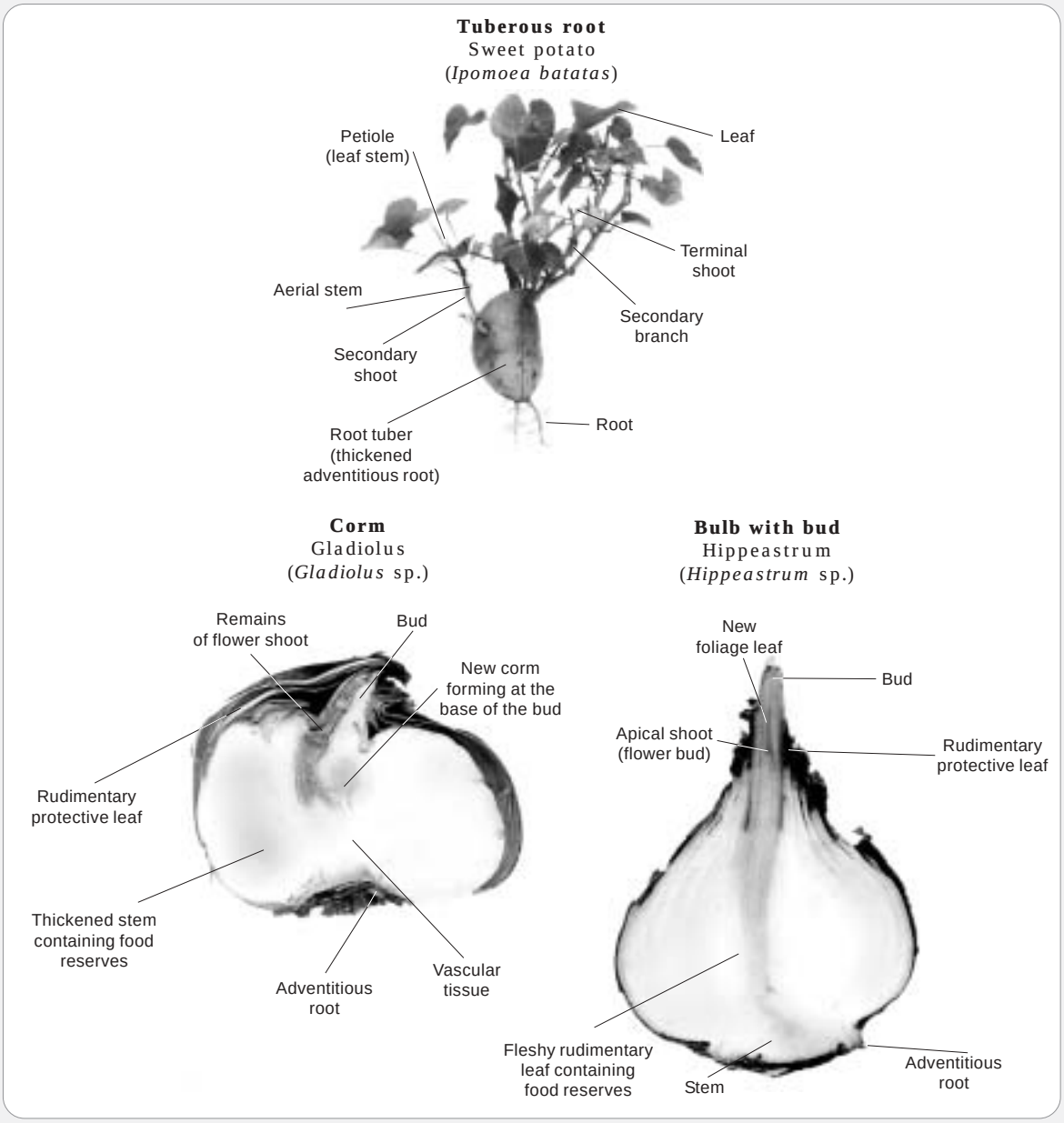

Figure 5. Tuberous root and longitudinal sections of a corm and bulb, showing principal components (from Kindersley 1994).

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Contributors to this Lesson

Benjamín Pineda, Alba Marina Torres, Daniel Debouck, Carlos Iván Cardozo, Rigoberto Hidalgo, Mariano Mejía, Graciela Mafla, Arsenio Ciprián, Manuel Sánchez, Carmen Rosa Bonilla, and Orlando Toro.

Next Lesson

In the next lesson, you will become familiar with the principal procedures for verifying the biological status of germplasm, both seeds and plant parts.

Lesson 2 Verification Procedures

Objectives

- To describe the procedures most used to evaluate indicators for germplasm viability and vigour
- To review some concepts used to measure germplasm viability and vigour

Introduction

The germplasm's biological or physiological status is verified either after conditioning or through periodic samplings during conservation under storage. It is a major activity for quality control. As mentioned in the previous lesson, applying it demands knowledge of the species and their peculiarities with regard to germination and the presence or absence of dormancy; the environmental and endogenous conditions that influence the behaviour of seeds or propagules; and the evaluation procedures and methods to conduct and their periodicity. Some of these requirements have already been discussed in the previous lesson. In this lesson, we consider procedures and methods for evaluating indicators of germplasm viability and vigour.

First, we point out that periodic evaluation of germplasm viability is indispensable for ascertaining that the materials conserved *ex situ* are remaining in good condition. These evaluation procedures are regulated and standardized by institutions such as FAO and IPGRI (now Bioversity International) to help germplasm bank managers guarantee, through specific tests, the biological integrity of the PGRs under their responsibility (Box 1).

Viability Indicators and their Evaluation

The **indicators** most used to determine viability are **germination** and **vigour**. For their assessment, different methods have been designed, whose application depends on the type of seeds to evaluate, and the availability of resources, facilities, and installations.

Before implementing tests to evaluate the viability and vigour of a given species, information should be collected on the characteristics of that species. We must have data on the habitat where the germplasm was originally collected, and likewise on seed type (orthodox or recalcitrant), age, and the conditioning and storage conditions to which the materials had been submitted. A sufficient supply of seeds is also advisable to permit additional experiments, should not enough information be available on the material. Many different methods are available to test seed viability, of which the most accurate and reliable is the germination test. Others include biochemical tests, which have the advantage of being quicker, but are not as accurate as the germination test (Rao et al. 2006).

