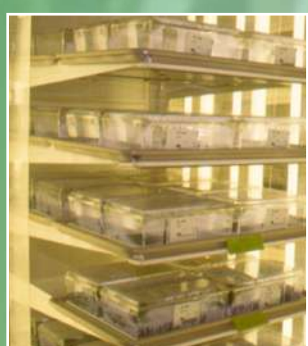
ISTA Handbook on Seedling Evaluation

4th Edition

International Seed Testing Association (ISTA)

ISTA Handbook on Seedling Evaluation
Fourth Edition 2018

By the ISTA Germination Committee

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and the ISTA Germination Committee
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Preface to the third edition, 2003

Present day qualitative seed germination analysis is carried out in much the same way as it was when the first analyses took place at the end of the 19th century; a simple germination test on 400 seeds provides reliable information on a seed lot of several tonnes.

Over many years, 'seedling evaluation' has played a fundamental role in the evaluation of a seed lot's potential. Normality criteria, in other words acceptable quality, evolve regularly. Quality criteria either become more severe in order to adapt to farmers' technical requirements, or genetic progress, and advances in production technology allow for the acceptance of minor faults that had previously meant rejection, but that experimental research has proven to be innocuous.

This new version of the *ISTA Handbook on Seedling Evaluation* updates seedling anomalies that may occur during germination tests and gives guidance on their evaluation.

This version also presents some innovations: some of these were initiated by Regula Schmitt, others by Ronald Don and the ISTA Germination Committee.

An educational approach dominates the early sections, in response to the demands of analysts and teachers wanting basic biological and anatomical information on seeds. This information forms the key to understanding the ways in which germination takes place and also the origin of certain anomalies.

Section 8: Seedling evaluation, tries to provide parameters for the assessment of anomalies. For example, what is an 'inadequate root system' or a 'deep hypocotyl split'? Whatever the definition, it is the analyst who has the final say, and it is up to him or her to take the final decision that can sometimes have serious economic consequences.

The descriptions *sensu stricto* benefit from technical progress made concerning illustrations, colour photographs and layout.

Finally, a new loose-leaf presentation format will facilitate updating the Handbook and adding new information to it.

While waiting for developments in the use of molecular markers or other laboratory tests, germination tests and the visual appraisal of seedling anomalies probably have a very long future.

Acknowledgements (third edition, 2003)

It is Ronald Don of the Official Seed Testing Station for Scotland in Edinburgh who undertook the writing of the Handbook in its present form.

Ronald Don has given this Handbook a new look that should satisfy the needs of seed analysts as well as laboratory seed analysis trainers. He has practically rewritten all the general sections, relying on his long, enthusiastic experience as an analyst himself as well as a trainer of analysts from all over the world.

The collective revision of the Handbook by the Germination Committee (2001–2004[1]) has led to the consolidation and validation of the contents; this will help maintain the Handbook's position as the principal reference work in its field.

The Flower Seed Testing Committee (2001–2004[2]) has shared its knowledge and greatly helped in the description of flowering species.

The Forest Tree and Shrub Seed Committee[3], headed by Zdenka Prochazkova, has greatly improved the descriptions and illustrations of several genera, such as *Pinus*. The experience and the knowledge of the members of this Committee will bring more and more input to the Handbook in the near future, taking the opportunity of the loose-leaf presentation formation.

Several laboratories, technologists and analysts have contributed to this work in bringing to it their experience, and also by supplying pictures and photographs (Valerie Blouin[4], Sylvie Ducournau[4], Philippe Garreau[4], Gillian McLaren[5], Pierre Soufflet[3]). Véronique Binoit has participated in secretarial work, contributing to the considerable task of organising the layout and graphics, and the co-ordination with Bettina Kahlert of ISTA; without Bettina and Gillian, this work would never have seen the light of day.

On behalf of ISTA and all those analysts who will use this *Handbook on Seedling Evaluation*, I wish to thank everyone who has been involved in this work, including those whose names are not mentioned here.

Joël Léchappé
Chair of the Germination Committee 2001–2004

1 Members: Iren E. Barla-Szabo, Ronald Don, Krystyna Kolasinska, Joël Léchappé, Lea Mazor, Günter Müller, Enrico Noli, Håkon Tangerås, Grethe Tarp, Anny van Pijlen and Loren Wiesner.
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Preface to amendments, 2006

The ISTA Germination Committee has worked on the revision of the *ISTA Handbook on Seedling Evaluation*, Third Edition 2003, since the ISTA Congress 2004, held in Budapest, Hungary. The revision is included in the *ISTA Handbook on Seedling Evaluation*, Third Edition 2006, or is available separately as Amendments 2006 to the *ISTA Handbook on Seedling Evaluation*, Third Edition 2003.

The revision consists of three different parts:
1. The revision of *Section 4: Laboratory conditions for seedling evaluation* to add the new substrate definitions for growing media and organic growing media. 'Growing media' as a generic term for all substrates – paper, sand and other media – was introduced into the *International Rules for Seed Testing,* Edition 2006 (ISTA Rules). The Handbook is now updated accordingly.
2. The revision of *Section 12: Seedling Type D – Seedling Group A-1-2-3-1 (Lolium)* to include guidance on the evaluation of primary roots.
   A comparative test using photographs of grass seedlings has shown that ISTA member laboratories do not appear to evaluate defects of primary roots in the same manner (especially when the primary root is short). To assist uniformity of evaluation, guidance is added to the Handbook on the evaluation of primary roots of grasses, and the extent to which secondary roots can be taken into account.
3. The addition of an *Appendix 5: Illustrative standard operating procedures* to reflect quality assurance requirements.

Temperature control and measurement is an important factor in the germination laboratory and other areas of seed testing. To assist laboratories in meeting the specifications for temperature control and measurement, given in the ISTA Rules, an illustrative SOP is included in the Handbook: *Germination procedures – temperature measurement and control in the germination laboratory*.
To assist laboratories in ensuring that the germination media which they use meet the specifications given in the ISTA Rules, the following four illustrative SOPs are included in the Handbook:
- *Germination procedures – growing media specification checks: water retention*;
- *Germination procedures – growing media specification checks: pH*;
- *Germination procedures – growing media specification checks: conductivity*; and
- *Germination procedures – growing media specification checks: cleanliness and innocuity*.

The illustrative SOPs included in the Handbook are for guidance purposes only. They illustrate procedures that the ISTA Germination Committee considers to meet the requirements of the ISTA Rules and the ISTA Seed Testing Laboratory Accreditation Standard. Accredited laboratories are expected to comply with the requirements and to document similar procedures adopted in their laboratory.

Acknowledgements (amendments, 2006)

All ISTA Germination Committee members (2004–2007[6]) have contributed to the production of these amendments through their comments and suggestions on the numerous drafts and proofs.

Particular thanks are due to:
- Joël Léchappé, who was the inspiration behind the changes in growing media definitions and specifications;
- The French and Scottish Committee members, who developed the illustrative SOPs on checking germination media specifications;
- The Scottish Committee members, who developed procedures for temperature control and measurement;
- The French, Japanese, Scandinavian and Scottish Committee members, who trialled the illustrative SOPs.

Finally, these amendments would not exist without the input of Bettina Kahlert. She co-ordinated the production of the amendments, organised their layout and graphics and carried out final proofing with Gillian McLaren.

Ronald Don
Chair of the Germination Committee 2004–2007

6 Ronald Don, Sylvie Ducournau, Kari Fiedler (joined the Committee in 2005), Doris Groth (retired from the Committee in 2006), Krystyna Kolasinska, Joël Léchappé, Lea Mazor, Gillian McLaren, Günter Müller, Enrico Noli, Takayuki Okuda (joined the Committee in 2006), Zdenka Procházková, Pamela Joan Strauss, Håkon Tangerås, Grethe Tarp, Anny van Pijlen and Loren Wiesner (retired from the Committee in 2005).

Preface to amendments, 2009

The ISTA Germination Committee has worked on a revision to the *ISTA Handbook on Seedling Evaluation*, Third Edition 2006, since the ISTA Congress 2007, held in Iguaçu Falls, Brazil. The revision is included in the *ISTA Handbook on Seedling Evaluation*, Third Edition 2009, or is available separately as Amendments 2009 to the *ISTA Handbook on Seedling Evaluation*, Third Edition 2006.

The revision consists of three different parts:

1. The addition of *Section 4.6.4: Assessment of fresh seed*. This new section gives a flow chart with step-by-step instruction on the assessment of ungerminated seeds at the end of a germination test.
2. The revision of *Appendix 5: Illustrative standard operating procedures*, to include a flow chart that gives step-by-step instructions regarding the calculation of the water-holding capacity of growing media.
3. The addition of an *Appendix 6: 50 % rule for the evaluation of foliated cotyledons*. This gives guidance on the evaluation of seedlings with damaged, necrotic, decayed and discoloured tissue. Guidance on when actual cotyledon size or estimated cotyledon size should be used when applying the 50 % rule is provided, as well as diagrammatic and photographic examples of the application of the 50 % rule. This Appendix should be of particular use to those evaluating seedlings with physiological necrosis.

The amendments included in the Handbook are for guidance purposes only. They illustrate procedures that the ISTA Germination Committee considers to meet the requirements of the ISTA Rules and the ISTA Seed Testing Laboratory Accreditation Standard. Accredited laboratories are expected to comply with the requirements and to adopt similar procedures in their own laboratories.

Acknowledgements (amendments, 2009)

All ISTA Germination Committee members (2007–2010[7]) have contributed to the production of these amendments through their comments and suggestions on the numerous drafts and proofs.

Particular thanks are due to:

- Takayuki Okuda, who was the inspiration and driving force behind the appendix on the 50 % rule for the evaluation of foliated cotyledons, and also provided the excellent diagrams and photographic images for this appendix;
- The French and Scottish Committee members, who developed the flow chart giving step-by-step instructions on the calculation of the water-holding capacity of germination media; and
- Anny van Pijlen, who drafted the flow chart giving guidance on the evaluation of ungerminated seed at the end of a germination test.

Finally, these amendments would not exist without the input of Jonathan Taylor of the ISTA Secretariat. Jonathan co-ordinated the production of the amendments, organised their layout and graphics and carried out final proofing with Takayuki.

Ronald Don
Chair of the Germination Committee 2007–2010

7 Ronald Don, Ignacio Aranciaga, Sylvie Ducournau, Kari Fiedler, Krystyna Kolasinska, Joël Léchappé, Lea Mazor, Gillian McLaren, Günter Müller, Enrico Noli, Takayuki Okuda, Zdenka Procházková, Håkon Tangerås, Grethe Tarp and Anny van Pijlen.

Preface to amendments, 2013

The ISTA Germination Committee has been working on a revision to the *ISTA Handbook on Seedling Evaluation*, Third Edition 2009, since the ISTA Congress 2010, held in Cologne, Germany. The revision is included in the *ISTA Handbook on Seedling Evaluation*, Third Edition 2013, or is available separately as Amendments 2012 to the *ISTA Handbook on Seedling Evaluation*, Third Edition 2013.

The revision was proposed following major changes made in the ISTA Rules to *Chapter 5: The Germination Test*, voted on at the ISTA Congress in Cologne. It includes:

1. The addition of sections concerning counting equipment and germination apparatus in *Section 4: Laboratory conditions for seedling evaluation*. It was decided at the Congress in 2010 to remove these parts from the ISTA Rules because they were considered as practical guidelines and therefore more suited to the Handbook. It was also the opportunity to add photos of the equipment and to give more detailed information on how to use it. Following a request to include a suggested method for evaluating counting equipment in the Handbook, a method has been proposed to check that counting equipment does not influence germination results. This method is described in *Section 4.1.3: Germination apparatus*.
2. The revision of *Appendix 2: Index of seedling groups*, regarding the classification of seedling types for *Vigna subterraneae* and *Vigna angularis*. These two species had previously been classified as Seedling Type F (epigeal germination) whereas, in fact, they are of Seedling Type G (hypogeal germination).
3. The revision of A*ppendix 3: Index of seedling abnormalities*, to harmonise the list of seedling abnormalities in the Handbook to the list of abnormalities of the ISTA Rules.
4. The revision of *Section 12: Seedling Type D – Seedling Group A-1-2-3-1* and *Section 13: Seedling Type D – Seedling Group A-1-2-3-2*, to include abnormal type 'scutellum detached from the endosperm' in the corresponding sections and to add photos illustrating this defect. Supplementary remarks on these sections have been completed with indications on how to evaluate 'detached endosperm' in Section 12 and on how to evaluate 'trapped coleoptiles' and 'detached endosperm' for *Zea mays* and *Sorghum* spp.