Evaluation of viability involves a series of stages from preliminary testing to the application of highly elaborate procedures. The first stage is to establish a **preliminary germination test** in a suitable environment and under recommended conditions of light and temperature, preferably those of the habitat where the germplasm had originally been collected. The results obtained from this test will orient the next procedures.

Box 1

FAO and IPGRI guidelines for gene banks

Viability monitoring

26. Genebank managers have the responsibility to provide conditions which will maintain the viability of each accession held within the genebank above a minimum value. Hence accession viability must be monitored. The preferred standard is that this obligation extends not just to the genebank, which can be considered the originator of the accession, but also to those genebanks holding a duplicate of the accession.

27. Viability will usually be assessed by means of a germination test, although other test procedures (such as the topographical tetrazolium test) may be required in order to clarify whether the non-germinating seeds in these tests are non-viable or whether their dormancy has not been broken during the test. Empty seeds not already removed before storage should be removed before beginning the germination test. An IBPGR handbook (Appendix II, IBPGR, 1985) is available which provides both general and specific advice on the conduct of germination tests and appropriate dormancy-breaking procedures.

28. The minimum standard is that accession viability monitoring tests be carried out at, or soon after, receipt and subsequently at intervals during storage. The initial germination test should be carried out on a minimum of 200 seeds drawn at random from the accession.

29. The period between viability monitoring tests will vary among species and will also depend upon the seed storage conditions. Genebanks should regularly conduct monitoring tests. Under the preferred storage conditions for base collections, the first monitoring test should normally be conducted after 10 ye ars for seeds with high initial germination percentage. Species known to have poor storage life or accessions of poor initial quality should be tested after 5 years. The interval between later tests should be based on experience, but in many cases may well be greater than 10 years. Note that where the preferred conditions of storage are not being met, then monitoring may need to be more frequent. Where a genebank has been operating for some years under the preferred conditions and has obtained sufficient information from their own monitoring tests on the range of material they work with to justify more extended monitoring intervals then this should be done.

30. The objective of the viability monitoring test is to decide whether regeneration is required. It is recommended that, in order to save seeds, 50 - 100 seeds be drawn at random from the accession for each monitoring test. The simplest method of determining whether substantial loss in viability is occurring, and distinguishing between this and the fluctuation in test results which is largely a consequence of sampling error, is to plot the results of successive monitoring tests against the period of storage and to see whether a progressive trend of loss in viability can be detected. Where such an indication is obtained, it is recommended that, provided sufficient seeds are available, a further sample of 100 seeds are drawn at random for a further viability monitoring test to reduce the probability that regeneration is initiated prematurely. Once it has been decided that an accession should be regenerated, further germination tests should be suspended to save valuable seeds.

31. It is essential that genebanks have, or have access to, sufficient laboratory equipment to enable viability monitoring tests to be carried out in a regulated, uniform and timely manner. In some cases the particular problems of the species maintained will require the provision of more specialized equipment, e.g. X -ray equipment to test for empty seeds and/or insect-damaged seeds.

(Continued)

Box 1. (Continued.)

32. Initial germination testing and viability monitoring during storage requires adequate facilities to carry out these tests according to the conditions described in paragraphs 27 to 31. It is acceptable that a base collection should have access to suitable seed testing facilities and it is preferred that these should be at the same site as the base collection.

33. In the case of active collections, it is suggested that monitoring every 5 years will normally be satisfactory. However, this should be adjusted up or down depending upon the species stored, initial viability, and the storage environment. Where base and active collections are maintained side -by-side within the National Agricultural Research System under the preferred conditions for base collections then the advice for base collections should be followed for the active sample and in most cases it will not be necessary to sample from the base collection until the results for the active collection sample suggest this is necessary, or the latter becomes depleted. Note that this comment only applies in situations where the base and the active collections represent the same original seed sample which has simply been divided at random into the base and active samples.

34. There is no non-destructive viability monitoring test currently available. It is recommended that where the number of seeds within an accession is limited, and regeneration is feasible, the seedlings produced during accession viability monitoring tests should be grown out to provide a fresh stock of seeds (e.g. for distribution) providing, of course, that the number of seedlings available is sufficient for regeneration.

SOURCE: FAO and IPGRI (1994).

Should the seed not germinate, then tests must be carried out to discover the reason why (see *Module 3, Submodule C, Lesson 1*) and the recommended treatments then carried out (Table 1). In such a case, **respirometric or biochemical activity tests** must be conducted (Vázquez Y et al. 2004) before carrying out further procedures that may be unsuccessful.

An example of such a test is that of soaking seeds in a solution of 2,3,5-triphenyltetrazolium chloride (TTC). Viable seed, that is, seeds with biochemically active embryos become red on soaking. The test is based on the activity of the dehydrogenase enzyme systems linked to respiration in living things. For seeds, these systems are associated with the viability of the embryo and its consequent loss when no enzymatic activity exists. As the tissues of viable embryos respire, through oxidation and reduction, they liberate hydrogen ions. The hydrogen then combines with the TTC (Figure 1), which is normally colourless, producing, through reduction, a formazane, which is an insoluble nondiffusive pigment that colours tissues (Baskin and Baskin 1998a; Delouche et al. 1971).

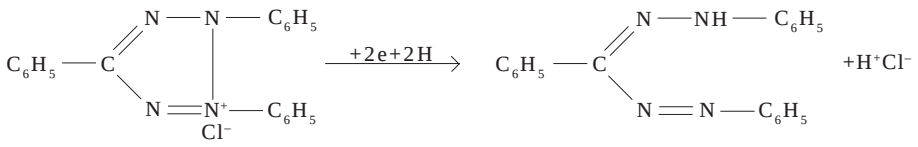

Figure 1. Reaction of 2,3,5-triphenyltetrazolium chloride with hydrogen that is released enzymatically by viable embryos to obtain formazane (from Delouche et al. 1971).

Table 1. Testing and treating nongerminating seeds: possible reasons why seed may not germinate, the corresponding treatments for correction, and the natural conditions that the treatments simulate.