Acknowledgements (amendments, 2013)

All the ISTA Germination Committee members (2010–2013[8]) have contributed to the production of these amendments through their comments and discussions.

With special thanks to:

- Dutch and French laboratories for providing photos of laboratory equipment;
- The ISTA Statistics Committee for its help in reviewing the method for evaluating counting equipment;
- Augusto Martinelli, who carefully identified all the remaining discrepancies between the ISTA Rules and the ISTA Handbook, and who provided comments and photos on specific seedling abnormalities;
- Joël Léchappé, who as liaison officer for the Germination Committee has always supported the work done by all the members;
- The ISTA Secretariat and especially Jonathan Taylor for his professional reviewing and production of these amendments.

Sylvie Ducournau
Chair of the Germination Committee 2010–2013

8 Sylvie Ducournau, Anny Van Pijlen, Ignacio Aranciaga, Ronald Don, Kari Fiedler, Fabio Gorian, Andrea Jonitz, Krystyna Kolasinska, Augusto Martinelli, Lea Mazor, Gillian McLaren, Harry Nijenstein, Takayuki Okuda, Håkon Tangerås and Rita Zecchinelli.

Preface to fourth edition, 2018

The ISTA Germination Committee has worked on the revision of the *ISTA Handbook on Seedling Evaluation*, Third Edition 2003, since the ISTA Congress 2013, held in Antalya, Turkey. The revision is included in a new electronic edition of the *ISTA Handbook on Seedling Evaluation*, Fourth Edition 2018.

The revision consists of changes or precisions in different sections of the Handbook.

1. In *Section 4: Laboratory conditions for seedling evaluation*, the following have been included:
   a. An illustrative description of a counting method 'by hand';
   b. An illustrative description of methods for seed disinfection prior to germination;
   c. An illustrative protocol for the use of sealed polythene envelopes for breaking seed dormancy;
   d. Precision about the storage duration and temperature of KNO_3 and GA_3 solutions.
2. In *Section 18: Seedling Type F – Seedling Group A-2-1-2-2*, precision has been added for:
   a. The evaluation of loops and spirals on the hypocotyl of *Phaseolus vulgaris*;
   b. The evaluation of the root system of ornamental and flower species of *Lupinus*;
   c. The evaluation of the root system of *Glycine max*.
3. In *Section 20: Seedling Type E – Seedling Group B-2-1-1-1*, *Section 21: Seedling Type G – Seedling Group B-2-2-2-2*, *Section 22: Seedling Type H – Seedling Group B-3-1-1-1*, colour photos of species that were already in the Handbook, and photos of species that were not in the Handbook, have been added.
4. In *Appendix 5: Illustrative standard operating procedures*, some precision or corrections have been added:
   a. In *A5.3 Germination procedures – growing media specification checks: water retention*, formulae have been corrected, the draining duration has been modified and the objective of the water retention measurement for germination testing has been clarified;
   b. In *A5.6 Germination procedures – growing media specification checks: cleanliness and innocuity*, precision about the time for evaluation has been added and the list of the sensitive species was extended.

Acknowledgements (fourth edition, 2018)

All ISTA Germination Committee members (2013–2018[9]) have contributed to the production of this edition of the Handbook through their comments and suggestions on the numerous drafts and proofs.

Particular thanks are due to:

- Augusto Martinelli, who proposed to revise the evaluation of loops and spirals on the hypocotyl of *Phaseolus vulgaris*, and who has always provided very useful comments on all the proposals;
- Rita Zecchinelli, who proposed the precisions about the root system evaluation of the ornamental and flower species of *Lupinus* and who has provided all of the relevant photos;
- Ignacio Aranciaga, who led a working group on *Glycine max* seedling evaluation that has resulted in a complete set of guidelines being available on the ISTA website. The root system evaluation of *Glycine max* seedlings comes from these guidelines and have been added to Section 18;
- Gillian McLaren, who proposed a very well-illustrated and clear protocol on the use of sealed polythene envelopes for dormancy breaking treatment of *Trifolium* species and provided the relevant photos;
- Fabio Gorian, who revised all of the proposed new photos for the tree and shrub Sections 20 to 22;
- Angelique Delanoue, from GEVES-SNES in France, who has taken many of the photos in Sections 4, 20, 21 and 22;
- Joël Léchappé who, as the liaison officer for the Germination Committee, has always supported the work done by all members;
- The ISTA Secretariat and especially Nadine Ettel for her consistently efficient contribution to the revision of this Handbook;
- And finally, a very big thank you to Vanessa Sutcliffe of HeartWood Editorial, for transforming our paper Handbook into an electronic version, which I hope will allow it to be further distributed.

Sylvie Ducournau
Chair of the Germination Committee 2010–2018

9 Gillian McLaren, Pernilla Andersson, Ignacio Aranciaga, Sarah Dammen, Lucile Daron, Meriam Dekalo-Keren, Ronald Don, Hortense Faucher, Lesly Gonzales Galaz, Fabio Gorian, Aidin Hamidi, Christine Herzog, David Johnston, Andrea Jonitz, Jin Wook Kim, Augusto Martinelli, Lea Mazor, Harry Nijenstein, Takayuki Okuda, Rita Zecchinelli.

Section 1: Life processes to seed and fruit

1.1 Life on Earth

There is little practical difficulty in deciding whether common objects are alive or not but providing a precise definition of life is difficult. Although we realise that living organisms, such as man, possess many characteristics not shared by minerals, no single characteristic will sharply distinguish living from non-living materials. Living matter is unique on account of the sum of its properties and not because of any one of them.

Living matter undergoes continuous internal changes, revealed externally by the gaseous exchanges of **respiration**. **Irritability**, the property of responding to a wide range of external influences is very marked. **Assimilation**, absorption and conversion of foreign substances into its own structure produces **growth** and this can lead to **reproduction**.

Every organism must, in order to be able to live, have a suitable niche within the environment, and must be capable of sustaining its life processes. In order to sustain life processes, an organism:

- needs an internal control system;
- must separate its structure from that of the environment; and
- must be able to take in, assimilate and utilise substances.

Additionally, every organism depends on energy. Animals are **heterotrophs**; i.e. they must absorb their foodstuffs already manufactured, as they are incapable of manufacturing food from its elements. Plants, on the other hand, are **autotrophs**; i.e. they utilise the radiant energy of the sun to manufacture their foodstuffs and are therefore the supplier of energy for man and animals.

1.2 Systematics of the Plant Kingdom

1.2.1 Structure

The basic element of plant systematic is the **species**. Individuals that possess a large number of features in common and are capable of breeding with each other belong to the same species. The basic element 'species' can be subdivided[1] as well as grouped. Species with a number of common characteristics are grouped into **genera**, these according to similar criteria into **families**, families into **orders**, orders into **classes**, classes into **divisions**. If necessary, each group may be subdivided into subgroups (e.g. subfamilies, subclasses, and subdivisions).

1.2.2 Nomenclature

All systematic groupings are designated by scientific, mostly Latin, names according to the binary nomenclature introduced by the great botanist Carl von Linné (**Linnaeus**) in 1753. Accordingly the species name, often adjectival and always written with a small initial letter, is added to the genus name. For example, within the genus *Poa* (meadowgrass) wood meadowgrass is called *Poa nemoralis* and annual meadowgrass is called *Poa annua*. The complete scientific name also includes the author's name, which is frequently abbreviated, e.g. 'L.' for Linnaeus[2].

The family names all bear the ending **-aceae** and are not part of the scientific name.

1.2.3 Classification of the seed plants

Seed testing deals with the **division of seed plants** only; the other divisions of the Plant Kingdom, such as ferns, mosses etc., are therefore not covered in this context. To simplify matters genera and species covered by the tables of the *International Rules For Seed Testing*[3] are referred to as ISTA genera and ISTA species or simply ISTA plants.

The division of seed plants is subdivided into the two subdivisions:

- **Gymnosperms** ('naked-seeded plants'); and
- **Angiosperms** ('covered-seeded plants').

1.2.4 Gymnosperms

The **gymnosperms** are more archaic than the angiosperms and therefore show more simple structures in many respects. 'Flowers' of coniferous gymnosperms are strobili (small cones): a central axis bearing few to numerous scales. There are male (staminate) cones and female (ovulate) cones. The ovules lie open ('naked') on unfused scale-like carpels, and the pollen grains, nearly always brought by the wind, have free access and pollinate them directly.

Among the gymnosperms it is mainly the **class of conifers** that seed testing deals with[4]. Gymnosperm seedlings are recognised by their many long and narrow cotyledons, which form a whorl around the terminal bud.

1 A species can be subdivided into subspecies, varieties and formae. Systematically even lower are cultivars, the characteristics of which have been selected by the breeder; they usually have fantasy names.
2 To simplify the spelling of Latin names in this handbook, the naming of species is restricted to the genus and the species with no reference given to the author.
3 From now on referred to as ISTA Rules.
4 The tables of the ISTA Rules also include *Ginkgo*, which is a class of its own.

1.2.5 Angiosperms

The **angiosperms** are the dominant seed-bearing plants of the present day and, estimated to consist of about 250,000 to 300,000 species. Most ISTA plants are angiosperms. Angiosperms are different to gymnosperms in that the carpels are fused at the margins and build a **receptacle** to enclose the **ovules**. They also show **double fertilisation**. Another prominent characteristic of angiosperms is a complex reproductive structure called the flower.

The angiosperms are divided into two classes:

- **Monocotyledons** (seedlings have one cotyledon); and
- **Dicotyledons** (seedlings have two cotyledons).

1.2.6 Monocotyledons

Monocotyledons are characterised by the presence of **one single cotyledon** in embryo and seedling, which is often transformed into a structure with special functions. The seeds usually contain **endosperm** and most show **hypogeal** germination. Other characteristics, such as parallel leaf venation or the typical three-piece-circles within the flowers, are of no significance to the germination test.

The largest and most important group of monocotyledons among ISTA plants is the **group of forage grasses and the cereals (Poaceae)**. Beside the Poaceae there are few families and genera represented in the ISTA Rules.

1.2.7 Dicotyledons

The majority of ISTA plants are **dicotyledons**. Embryos and seedlings of dicotyledons have **two cotyledons** (some rare exceptions exist), which are frequently leaf-like and green. The seed reserves for germination and young seedling growth are stored in the endosperm or frequently in the embryo itself. The majority of dicotyledons show **epigeal** germination. Other characteristics, such as the net-like venation of the leaves and the structure of the flowers with parts in twos, fours or fives, are of no significance to the germination test.

Among the ISTA plants the dicotyledons belong to a large number of different plant families. Some are represented by many genera, some by only one or a few genera.

1.3 Vital processes of seed plants

The most important life-sustaining processes of plants are:

- **photosynthesis**;
- **respiration**;
- **growth**; and
- **reproduction**.

1.3.1 Photosynthesis

The continuation of life on earth thus depends on photosynthesis by plants. It is the most important process of the global biological Carbon and Oxygen cycles and plays a major role in the biospheric hydrological cycle. During the process of photosynthesis radiant energy is transformed by a series of extremely complex steps into chemical energy plants are able to utilise.

Light is absorbed by plant pigments such as **chlorophyll**[5], which are present in the **chloroplasts**[6]. Light energy trapped by the chloroplasts is used to produce substances rich in energy, such as sugars and starch (carbohydrates) from carbon dioxide (CO_2), which it absorbs from the atmosphere, and water (H_2O) taken in by the roots[7]. Carbohydrates are needed as energy for growth or for the synthesis of other essential compounds. They may also be stored for future use in seeds, tubers or various other storage structures. **Oxygen** is a product of the photosynthetic processes and is released into the atmosphere[8].

5 Chlorophyll is contained in the chloroplasts and gives the plant its green colour.

6 Chloroplasts are specialised organelles found mainly in the cells of leaves and are the site of photosynthesis.

7 Photosynthesis may be summarised as follows:

$$\text{carbon dioxide} + \text{water} \xrightarrow{\text{Light energy}} \text{carbohydrate} + \text{oxygen}$$

or

$$CO_2 + H_2O \xrightarrow{\text{Light energy}} (CH_2O)_n + O_2$$

8 Overall, plants produce (by photosynthesis) more oxygen than they consume (*in respiration*). They are therefore vital in maintaining the balance between oxygen and carbon dioxide in the atmosphere.

1.3.2 Respiration

The processes of respiration and photosynthesis are practically the opposite of each other[9]. Respiration is a continuous process in all living organisms. Respiration in flowering plants consists of a long chain of reactions during which sugars and other substances rich in energy, built up by photosynthesis, are decomposed. Oxygen absorbed from the atmosphere or obtained from the process of photosynthesis plays an important role. The energy stored in sugars is recovered for the use by plants in its vital processes. **Carbon dioxide** and water are by-products of respiration and are released into the atmosphere or used in the process of photosynthesis.