Reason	Observed symptom	Applicable treatment	Observations	Natural conditions simulated by treatment
Hard seed coat	Impermeability to water; Impermeability to gases; Physical barrier to embryo expansion	Mechanical scarification by incision or scraping	Locate the embryo in the seed so not to damage it	Abrasion and wearing away of the seed coat by the action of soil particles, or partial rupture by other agents or microbial attacks
		Scarification with sulphuric and hydrochloric acids	Test with different concentrations (from 10% to 100%) and exposure times to the acids	Passage through the digestive tracts of animals, with different digestion times
		Thermal scarification by immersion in hot water at different temperatures up to 100°C	Maintain constant temperatures or cool gradually	Thermal effect of fires over damp soil and the time taken to cool
		Thermal scarification by dry heating to as high as 100°C	Base temperatures on those reported for different soil depths during fires; take great care with times of exposure	Effect of high temperatures on vegetation during fires over dry soil
		Variable temperature periods (thermoperiods)	Thermoperiods can be achieved in incubators or germination chambers, or with any of the previous methods. To determine the amplitude of thermal fluctuation, consider what would occur in the species' habitat	Temperature variations that occur during the day, with the highest temperatures corresponding to those registered in the soil at the highest insolation

(Continued)

Table 1. (Continued.)

Reason	Observed symptom	Applicable treatment	Observations	Natural conditions simulated by treatment
Embryo's anatomical or physiological immaturity	Germination does not start	Postmaturation time; Cold temperatures; Hormones (i.e., physiological)	The embryo may mature over time; Apply stratification (chilling under moisture); Use exogenous gibberellins (gibberellic acid) in concentrations from 500 to 1500 ppm or more, according to the depth of dormancy	Environmental conditions of temperate or similar zones
Inhibited embryonic growth	Germination does not start because of inhibitors present in the seed or lack of external stimuli	Time, washing Light, temperature, changes in humidity	The inhibitors may degrade over time or be eliminated with abundant washing Different spectral colours (red, blue, far-red, white light). Mineral salts (potassium nitrate in concentrations of 0.1%–1%) alter cellular permeability, replacing, for example, the role of light in germination. Hyperosmotic solutions (polyethylene glycol or Carbowax 6000 in solutions with osmotic potentials of 10 to 15 bars) modify the seed's internal permeability. Use gibberellins in concentrations from 250 to 1000 ppm; auxins (IAA, 2,4-D); cytokinins (kinetins); or combinations (e.g., kinetin + Ethephon, i.e., ethylene + gibberellic acid)	Environmental conditions of tropical, subtropical, or similar areas

(Continued)

Table 1. (Continued.)

Reason	Observed symptom	Applicable treatment	Observations	Natural conditions simulated by treatment
Quiescent seed	Does not start germination, because resting	Water and adequate environment		Conditions that expose the seed to damp soil
Reversible physiological change	Does not start germination	Time, hormones, other stimuli, light, temperature, changes in hydration	Physiological activity can recover over time. Gibberellins in concentrations of 250 to 1000 ppm. Auxins (IAA, 2,4-D); cytokinins (kinetins); or combinations (e.g., kinetin + Ethephon (ethylene + gibberellic acid). Mineral salts (potassium nitrate in concentrations from 0.1% to 1%) alter cellular permeability, replacing, for example, the role of light in germination. Different spectral colours (red, blue, far-red, white light). Hyperosmotic solutions (polyethylene glycol or Carbowax 6000 in solutions with osmotic potential of 10 to 15 bars) modify the seed's internal permeability	Micro-environment of seeds under given conditions
Irreversible physiological change: damaged embryo, partial death, total death		The change is of such magnitude that no treatment would give results		

SOURCE: Vázquez Y et al. (2004).

The reaction occurs within the cells and, as the pigment is not diffusive, a clearly distinguishable delineation appears between breathing (viable) and nonbreathing tissue (dead). Breathing tissue acquires a red or pink colour while the second retains its natural colour (Figure 2). The position and size of the necrotic areas in the embryo, endosperm, and/or gametophytic tissue determine if the seed can be classified as viable or nonviable.

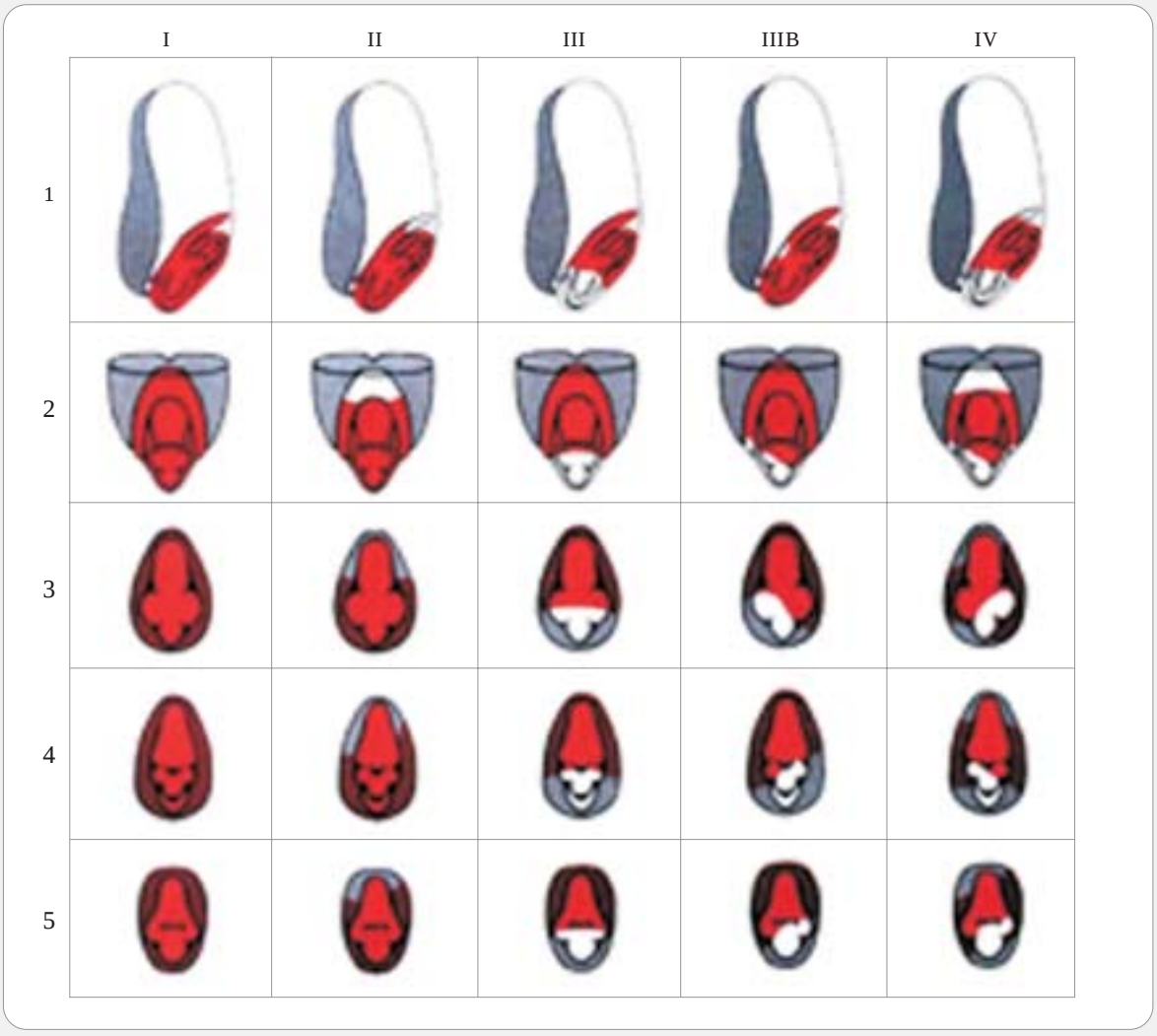

Figure 2. Guide for evaluating the reaction of seed tissue to 2,3,5-triphenyltetrazolium chloride (TTC) in cereals. The stylistic figures in column I show viable and therefore entirely coloured embryos. The other columns show the maximum area of flaccid or necrotic (and therefore uncoloured) tissue permitted for viable seeds. The exception is column IIIB, Row 1, which shows a nonviable seed. It has necrotic (uncoloured) tissue in the centre of the scutellum, indicating heat damage. Row 1 corresponds to wheat (I), common rye (II), barley (III and IIIs), and oats (IV) when bisected for evaluation; Row 2, cross-sections of oat seeds; Row 3, sections of barley seeds prepared by the excised embryo method; Row 4, sections of common rye seeds prepared by the excised embryo method; Row 5, sections of wheat seeds prepared by the excised embryo method (from ISTA 1999).

According to the ISTA guidelines (1999), in the TTC test or topographical tetrazole test, viable seeds become completely stained, whereas the dead seeds do not. However, partial staining can occur because of the variable proportions of necrotic tissue in different areas of the embryo, indicating that not all seed tissues are dead.

The recommended sample size for this test is four replications of 100 seeds each. Should seed availability be low, the *International Standards for Genebanks* (FAO and IPGRI 1994) recommend using a minimum of two replicates with 100 seeds per replicate. If the test results show that germination is below 90%, an additional 200 seeds should be tested, using the same method. Overall seed viability is then taken as the mean of the two tests (Rao et al. 2006). However, this test can be carried out only on those firm seeds that did not germinate in the standard germination test. The TTC test is usually applied to dormant seeds, although it can be used with any seed.

Determining Germination and Vigour

To implement viability tests, the following activities should be conducted:

- Preconditioning procedures established
- Test types defined:
  - Germination in sand, soil, or other substrate
  - Germination on germination paper
- Sites determined for conducting the tests:
  - Germinators or other infrastructure
  - Laboratory, growth rooms, incubators
- Procedures established for executing the tests:
  - Reception and preparation of samples, and verification of identification
  - Preparation of substrates and tools needed for the tests
    - Germination paper, sand, soil, other substrates, identifiers, trays, containers
    - Reagents
    - Adequate in-depth planting and spacing in trays; placement on germination paper and/or in trays
    - Placement of trays, containers, or germination paper in the site or equipment suitable for germination
    - Agronomic attention and care to ensure the test's success (e.g., humidity, temperature, and light)
- Type of data to collect determined and their evaluation for decision-making

Preconditioning

Often, to make seeds germinate and succeed in the viability test, the seeds' internal conditions must be favourable for the process. This means that barriers of a physical, chemical, physiological, or other nature must disappear to permit germination (see *Module 3, Submodule C, Lesson 1*). If this has not happened, then preconditioning procedures must be carried out, like those described below or in guidelines for testing germination of the most common crop species, as suggested by Rao et al. (2006).

Mechanical scarification. The aim is to modify hard or impermeable seed coats. The seed coat is ruptured, scratched, or mechanically altered to make them permeable to water

or gases. Although seed extraction and cleaning during harvest probably does a certain amount of scarification, most seeds with hard coats show improved germination with additional artificial treatment. Rubbing seeds with sandpaper, scratching them with a file, or incising them with blades, as according to case, would be sufficient. Manual scarification on any part of the seed coat is effective, but the micropylar region should be avoided, as it is the most sensitive part of the seed and is where the radicle is located (Rao et al. 2006). Scarification should not be so extensive that it damages the seeds. To determine the optimal time for germination, a few seeds may be sown in a test plot, soaked to observe swelling, or examined under a magnifying glass. Under the last, the seeds should appear dull in colour, but not so cut up that the seed's internal parts are exposed (Hartmann and Kester 1971).

Soaking in water. Reasons for soaking seeds in water include modifying the hard coats, removing inhibitors, softening the seeds, and reducing germination time. Sometimes, this treatment overcomes seed-coat dormancy or, in other cases, stimulates germination.

For some seeds, their impermeable coats can be softened by placing them into hot water (170°–212°F or 77°–100°C) at four to five times their volume. The fire is removed immediately and the seeds left to soak for 12 to 24 h in the gradually cooling water. Then, using suitable screens, the swollen seeds are separated from those that did not swell. The latter are once again subjected to the same treatment or to another method. In some cases, seeds can be boiled for a few minutes but this procedure is too hazardous, as exposure to such high temperatures can damage the seeds.

In certain cases, the inhibitors present in the seeds can be lixiviated by washing or soaking them in water. Seeds that ordinarily germinate slowly can be soaked before being put out to germinate, thereby shortening emergence time (Hartmann and Kester 1971).

Scarification with acid. Scarification with acid helps modify hard or impermeable seed coats. Soaking in concentrated sulphuric acid is an effective method but the acid must be used with care as it is very corrosive. It reacts violently with water, considerably elevating temperatures and producing splatters. Protective clothing should be worn to protect the operator's skin and eyes. Dry seeds are placed in glass or earthen containers and covered with concentrated sulphuric acid (specific weight 1.84) at a ratio of one part seed to two parts acid.