1.3.3 Plant growth

Cells are the structural units of all plants, and growth of plants is the result of both:

- **cell division**; and
- **cell elongation and expansion**.

Vegetative growth by cell division occurs only in certain tissues (**meristems**) capable of cell division. These are mainly in root tips and in the innermost part of the terminal or apical bud. The division of a cell results in two new (daughter) cells that are genetically identical to the original (mother) cell. Though the number of cells increases, there is little growth by cell division alone, since the resulting cells are very small. Visible growth of plants is the result of the cell elongation and expansion that follows cell division.

Cell elongation and expansion includes a number of complex processes, which may be summarised in three main steps:

1. The cell wall becomes extensible;
2. The cell increases in size as a result of water up-take; and
3. The extended cell wall is strengthened and stabilised by deposition of new wall material.

1.3.4 Reproduction

Life is constantly endangered on all sides and in many respects (e.g. by competition, predation, disease, and by adverse environmental conditions such as drought or frost, etc.). In addition, the life of every individual is finite: even if death by some other mechanisms does not occur, death by old age is a certainty. Reproduction is a fundamental characteristic of all living organisms ensuring that a species carries on beyond the individual. It is the ability to produce new individuals to continue the species.

In plants the reproductive rate is frequently very high, i.e. large numbers of new individuals are produced. This allows a species to colonise new areas or compete successfully with other species.

The plant can propagate in one of two fundamentally different ways, by:

- **vegetative** or **asexual reproduction**; and
- **sexual reproduction**.

Both ways of propagation have advantages and disadvantages, and many plants produce their offspring both vegetatively and sexually.

1.3.5 Vegetative reproduction

In vegetative reproduction certain parts or structures (vegetative propagules) become detached from a plant and subsequently develop into new plants, whilst the old plant lives on. This mode of reproduction can take place naturally or it can be artificially induced by man, then known as **artificial propagation**.

Many different forms of vegetative reproduction occur in nature: **bulbils**, small bulb-like structures that are formed in the leaf axils of plants (e.g. in *Lilium bulbiferum*) or are derived from an inflorescence (e.g. in *Poa bulbosa* or *Allium* spp.). The bulbils drop to the ground and grow into new plants. Other examples of vegetative propagules are the **bulbels**, small bulbs arising from the base of a larger bulb (e.g. in garlic (*Allium sativum*)); rhizomes (e.g. in couch grass (*Elytrigia repens*)), **runners** (e.g. in strawberries (*Fragaria* spp.)) and tubers (e.g. in potatoes (*Solanum tuberosum*)). Cuttings, grafting and tissue culture are examples of artificial propagation.

The advantage of vegetative reproduction is the production of comparatively well developed propagules, which have a better chance of survival than seeds with their limited food reserves. Unlike the seed, which is independent as soon as it is shed, vegetatively produced propagules often depend on the parent for water, food and nutrients until they become fully established. Vegetative reproduction plays no part in the evolution of a species, because all offspring are genetically identical with the parent. Plants produced in such a way therefore lack the genetic variability that enables a species to evolve, adapt to, and survive changing conditions.

9 Respiration may be summarised as follows:

carbohydrates + oxygen → (chemical) energy + carbon dioxide + water

or $(CH_2O)_n + O_2 \rightarrow \text{energy} + CO_2 + H_2O$

1.3.6 Sexual reproduction

The reproductive phase is an extremely important aspect of plant life. The production of flowers and their subsequent fertilisation and seed formation is called sexual reproduction. It involves the fusion of male and female **gametes** (sex cells) to produce a new individual. Gametes are special cells, which are fundamentally different from all other cells of the plant body. They may originate either from the same or from two different plants, and their fusion involves a mixing of genetic material and the formation of new combinations of genes. Since each individual has a slightly different genetic make-up, the properties of the parent plants are combined anew and the variation[10] introduced enables species to evolve, adapt to, and survive changing conditions.

The disadvantage of sexual reproduction is its complexity and the risk of latent mutants. Genetic combinations, which produce weak or non-competitive individuals, cannot withstand the pressures of natural selection and become extinct.

1.3.7 Life cycle

It is possible to summarise the life cycle of a flowering plant in the following way:

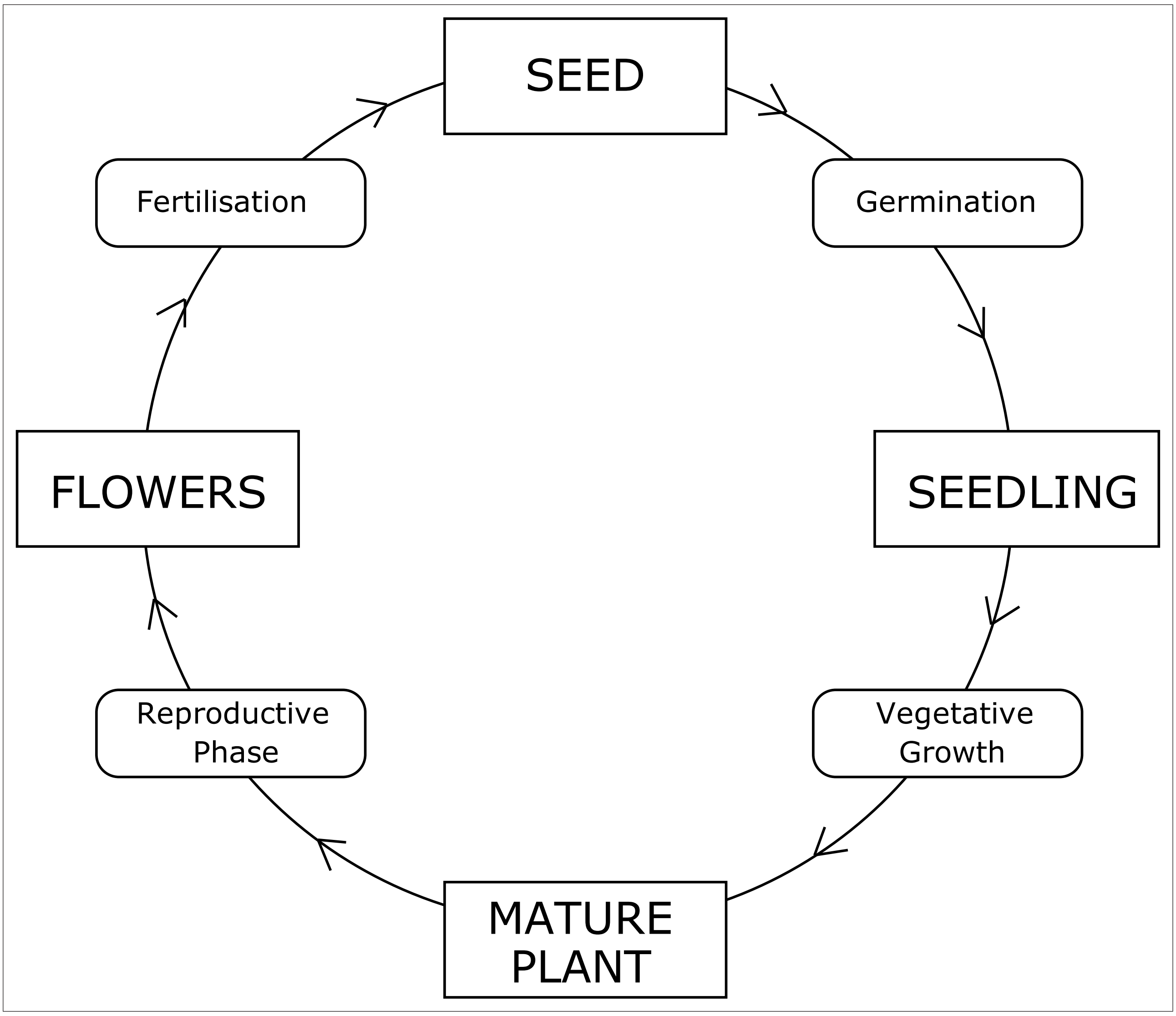


Figure 1.1 Life cycle of a flowering plant.

10 The various species have been derived from a multitude of genetic variation.

1.3.8 Flower structure

All seed plants bear their reproductive organs within flowers. Whereas mosses and ferns are dependent on moist surroundings for their reproduction, the flower enables reproduction in drier environments. The function of the flower is to produce and facilitate the meeting of male and female gametes. How this meeting occurs depends in part on the flower structure. All angiosperm flowers follow the same basic plan, and their structures are related to the mode of pollination. In temperate regions pollen is transferred mainly by **insects** (bees, beetles etc.), that visit flowers in search of food or by the **wind**[11].

1.3.9 Insect-pollinated flowers

A typical **insect pollinated** flower has the following structures:

- the **floral axis** or pedicel;
- the **sepals** (green in most species);
- the **petals** (coloured in most species);
- the **stamens** or **androecium**, the male (♂) flower part; and
- the **carpel(s)** or **gynoecium**, the female (♀) flower part.

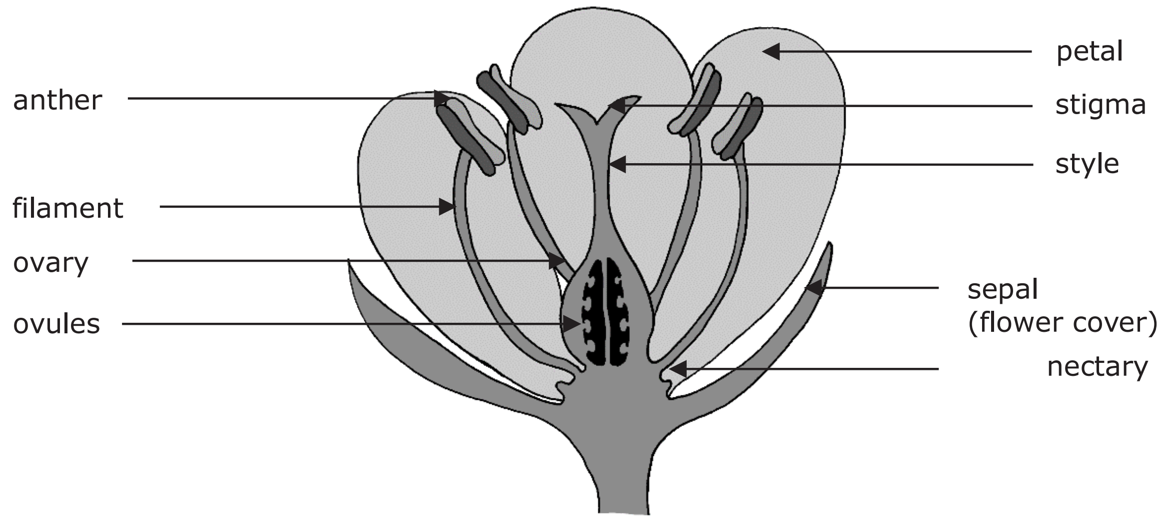


Figure 1.2 Structure of a typical insect pollinated flowering plant.

The various kinds of leafy structures of the flower are usually arranged in circles or whorls at the upper end of the floral axis. **Sepals** and **petals** form the outer circles and protect the young sexual organs when the flower is still closed. Later they have a role in the attraction of pollinators.

The innermost part of the flower at the top of the floral axis is the **gynoecium** consisting of one or more **carpels**. In angiosperms the carpels are fused to form a receptacle[12] containing the ovules[13] within the **ovary**. The pistil represents the entire female sex organ and is composed of:

- the **ovary**, which is the hollow basal region of a carpel and contains one or more ovules; it may consist of one or several connate carpels;
- the **stigma** (or two or several stigmata[14]), which is the surface to which the pollen grains adhere; and
- the **style**, which is the connection between ovary and stigma.

11 The gymnosperms (conifers) are pollinated entirely by the wind.
12 In its typical form the carpel resembles a folded or rolled leaf blade with the margins fused.
13 The more archaic gymnosperms (conifers) have their carpels open and the ovules lying 'naked' on them.
14 The number of stigmata corresponds to that of connate carpels.

The ovary develops into the fruit after fertilisation.

The ovule consists of the **nucellus**, which is enclosed by the **integuments** (usually two). In the course of flower development several divisions of the cell nucleus take place in the nucellus. They result in an eight-nucleate embryo-sac ready for fertilisation. One of the eight nuclei becomes the **egg cell** (female gamete), two fuse with the **polar nuclei** to become the endosperm nuclei which develops into the endosperm. The five remaining nuclei are of minor significance.

The ovule develops into the seed after fertilisation.