To achieve uniform results and prevent the accumulation of dark and resinous material that is sometimes present in seeds, the mixture can be gently stirred at suitable intervals. Because stirring the seeds may elevate temperatures, vigorous stirring of the mixture should be avoided to prevent damage to the seed and minimize splattering of the acid. The most desirable range of temperatures is 60° to 80°F (15°–27°C). With higher temperatures, the contact period is shortened and lengthened with lower temperatures.

The duration of the treatment should be carefully standardized. At the end of the treatment, the acid is drained away and the seeds washed. The acid used should be thrown away onto ground that is not in use, never into drain pipes. All possible speed should be used when washing the seeds. Abundant water is needed to dilute the acid, reduce temperatures, and prevent splattering. Washing for 10 min under running water is considered sufficient. The wet seeds can be planted immediately or dried and stored for later planting (Hartmann and Kester 1971).

Chilling under moisture (cold stratification). The main goal of this treatment is to expose seeds to the low temperatures that are frequently needed to obtain prompt and uniform germination. This treatment is necessary for the seeds of many tree and shrub species to encourage physiological changes in the embryo (postmaturation).

Seeds are put in refrigerators or, during winter, outside in covered boxes or in holes, 15 to 30 cm deep in the earth. Seeds are placed in containers on a moistened germination substrate and kept at 3° to 5°C in a refrigerator for a minimum of 7 days (Rao et al. 2006). The time needed to complete postmaturation depends on the class of seeds and sometimes on the individual plots. For most seeds, the necessary stratification period ranges between 1 and 4 months. During this period, the seeds should be examined periodically. If they are dry, they need to be moistened again. At the end of the postmaturation period, some seeds may germinate in storage.

To sow them, the seeds are removed from their containers and separated from the medium, with care being taken not to damage the moist seeds (Hartmann and Kester 1971).

Combining two or more pregermination treatments. Two or more treatments are combined to either overcome the effects of an impermeable seed coat and dormant embryo (double dormancy) or encourage the germination of seeds with complex embryo dormancy. The combination of mechanical or acid scarification or soaking in hot water with chilling under moisture is effective for seeds that have both hard impermeable coats and embryo dormancy. Any of the three treatments can be used to modify seed coats (Hartmann and Kester 1971).

Planting time. Planting in a given time of the year can be used to encourage the postmaturation of dormant seeds and to comply with special requirements for germination. This procedure can help save a certain amount of time and use of special equipment that otherwise would have been needed. Seed is planted outside directly in the seedbed or cold bed at a time in the year when the natural environment provides the necessary conditions for postmaturation. If seeds are left in the seedbed for a long time, they must be protected from desiccation, adverse environmental conditions, animals, birds, diseases, and competition with weeds (Hartmann and Kester 1971).

Dry storage. Recently harvested seeds of many annual or perennial herbaceous plants do not germinate if they have not gone through a period of dry storage. This postharvest dormancy can last a few days or several months, depending on the plant species. Because dry storage is the usual method for handling and keeping most seeds of cereals, vegetables, and flowers, this dormant period is usually over by the time seeds arrive for viability tests. Otherwise, drying the seeds will facilitate germination (Hartmann and Kester 1971).

Chemical stimuli. Many recently harvested but dormant seeds respond to soaking in chemical stimuli such as potassium nitrate, gibberellic acid, thiourea, and sodium hypochlorite.

- **Potassium nitrate** is used at 0.2%, with the seeds placed in germination trays or in petri dishes, and the substrate is moistened with solution.
- **Gibberellic acid** (GA), a plant hormone, increases the germination rate of certain classes of dormant seeds, increases the speed of germination, encourages plant growth, and

overcomes stunting or dwarfism in dormant epicotyls. Seeds are soaked in an aqueous solution of gibberellic acid at variable concentrations (100 and 500 mg/litre), according to the species' response (Hartmann and Kester 1971).

- **Thiourea** [CS $(NH_2)_2$] has been used experimentally to promote germination in some dormant seeds, particularly those that do not germinate in darkness or at high temperatures, or require cold-moisture treatment. Aqueous solutions, ranging from 0.5% to 3%, are used. As thiourea somewhat inhibits growth, seeds should not be soaked for more than 24 h. They should then be rinsed with water (Hartmann and Kester 1971).
- **Sodium hypochlorite** is used to encourage the germination of seeds like those of rice. It blocks the effect of inhibitors dissolved in the water found in the husk. A commercial concentrate of sodium hypochlorite is used at a ratio of 1 part of the chemical to 100 parts of water (Hartmann and Kester 1971).

Germination tests

A germination test consists of exposing seeds to favourable environmental conditions such as moisture, temperature, oxygen, and, in certain cases, light, to ensure the embryo resumes active growth. Hence, different media or substrates are used (Table 2), as well as installations and equipment to obtain the expected results (Figure 3).

Standards and norms have been established for germination tests in terms of duration, number of seeds, drying levels, incubation temperatures, and assessment (ISTA 1999). However, these standards should be applied with care, making adjustments as according to germplasm type.

Table 2. Culture media and substrates used in germination tests.

Medium or substrate	Observations
Agar-agar 1%	A medium with stable moisture content and low contamination. It can be used in the field or laboratory. Very useful under shady conditions, as it conserves moisture for longer. Care must be taken when working under direct sunlight, as it dehydrates and condensed water accumulates in the petri plate. The medium facilitates radicle emergence and seedling transplant.
Filter paper	Provides good support, but care must be taken to prevent excessive drying.
Paper towels	More economical to use, but needing the same care as filter paper to prevent excessive drying.
Vermiculite, perlite, and similar substrates	Useful for large seeds only. Conserve moisture for more time than does paper, but moisture levels still need watching. Depth is easy to control.
Soil	As useful as perlite and similar substrates. Has the advantage of contributing nitrogenous compounds that stimulate germination.
Sand	This medium is very popular but must be washed thoroughly to eliminate salts before use. Drains more easily than soil and so needs a controlled water supply.