The **androecium** is the male part of the flower and it encircles the gynoecium. It is composed of a number of **stamens**. The stamen consists of a narrow stalk (**filament**) which bears, at its apex, an **anther**. **Pollen** grains develop in the anthers and are held there prior to pollen release. The nucleus in the pollen grain also undergoes several divisions, so that the ripe pollen grain contains two **male gametes** or **sperm cells**. Two covers enclose pollen grains, an inner plasmatic and an outer sculptured one.

1.3.10 Wind-pollinated flowers

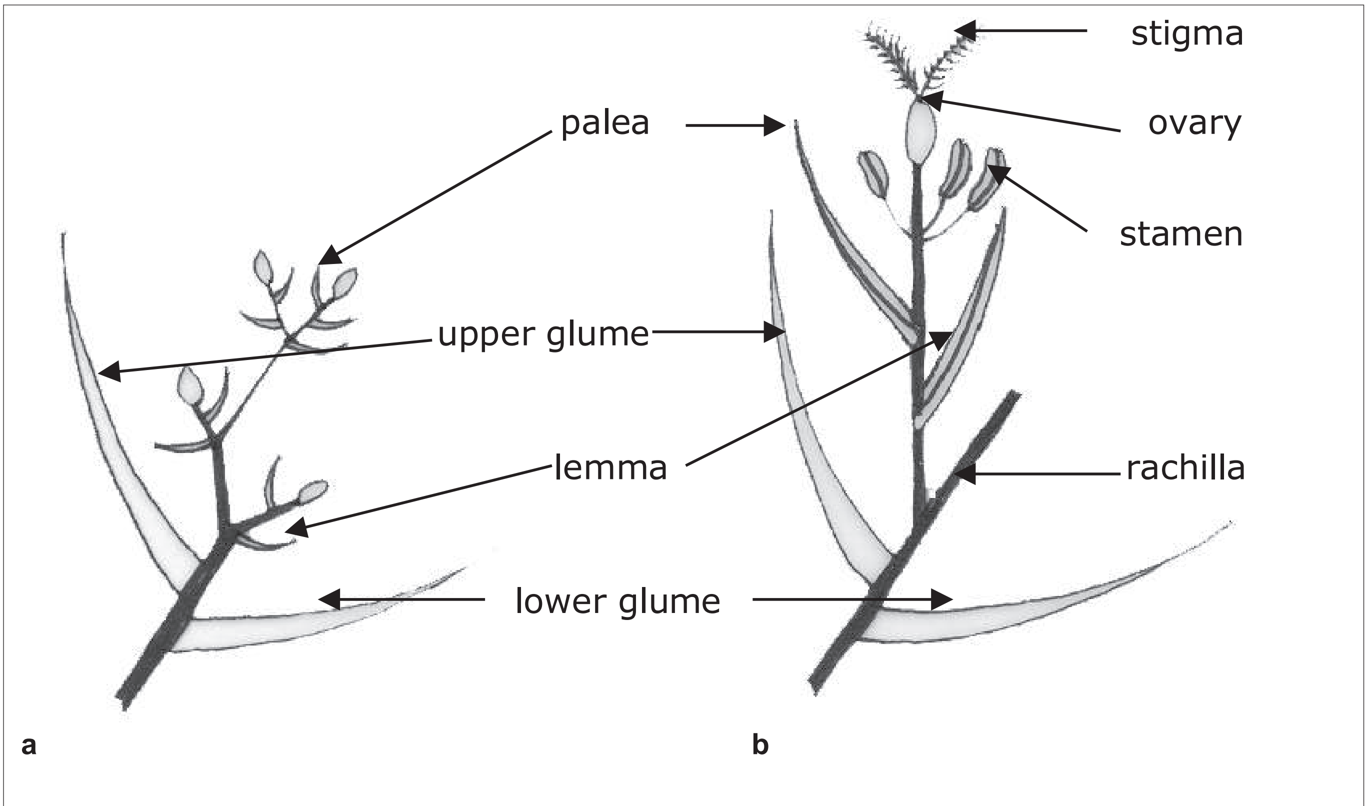


Figure 1.3 Diagrammatic representation of (**a**) spikelet and (**b**) flower of *Festuca* sp.

Though following the same basic structure as insect-pollinated flowers, **wind-pollinated flowers** are often more simple, i.e. without showy sepals and petals. They are usually small, unremarkable and are often combined to form dense **inflorescences**[15]. They usually have no scent, but produce large amounts of light pollen from stamens, which like the stigmas, hang outside the flower.

15 The flowering shoot and arrangement of a plant's flowers on the floral axis; this is specific for individual species.

In seed testing the most important group of angiosperms[16] with wind-pollinated flowers are the Poaceae. The reproductive organs of a grass flower are an ovary, with two or three feathery stigmata, and three stamens. They are enclosed in leaf-like structures, the **lemma** and **palea**. A single grass flower is called a floret; two to several florets combine to form a spikelet and usually a great number of spiklets are arranged in the **inflorescence** (e.g. ear, panicle).

When mature the spikelet axis breaks at the points of fracture, so that the mature caryopsis[17], often remains enclosed in lemma and palea.

1.3.11 Pollination

The transfer of pollen grains to the stigma of the ovary is called pollination. We can distinguish between:

- **self-pollination** (with pollen from the same flower);
- **neighbour-pollination** (with pollen from a different flower on the same plant);
- **cross-pollination** (with pollen from a flower of a different plant); and
- **artificial pollination** (which occurs in the breeding of new cultivars and in the production of some F1 hybrids where man plays a part by transferring pollen to flower stigmas, or prevents self pollination).

In nature cross-pollination is usually more desirable since this produces genetic variation by combining genes from different sources. Although self-pollination is widespread among plants, cross-pollination usually produces better results, e.g. more viable seeds are produced than from self-pollination, and many plants show sophisticated arrangements to avoid self-pollination.

Man uses cross-pollination as one of the tools of plant breeding, but once a new cultivar of a species has been bred, self-pollination is preferred. To prevent cross-pollination between different cultivars, certification regulations state specific isolation distances between crops of different varieties. These distances being greater for insect pollinated crops than for wind pollinated crops.

1.3.12 Fertilisation

Fertilisation is the fusion of the nuclei of a male and a female gamete to form a zygote.

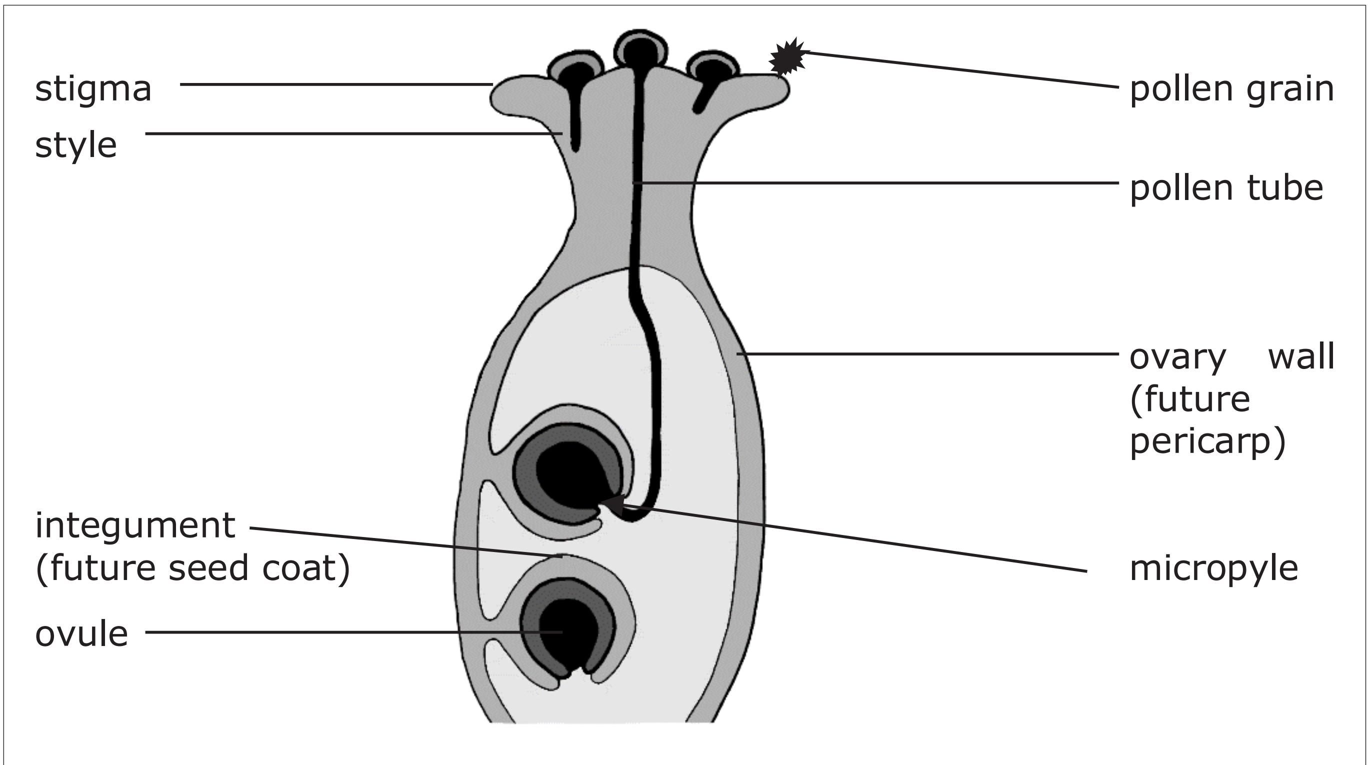


Figure 1.4 Diagrammatic representation of fertilisation.

16 All gymnosperms are pollinated by the wind too.

17 The grass fruit: a dry, one-seeded fruit with the fruit wall fused to the seed coat.

The pollen grain contains two male gametes or sperms. After the pollen grain has got onto the stigma, it germinates and produces a pollen tube. This tube grows down through the style into the ovary. Once in the ovary, the pollen tube enters an ovule through the micropyle[18] and thereby transports the two sperms into the embryo-sac and to the egg-cell within the ovule. There one of the sperms fuses with the egg-cell to form the zygote, which subsequently develops into the embryo. The second of the two sperms unites with the two polar nuclei of the embryo-sac. The product of this fusion is the endosperm nucleus, from which the endosperm develops. Thus in the angiosperms a double[19] fertilisation occurs, resulting in the embryo and the endosperm.

1.3.13 Formation of the seed

As a result of the fusion of the polar nuclei with one of the sperms, the endosperm develops by means of rapid cell division. It serves as nutrition for the embryo and soon fills up most of the embryo sac. Later the endosperm is either consumed by the growing embryo (resulting in **non-endospermic** seed) or it is transformed into a nutrient storing tissue, which is consumed only by the young seedling (**endospermic** seed).

At the same time the tissues of the nucellus usually degenerate and are completely absorbed. In certain families however, for example the Chenopodiaceae, the nucellus tissues are transformed into a nutritive storage tissue called the **perisperm**[20].

From the zygote the **embryo** gradually develops by cell division; it is at first spherical, later on rather worm- or thread-shaped. It is embedded in the endosperm, from which it draws nutrients. As the size of the ovule increases, the integuments expand and take on the role of seed coat or **testa**. Usually both integuments are involved in the formation of the seed coat: the inner one acting as a protective layer around the embryo and the outer often adapted for the dispersal of the seed and develops hairs, hooks, tubercles etc. on its surface. The surface characteristics of the seed coat are often species specific and may serve to identify seeds.

1.3.14 Seed

A seed is the result of the fertilisation of an ovule and morphologically consists of:

- an **embryo** plant that develops into a seedling during germination;
- **stored nutrients**; and
- a protective **seed coat** or testa.

Frequently, however, the unit tested in the laboratory and commonly termed ‘seed’ also contains accessory structures, such as the fruit wall (pericarp) or remains of floral structures (e.g. the cluster of *Beta* or the lemma and palea of forage grasses). **In seed testing and in this Handbook, no distinction is made between seed in the botanical sense and units retaining additional structures – all are called ‘seeds’.**

After fertilisation the embryo develops by cell division and cell differentiation[21]. The degree of differentiation of the embryo at the time of seed harvest depends on the species. In some species it is highly differentiated, and its essential structures can clearly be observed (e.g. in *Phaseolus*), whereas the embryo of other species (e.g. *Daucus*) is little differentiated and merely thread-like. A well formed embryo generally consists of an axis, which bears one (**monocotyledons**), two (**dicotyledons**) or more (most conifers) **cotyledons** or seed leaves. It is terminated by the **plumule**, the embryonic shoot apex, which may be enveloped by leaf initials in highly differentiated embryos. The descending end of the axis forms the **radicle**, the embryonic root, with its meristem surrounded by the root cap.

By the time the embryo is fully developed the endosperm has either been:

- transformed into storage tissue for the food reserves of the seedling, e.g. *Allium* (Figure 1.5a) and *Zea* Figure 1.5b) – seeds of this type are called **endospermic**; or
- stored in the embryo itself, usually the cotyledon, e.g. *Phaseolus* (Figure 1.5e) and *Quercus* (Figure 1.5f) – seeds of this type are called **non-endospermic**.

18 A small opening left by the integuments.
19 The gymnosperms show a **single fertilisation**: the fusion of a male gamete with the egg-cell.
20 The nutrient tissue of the **gymnosperms**, called gametophyte tissue, consists of tissues that had been formed in the ovary *prior* to fertilisation.
21 Cell differentiation: those processes by which form and function of cells are controlled and transformed in such a way that specialised tissues and structures result.

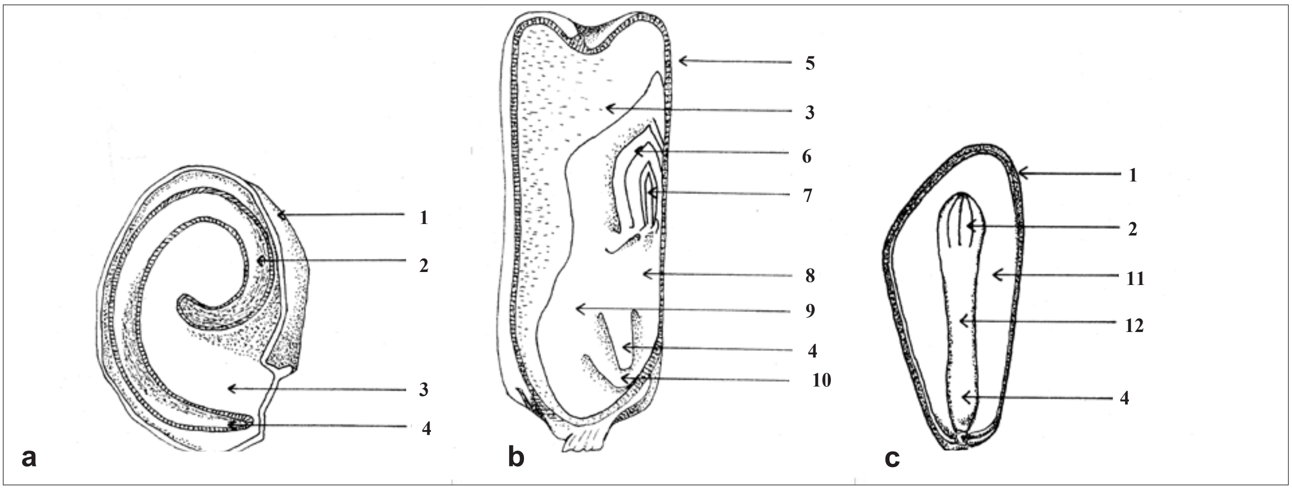
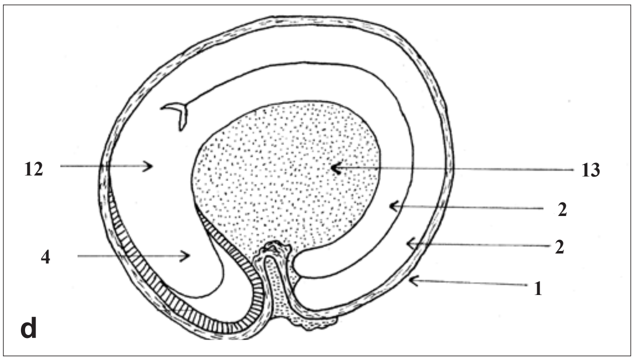


Figure 1.5 a Seed with nutrients stored in endosperm: *Allium* sp. **b** Seed with nutrients stored in endosperm: *Zea mays*. **c** Seed with nutrients stored in gametophyte tissue: *Pinus* sp.

In the majority of seeds the nucellus, the tissue forming the main mass of the ovule before fertilisation, is completely depleted by the growing embryo, but in a few instances, e.g. *Beta* (Figure 1.5d), it remains as a storage tissue for the food reserves and is called the perisperm. The food reserves sustain the early growth of the seedling, rendering it independent until it is in a position to produce its own. Embryo and storage tissues are enclosed by the seed coat (testa), which protects the seed against injury and loss of nutrients by leaching. It is therefore often hard and solid, but where the true seed remains enclosed within other structures, it may be merely membranous.



1 testa
2 cotyledon(s)
3 endosperm
4 radicle
5 pericarp
6 coleoptile
7 plumule
8 mesocotyl
9 scutellum
10 coleorrhiza
11 gametophyte tissue
12 hypocotyl
13 perisperm
14 primary leaves

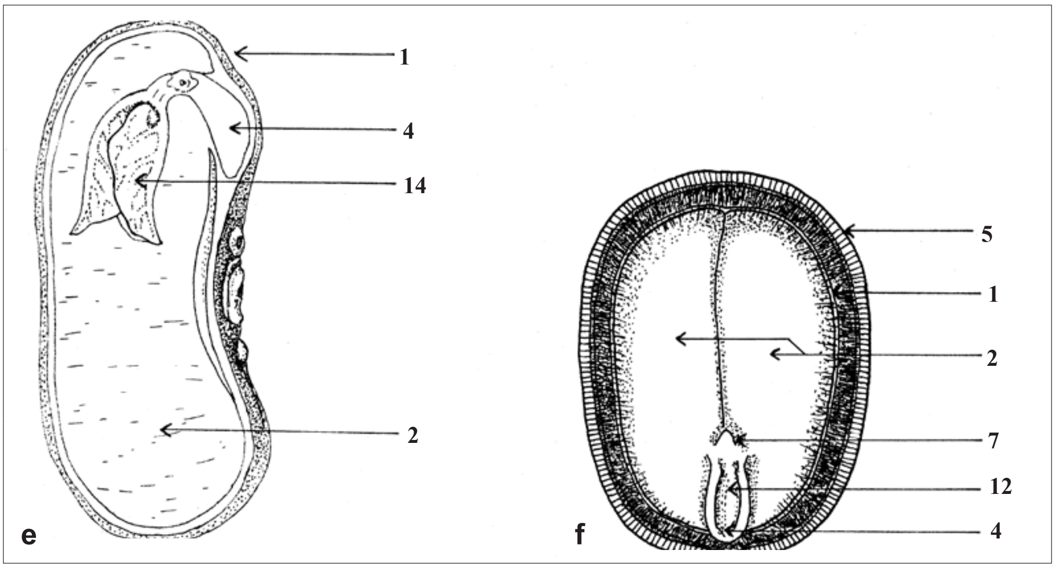


Figure 1.5 d Seed with nutrients stored in perisperm: *Beta vulgaris*. **e** Seed with nutrients stored in the cotyledons: *Phaseolus vulgaris*. **f** Seed with nutrients stored in the cotyledons: *Quercus* sp.

Section 1: Life processes to seed and fruit

The food reserves stored in the seed are mainly carbohydrates (sugars, starch), proteins and fat. They allow early growth of the embryo and the young seedling making it independent of external resources until it is in the position to provide itself with nutrients from the soil and with energy through photosynthesis.

1.3.15 Formation of the fruit

At the same time as the ovules ripen to form seeds, the ovary changes in a variety of ways to form the **fruit**, which protects the seed and may aid in the dispersal of the seeds. There are many different criteria by which fruits may be classified[22]. Generally, there are two types of fruit, dry or fleshy. Dry fruits are those which have a dry outer covering protecting the seeds, whereas fleshy fruits are those where the seeds are protected by a thick fleshy pulp. Examples of dry fruits are cereals, grasses, *Ranunculus* spp., *Taraxacum* spp. and *Papaver* spp. Examples of fleshy fruit are apples, tomatoes, gooseberries and plums.

We can further classify fruits by making the distinction between **true fruits** and **false fruits**. True fruits are formed from the ovary only, whereas false fruits are where another part of the flower is incorporated into the fruit structure, e.g. the receptacle may become part of the fruit. False fruits are particularly important in agriculture and are often specially bred and selected for their large edible receptacles, e.g. apples and strawberries.

One can also distinguish between **dehiscent[23] fruits**, which disperse the seeds from a follicle, pod or capsule, and **indehiscent[24] fruits**, where the seeds remain enclosed in the fruit and are dispersed with the fruit. Among the indehiscent fruits there are *fleshy* forms (e.g. berry, drupe) and *dry* (e.g. nutlet, achene, and caryopsis). Examples of agriculturally important crops with dehiscent fruits are the legumes and brassicas. Most other 'seeds' tested in the seed testing laboratory are really fruits. The simplest form of fruit is where the carpel wall hardens and this is often described as an achene, e.g. *Lactuca sativa*, *Ranunculus* spp., etc. In the grasses the fruit is called a caryopsis and the pericarp is fused to the testa or seed coat.

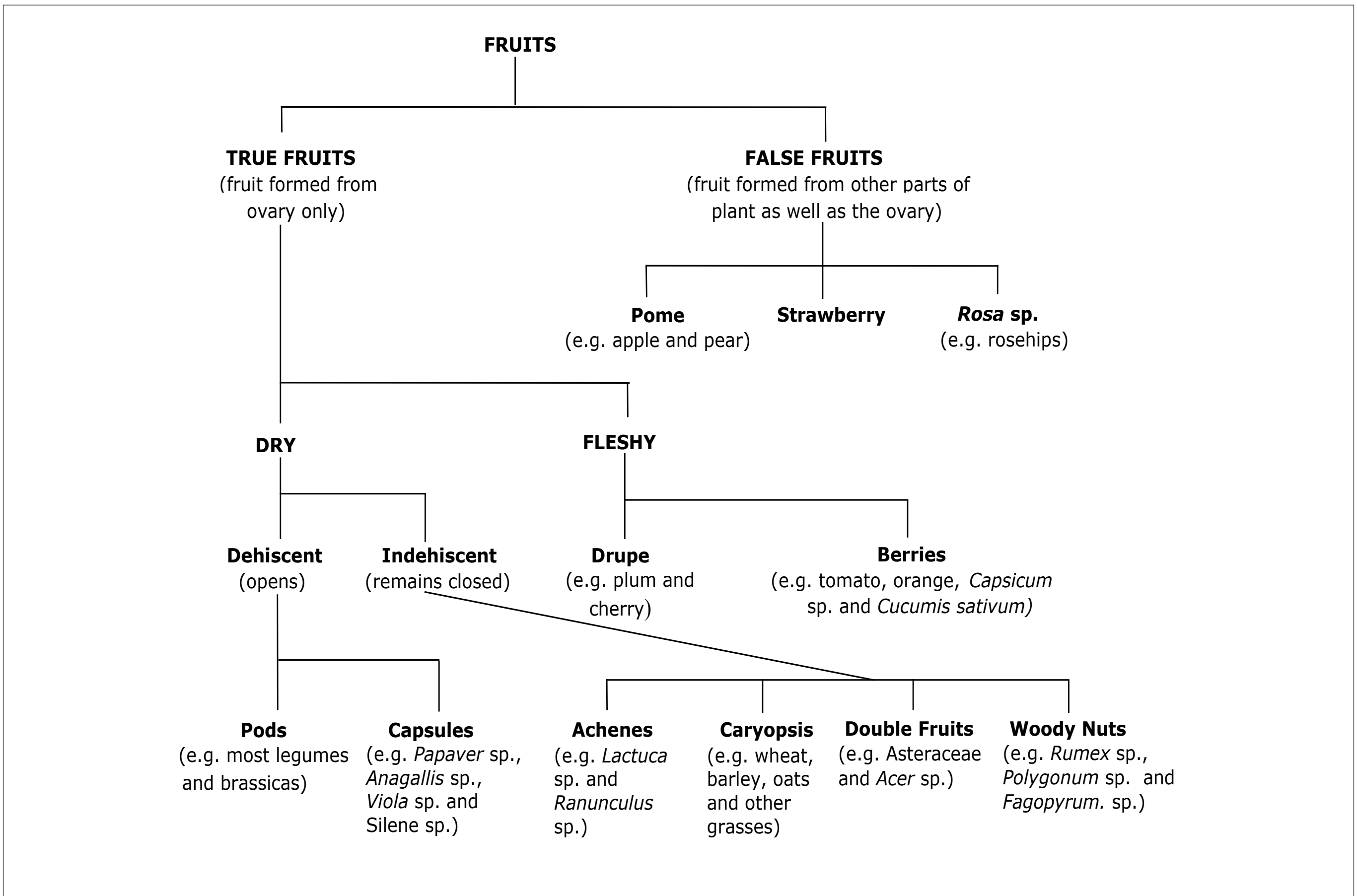


Figure 1.6 Key for use when classifying fruits.