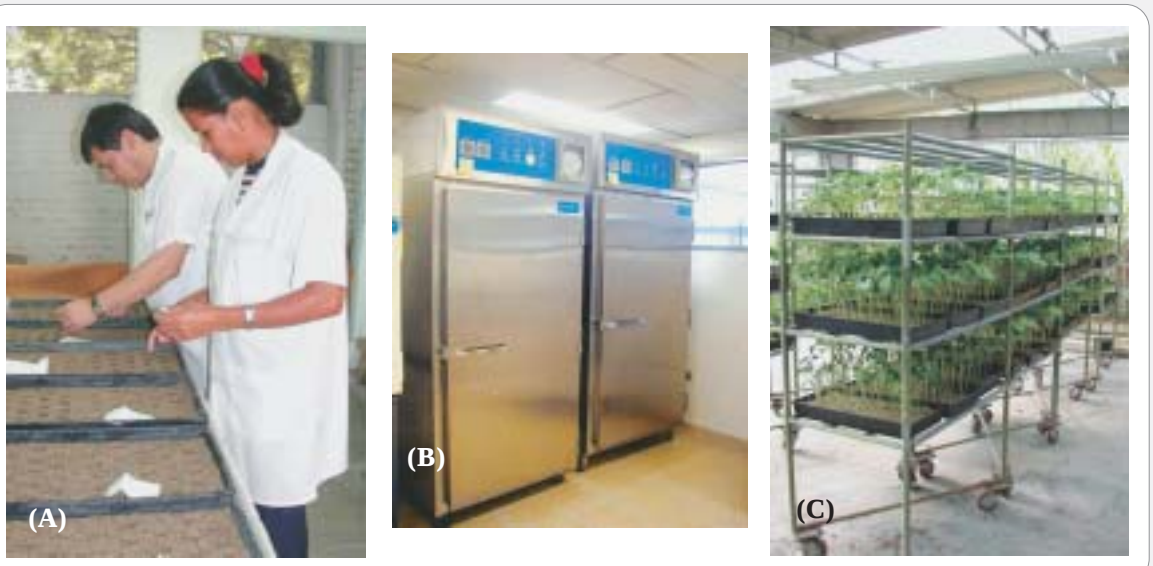

Figure 3. **(A)** Planting in trays containing sterilized sand, **(B)** germination chambers, and **(C)** shelving carrying trays of growing seedlings (photos by B Pineda, GRU, CIAT).

Detailed information on the various methods for determining seed viability can be found in the following publications: ISTA's *International Rules for Seed Testing* (1999) and its *Handbook on Seedling Evaluation* (2003), the manuals *Seed Technology for Genebanks* by Ellis et al. (1985) and *Seed Vigor Testing Handbook* by AOSA (1983), and Hong and Ellis' *Protocol to Determine Seed Storage Behavior* (1996). ISTA's *Rules* suggest a sample size of four replications of 100 seeds for the viability test. However, sample size will depend on the quantity of seed available and, in some cases, may be reduced. What is important is being able to make several replications rather than just one large sample. A frequently used number is three replications of 50 seeds each (Baskin and Baskin 1998b).

Relationships between vigour and germination

In some cases, tests for vigour are different from those for germination. However, during evaluations of germination, data are taken that also serve to estimate vigour, for example, germinative strength, germination speed, seedling emergence and development, uniformity of germination, and sensitivity to factors of environmental stress during germination and emergence. A close relationship between the two concepts can therefore be established (Figure 4).

Standard germination tests are carried out under conditions optimal for activating the embryo in seeds. During seedling development, observations pertinent to vigour are made. Some of these assess and classify seedling growth (Figure 5) and the percentage and speed of emergence. Others consider response to stress factors such as cold temperatures (10°C) and chilling (18°C), rapid aging, and osmotic pressure (AOSA 1983).