22 The variety of fruit forms is hardly limited, and there is no generally accepted system of classification. The attempt in Figure 1.6 is to classify the main fruit types and for each type give a common example.
23 Dehiscent: opening at maturity along definite lines or sutures.
24 Indehiscent: not opening at maturity.

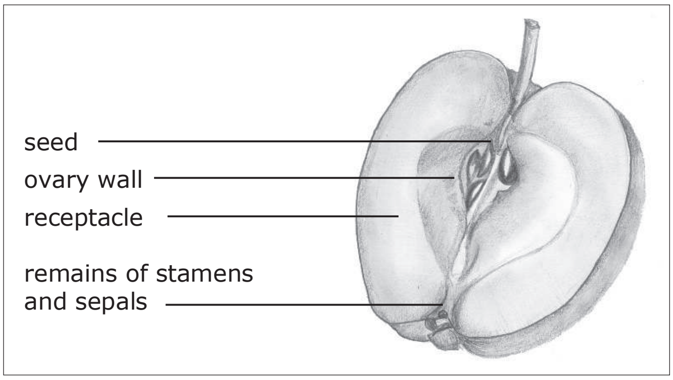


Figure 1.7 Cross-sectional diagram of an apple – a false fruit.

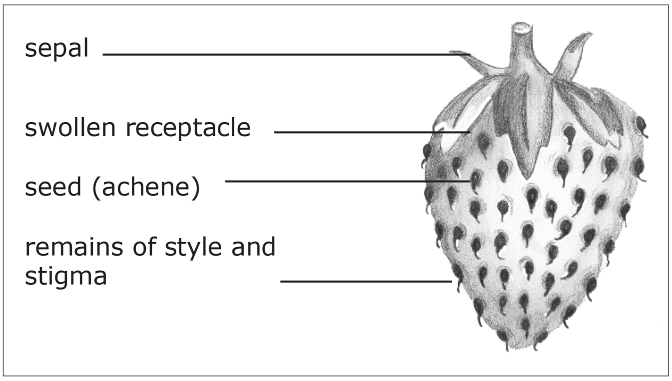


Figure 1.8 Diagram of a strawberry – a false fruit.

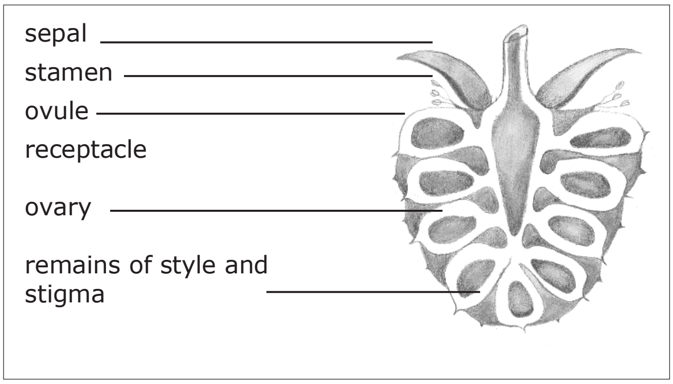


Figure 1.9 Cross-sectional diagram of a raspberry – a true fruit.

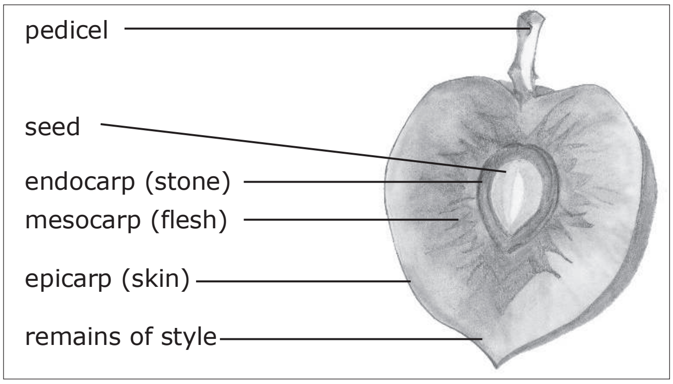


Figure 1.10 Cross-sectional diagram of a plum – a drupe.

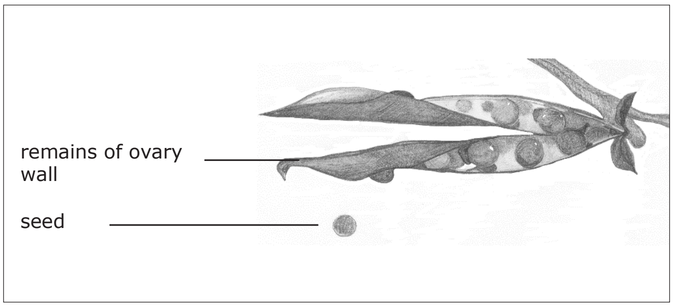


Figure 1.11 Diagram of the dehiscent fruit of *Vicia sativa.*

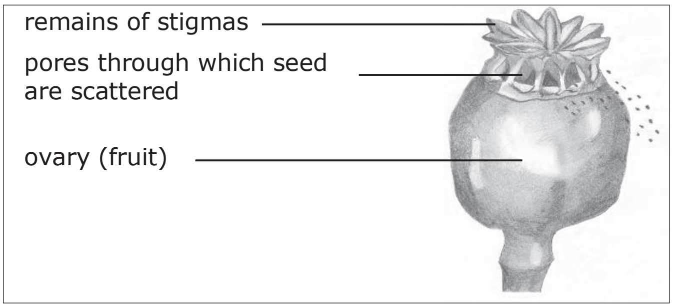


Figure 1.12 Diagram of the dehiscent fruit of *Papaver* sp.

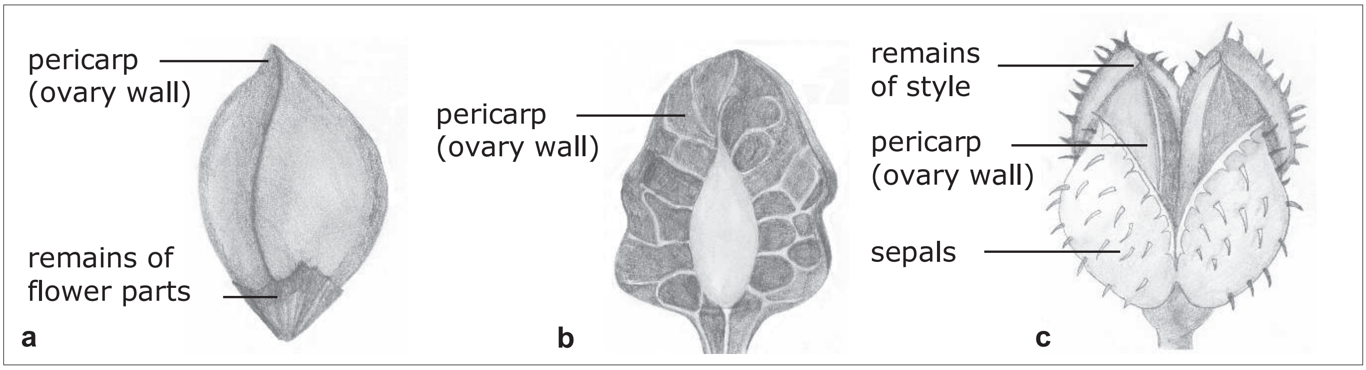


Figure 1.13 Nut-like indehiscent fruits of (**a**) *Polygonum aviculare*, (**b**) *Rumex* sp., (**c**) *Fagus* sp.

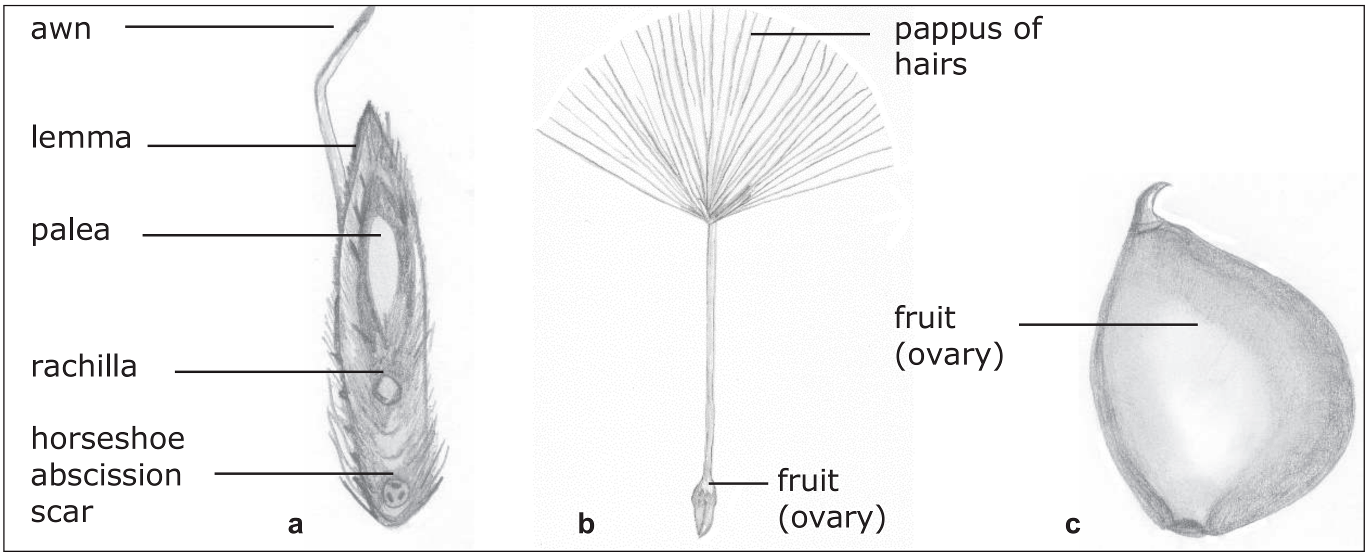


Figure 1.14 Indehiscent fruits of (**a**) *Avena fatua*, (**b**) *Taraxacum officinale*, (**c**) *Ranunculus* sp.

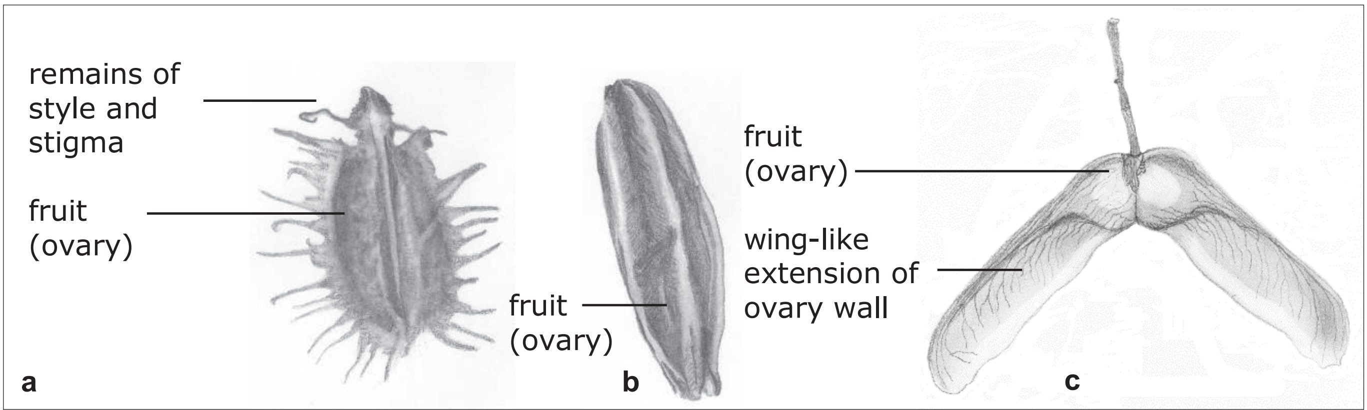


Figure 1.15 Double type indehiscent fruits of (**a**) *Daucus carota*, (**b**) *Carum carvi*, (**c**) *Acer* sp.

There are many kinds of fruit formed after the process of fertilisation – all with the same basic function, i.e. to protect the seed and aid dispersal.

1.3.16 Dispersal of fruits and seeds

When the seeds are mature, individual seeds or fruits are released from the mother plant. Many fruits and seeds are adapted in a particular way to aid dispersal over a considerable distance away from the parent plant. Dispersal reduces overcrowding around the parent plant and competition between members of the same species, and it allows the colonisation of new areas to take place.

Most fruits and seeds are dispersed by wind, insects or animals; although some like the vetch, *Vicia sativa* have explosive mechanisms to release seeds.

Agriculturally, seed dispersal is not a desirable characteristic since it decreases the seed yield. Man has tended to breed crops in which shedding from the mother plant is minimised, e.g. cereals, or he harvests crops before the fruits are fully ripe, e.g. legumes and brassicas.

Section 2: Germination

To the plant physiologist, **seed germination** is the active growth of the embryo that results in the rupture of the seed coat. In this Handbook seed germination also includes the emergence of the seedling which develops into the young plant.

Seeds of some species are capable of germination soon after fertilisation while others may be **dormant** and require a period of rest or post-harvest development before germination can occur. During this period of rest the seed is in a relatively inactive state and has a low metabolic rate. The seed is said to be in a state of **resting**.

The resting of the embryo ends with germination. Regardless of the length of time between maturity and the resumption of growth, seed germination is characterised by several general processes. Germination is the process that transforms the embryo of a seed into an independent photosynthesising plant. Before it can occur, the seed must be viable, provided with a suitable temperature, an adequate supply of water and oxygen, and in some cases the presence or absence of light is a requirement.

2.1 The germination process

The development of a dry seed into a new plant involves four groups of processes:

- the imbibition of water;
- the formation of enzyme systems;
- the initiation of growth, i.e. rupture of the seed coat and radicle emergence; and
- finally the growth and development of the seedling.

2.2 Imbibition: the absorbance and uptake of water

The process of water uptake in seeds takes place in three distinct phases as is clear from Figure 2.1.

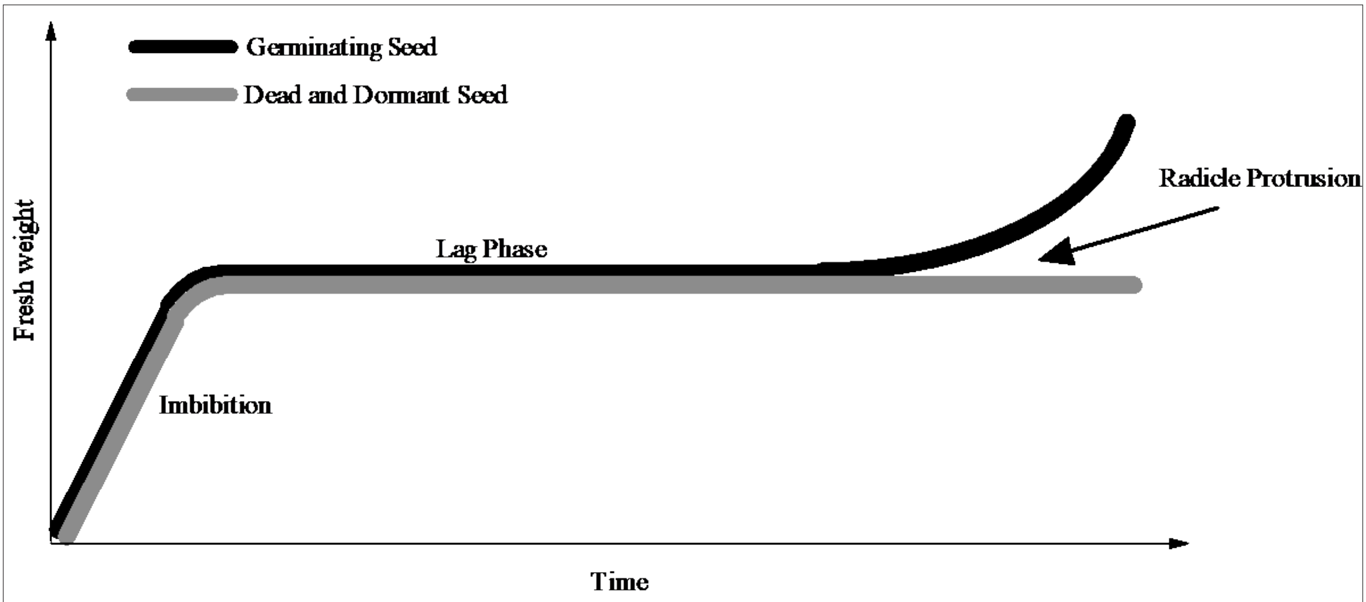


Figure 2.1 Water uptake as changes in fresh weight with time, of dead, dormant and germinating seed.

Seeds are **hydrophilic** and water, which is absorbed through the seed coat, diffuses through the seed tissues. Water softens the seed coat making it more permeable to respiratory gases and the entire seed swells as the cells become **turgid**. During the first few hours of imbibition there is a rapid increase in fresh weight. Moisture content, as expressed on a fresh weight basis, increases from about 5–20 % to 70–75 % with the seed regaining the equivalent amount of water to that lost during ripening. This initial period of fresh weight increase is one of hydration. Imbibition is a purely mechanical process in which water is absorbed: It occurs in both viable and dead seed.

2.3 Formation of enzyme systems and utilisation of food reserves

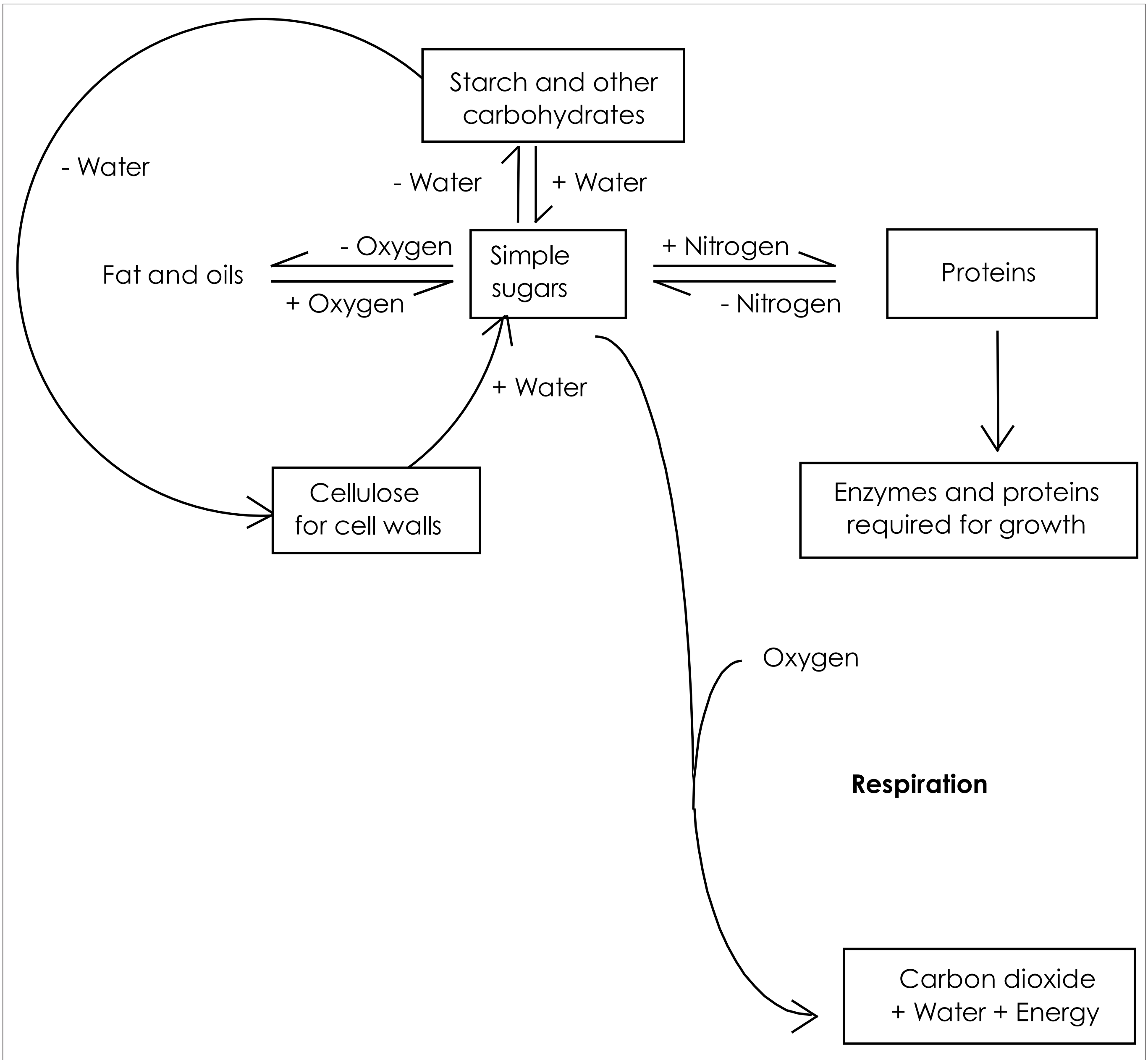


Figure 2.2 The utilisation of seed storage compounds.

Imbibition, the initial period of rapid fresh weight increase, is followed by a plateau period or lag phase where there is little change in fresh weight. During this period, the cells of the embryo form **vacuoles**. The metabolic systems necessary for growth, and the enzymatic component of the systems, are developed. These processes occur only in viable seed.

During the lag phase there is a general mobilisation of the seeds' food reserves. The three types of seed storage compounds, carbohydrates, fats/oils, and proteins are generally insoluble. This insolubility prevents them from being leached during the early stages of imbibition, but before they can be used for energy and growth they must be converted into a soluble form by **enzymes**[1]. These enzymes are activated or synthesised during the lag phase. Enzymes catalyse the reactions involved in the breaking down, assimilation and use of all three types of storage compounds.

The end of the lag phase is marked by **physiological germination**, i.e. the rupture of the seed coat and radicle protrusion.

1 Specialised proteins promoting, regulating and controlling the metabolic process without being permanently changed or destroyed. There are specific enzymes for each type of biochemical reaction.

2.4 Start of growth and radicle emergence

Early embryo growth is due to water uptake causing cell elongation and expansion. Radicle emergence sometimes takes place in dead seed due to this process. Subsequent growth of viable seeds is due to cell division and the synthesis of new material.

Ordinarily the root emerges before the shoot, giving the seedling a chance to establish early root connection with the moist soil, though in some species the shoot emerges first (e.g. *Cyclamen* sp.). Growth of the young seedling occurs at the expense of the storage tissues which gradually decrease as food reserves are depleted, and by the time the young seedling is able to synthesise its own food most of the storage tissues have been exhausted.

2.5 Growth and development of the seedling

From the emergence of the radicle, until the emergence of the seedling apex from the soil, the seedling's growth is under the ground. In the absence of light, growth is a process of exaggerated elongation, i.e. **etioliation** with the stem modified in several ways to facilitate progress through the soil.

In cereals and grasses these modifications include the enclosure of the growing point in a closed leaf cylinder, the coleoptile. Stem elongation can also occur in the mesocotyl, the internode between the seed and coleoptile, of some grasses and cereals, e.g. *Avena* sp. Dicotyledonous plants also have several modifications for under ground growth including the compaction of the stem apex into a hook and the lack of leaf expansion. As the seedling emerges from the soil it meets the light; the stem apex straightens and leaf enlargement begins.

The growth patterns of seedlings have been classified as being either **hypogeal**[2] or **epigeal**. In hypogeal germination the cotyledon(s) remain(s) below the ground, whilst the epicotyl elongates and emerges from the soil. In epigeal germination the **hypocotyl** elongates and drags the cotyledon(s) out and above ground where in some species they expand, turn green and function like leaves. A diagrammatic representation of epigeal and hypogeal germination is shown in Figure 2.3.