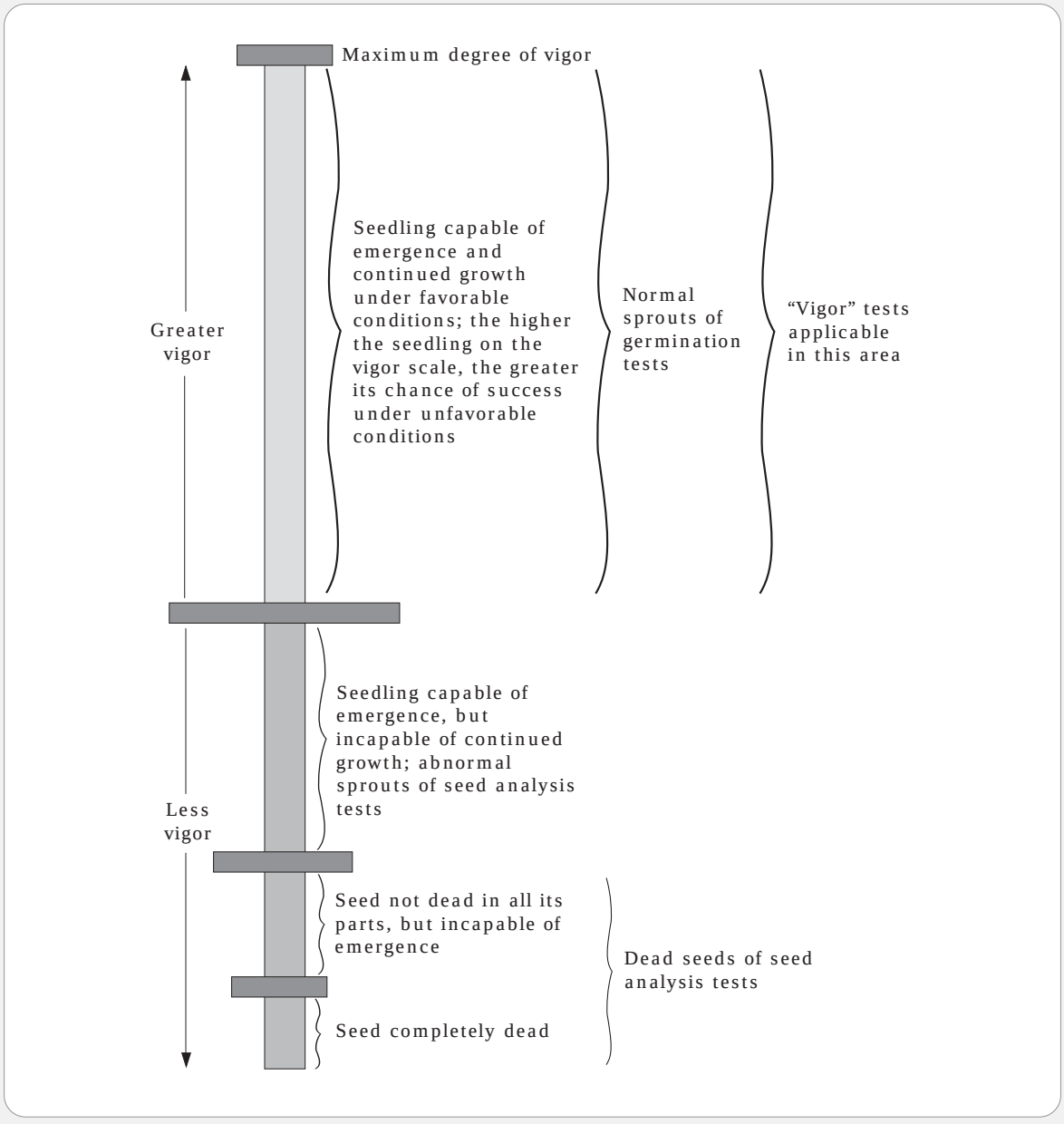

Figure 4. A graphic representation of the relationship between germination and vigour (from AOSA 1983).

The ISTA experts, to validate germination tests, make counts of and categorize seedlings as **complete normal seedlings**, **normal seedlings with mild defects**, and **abnormal seedlings**. In each case, parameters are defined according to the presence, absence, or level of defects in essential structures such as the radicular system (primary root, secondary or seminal roots), embryonic axis (hypocotyl, epicotyl, terminal bud, and the mesocotyl in Poaceae) (ISTA 1999).

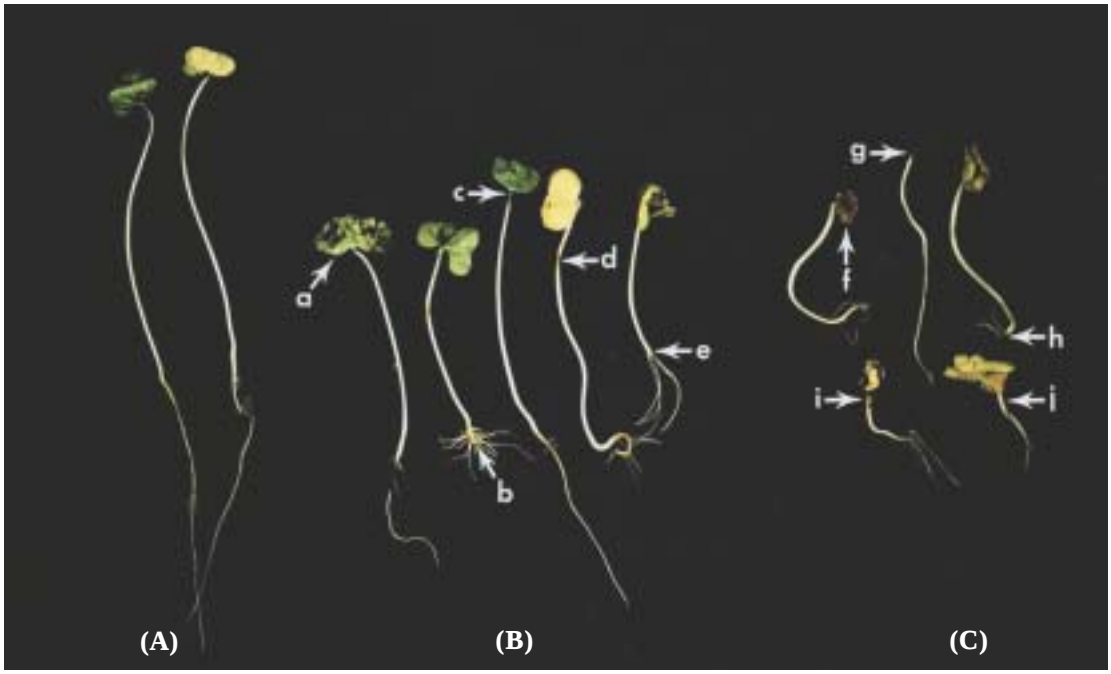

Figure 5. Classification of cotton seedlings in the final count in the vigour test. **(A)** Vigorous normal seedlings. **(B)** Normal seedlings, but less vigorous and with some defects: **a.** necrotic cotyledon; **b.** atrophied primary root; **c.** lack of one cotyledon; **d.** necrotic hypocotyl; **e.** absent primary root. **(C)** Abnormal seedlings: **f.** deteriorated cotyledons and epicotyl; **g.** lack of both cotyledons; **h.** weak secondary roots and absent primary root; **i.** damaged hypocotyl; **j.** shortened hypocotyl (from AOSA 1983).

Measuring Germination and Vigour

Germination percentage and germination rate

Measuring germination implies assessing two parameters: germination percentage and germination rate. **Germination percentage** should be related to time, indicating the number of seedlings produced over a given period. **Germination rate** can be measured, using several methods. One determines the number of days required to obtain a specified germination percentage. Another calculates the average number of days (MDG) needed for the plumule or radicle to emerge, as follows:

$$\text{Mean days} = (N_1T_1 + N_2T_2 \ldots N_xT_x)/\text{Total number of germinating seeds}$$

where,

N is the number of seeds that germinated within consecutive intervals of time; and
T is the time elapsed between the beginning of the test and the end of the given interval of measurement.

Viability is represented by the germination percentage, which expresses the percentage of seedlings that were actually produced of the number that could have been produced by a given number of seeds (i.e., $(N_{seedlings}/N_{potential}) \times 100$). The germination should be rapid and seedling growth vigorous. This is seed vitality, or **germinative capacity**, and can be represented by germination speed.

If the sequence of time is measured for the germination of a seed lot or for the emergence of seedlings in a seedbed, a typical pattern is usually found for the germination curve (Figure 6). An initial delay occurs as germination begins, then the number of seeds that germinate rapidly increases, followed by a reduced rate of appearance. When viability is less than 100%, determining the exact final point becomes difficult (Hartmann and Kester 1971).

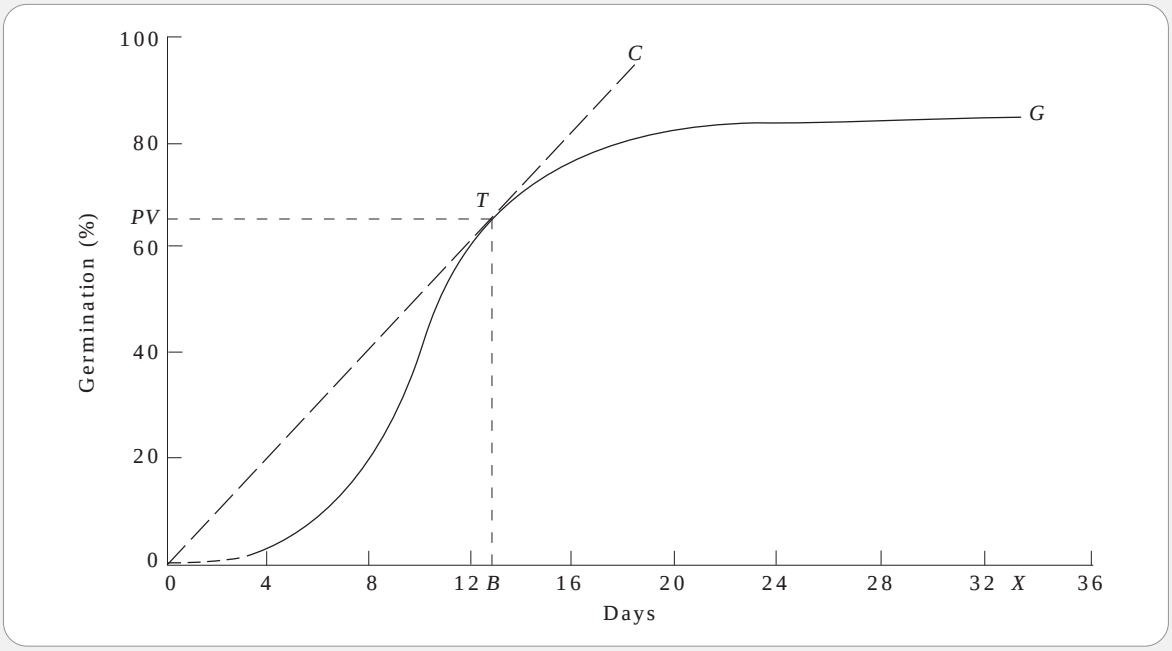

Figure 6. Typical germination curve of a seed sample. After an initial delay, the number of seeds that germinate increases and then diminishes. This type of curve can be used to measure germination. See text for explanation (redrawn from Czabator, cited in Hartmann and Kester 1971).

According to Hartman and Kester (1971), Kotowski has used the inverse of this formula multiplied by 100 to determine a coefficient of speed, whereas Czabator suggested another measurement for seeds of woody perennials where germination maybe slow; that measure includes both speed and percentage of germination, that is, the **germination value (GV)**. To calculate GV, the germination curve (Figure 6) should be obtained by periodically counting radicle or plumule emergence. The important values of the curve are the T value, where the speed of germination begins to diminish, and the G value, which is the final percentage of germination at the end of an X interval of measurement. (Several T values are, in fact, taken to indicate the times between the beginning of the test and the end of given intervals of measurement.) These points divide the curve into a fast and slow phase. Peak value (PV) is

the percentage of germination at T, divided by the number of days needed to arrive at T. The mean daily germination (MDG) is the final percentage of germination divided by the number of days the test is held. Therefore (Figure 6):

$$GV = PV \times MDG$$
$$GV = (68/13) \times (85/34)$$
$$GV = 5.2 \times 2.5 = 13$$

A test usually takes 10 days to 4 weeks, but may last as long as 3 months for seeds that are slow to germinate. A normal seedling usually has a well-developed root and stem, although the criterion 'normal seedling' varies among the different seed classes. Moreover, abnormal seedlings, and **hard**, **firm, dead,** or **rotten** seeds may occur. Abnormal seeds may result from:

- Diminished vitality because of age
- Poor storage conditions
- Damage, whether mechanical, or by insects or disease
- Poisoning from overdosage of fungicides
- Frost damage
- Nutrient deficiencies such as minerals (e.g., Mn and B in peas and beans)
- Poisoning from toxic materials sometimes found in metallic germination trays, substrates, or piped water

In general, firm seeds can be distinguished from the nonviable; firm seeds are solid, swollen, and free of moulds, or they show erratic germination. Any seed that does not germinate should be examined to determine possible reasons (Hartmann and Kester 1971).

Viability and vigour of vegetative planting materials

Although the concepts of **viability and vigour** have been developed and applied to seeds, they can be extended, with the necessary adjustments, by analogy to **vegetative planting materials** (propagules and plant parts). Basically, for the respective evaluations, plant parts (e.g., roots, stems, and leaves) or specialized plant organs (e.g., bulbs, corms, tuberous roots, rhizomes, and pseudobulbs) that have the capacity for regeneration would be examined for their viability, that is, for their capacity to regenerate healthy and vigorous plants. Parameters would include **germination percentage**, expressed as the proportion by number of propagules or reproduction units used that have produced seedlings. The vitality of the reproduction units, or **germinative capacity**, could be represented, as for seeds, by **germination speed** or **germination rate**.

Because little information is available on evaluating the biological or physiological status of vegetative planting materials, pertinent research is advisable. When working with *ex situ* conservation, the knowledge needed for the successful management of this type of PGRs should be generated and disseminated.

Evaluating the Lesson

After this lesson you should be familiar with the procedures most used to evaluate indicators of germplasm viability and to assess the respective tests. Before going on to the next lesson, consider the following problems:

- If you have worked in the verification of germplasm viability, comment on the difficulties you have encountered during tests and indicate how you resolved them.
- If you have not had experience or have not carried out viability tests, indicate how you would conduct those procedures with the materials in your bank. If you prefer, carry out a small practice experiment, describing what you did and what results you obtained.

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Contributors to this Lesson

Benjamín Pineda, Alba Marina Torres, Daniel Debouck, Carlos Iván Cardozo, Rigoberto Hidalgo, Mariano Mejía, Graciela Mafla, Arsenio Ciprián, Manuel Sánchez, Carmen Rosa Bonilla, and Orlando Toro.

Next Lesson

In *Lesson 1* of the next *Submodule D*, you will study aspects of plant health quality and its verification.