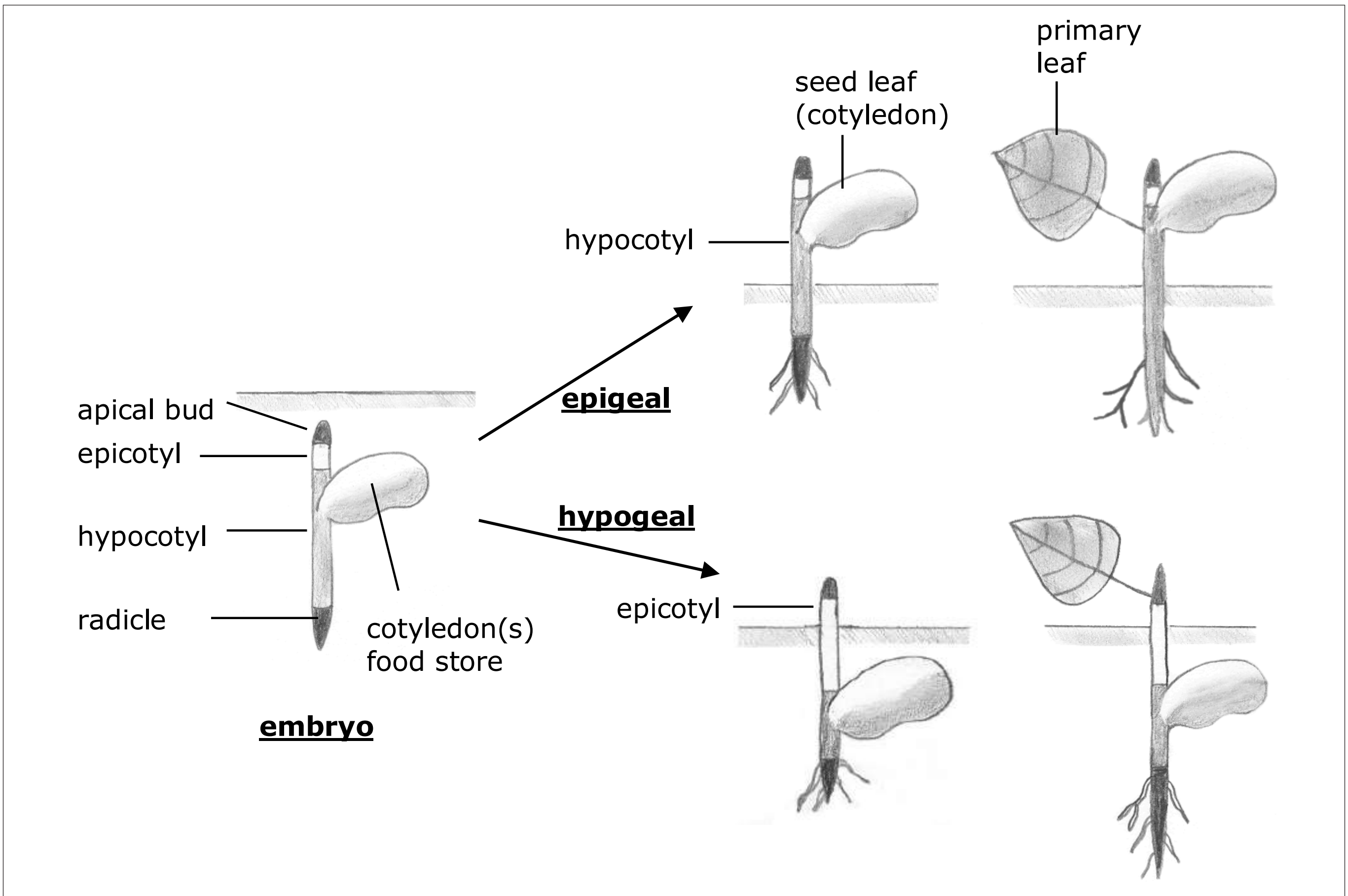


Figure 2.3 Diagrammatic representation of the two types of growth pattern of seedlings.

2 The terms epigeal (above soil) and hypogeal (in the soil) are misleading for they do not mean that the seed germinates above or in the soil, but that the cotyledon(s) is (are) raised above or remain in the soil during germination.

The two types of growth patterns are not related to seed structure and occur in both dicotyledons and monocotyledons.

2.6 Dicotyledons

Although the seed of pea (*Pisum sativum*) and bean (*Phaseolus vulgaris*) are similar in structure, their germination patterns are quite different.

The germination of *Phaseolus vulgaris* as shown in Figure 2.4, is epigeal. Water is imbibed by the seed through the seed coat and the micropyle. After radicle emergence there is a rapid extension of the hypocotyl, which forms a hook and 'elbows' the cotyledons out of the soil. The plumule and primary leaves are protected between the cotyledons. Once the cotyledons emerge from the soil the hypocotyl straightens, the epicotyl elongates, the cotyledons open and the primary leaves expand. Above ground the cotyledons become green and can carry out photosynthesis, but their major role is that of a food store, and as soon as the primary leaves expand, the cotyledons begin to senesce.

Figure 2.4 Epigeal germination: *Phaseolus vulgaris.*

The cotyledons of the germinating pea (*Pisum sativum*) seed act only as a food store and remain below the soil (Figure 2.5). After radicle emergence, the epicotyl elongates and the stem apex becomes compacted into a hook. This pushes the plumule above the soil where the stem apex straightens and leaf enlargement begins. In many dicotyledons the cotyledons, as well as acting as a food store, make a major contribution as the first photosynthesising structures. Figure 2.6 illustrates the germination of a *Brassica rapa* seedling.

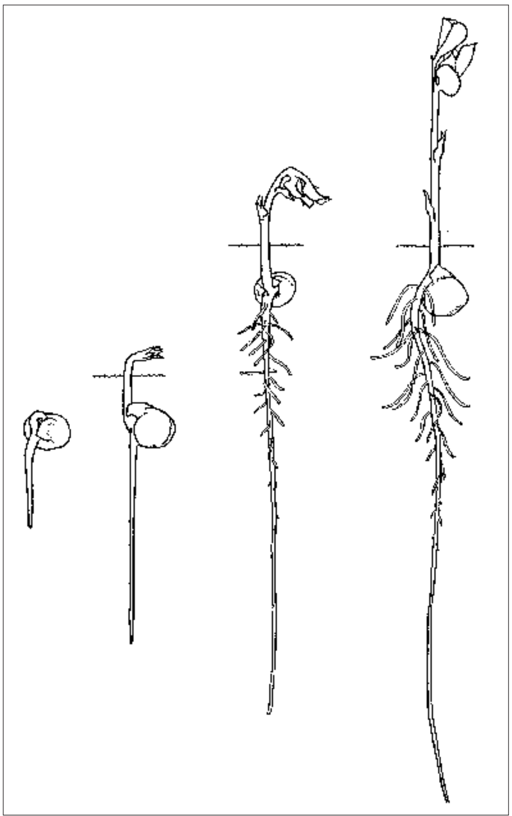


Figure 2.5 Hypogeal germination: *Pisum sativum.*

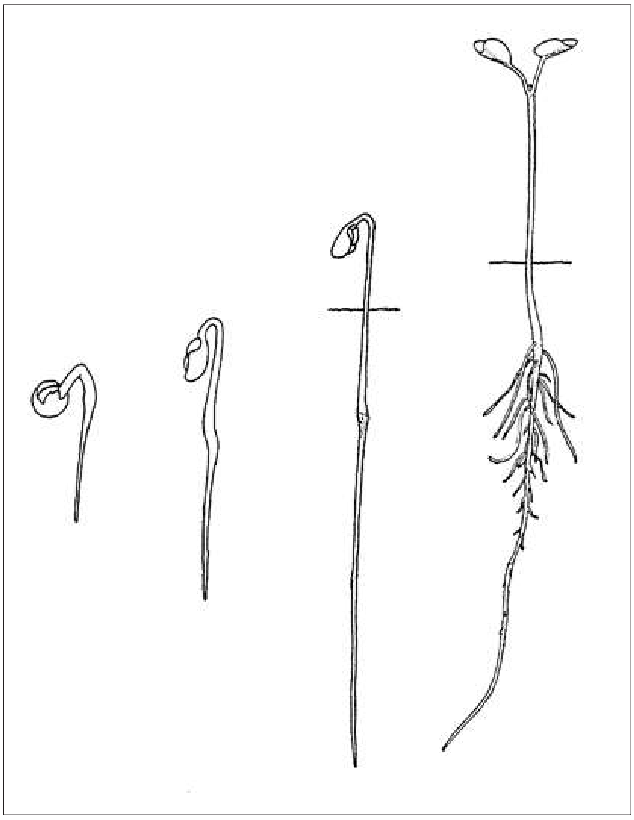


Figure 2.6 Germination of *Brassica rapa* (turnip) – epigeal dicotyledon.

2.7 Monocotyledons

The seed of monocotyledons are endospermic and most exhibit hypogeal germination. In Poaceae, the most important economic group of monocotyledons, part of the cotyledon has become adapted for a specialised digestive function during germination and is called the **scutellum**. Here, the onset of germination is marked by the rupture of the seed coat by the expanding **coleorhiza**. The primary root pushes through the coleorhiza followed almost simultaneously by the other seminal roots. This is followed by the elongation of the coleoptile with the primary leaf developing inside (Figure 2.7).

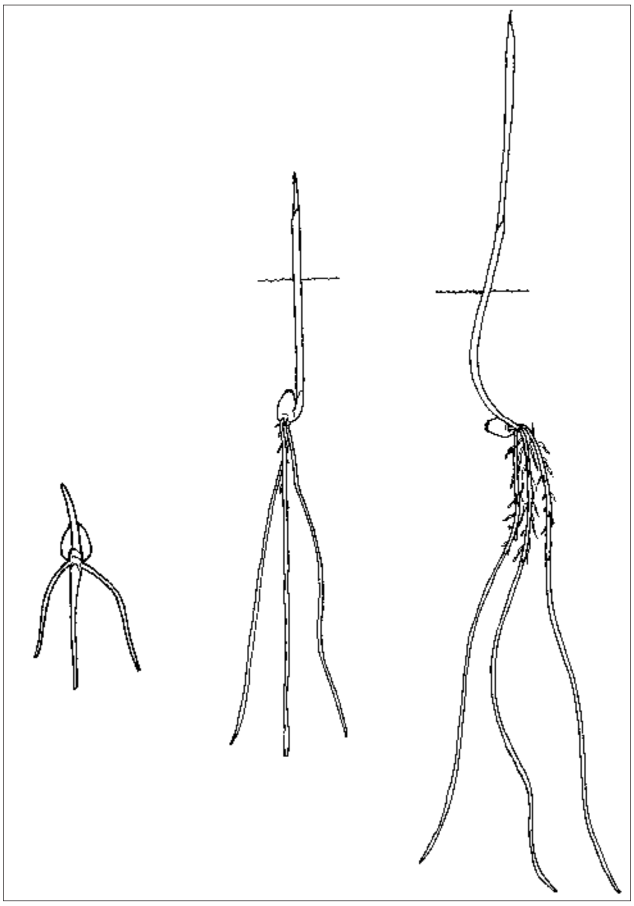


Figure 2.7 Germination of wheat (*Triticum aestivum*) – hypogeal monocotyledon.

The coleoptile, which protects the leaves as they pass through the soil, appears above ground intact, the primary leaf subsequently emerging through it. Mesocotyl development and elongation is dependent on the species and the depth of sowing.

Allium species are examples of monocotyledons with epigeal germination (Figure 2.8). At the start of germination the primary root emerges through the seed coat and elongates without producing lateral roots. The upper green part of the seedling consists of a cylindrical cotyledon with its tip embedded in the seed's endosperm. Towards the upper end, the cotyledon shows a sharp bend or 'knee' which aids emergence from the soil. The root develops adventitious roots and the primary foliage leaf emerges from an opening just above the hypocotyl.

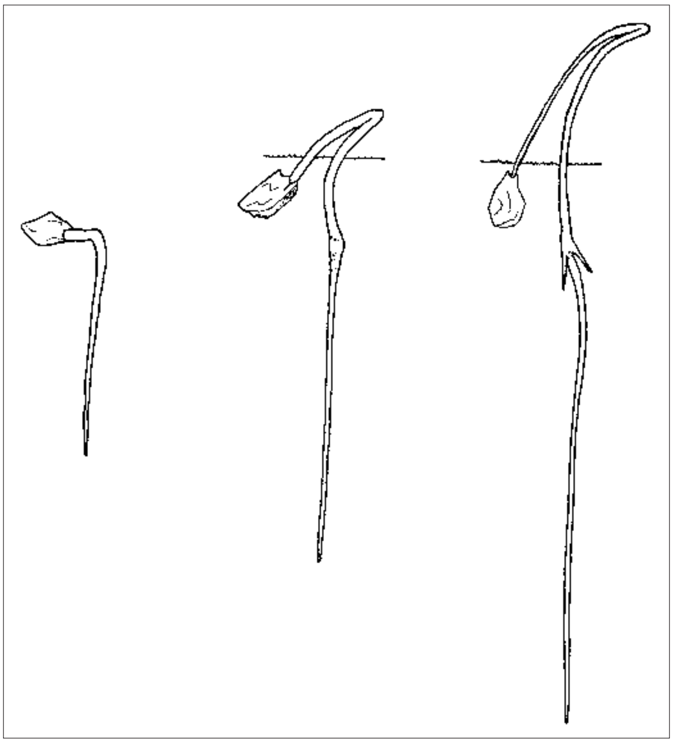


Figure 2.8 Germination of onion (*Allium cepa*) – epigeal monocotyledon.

2.8 Factors affecting germination

Both external and internal factors affect the germination process and can have a profound effect on the outcome. External factors include those that affect the germination environment, while internal factors can be related to the 'history' of the individual seed.

2.8.1 Environmental factors

For germination to proceed, the seed must be provided with water, oxygen and a suitable temperature. In some cases the presence or absence of light is also a requirement.

2.8.1.1 Water

Water is a basic requirement for seed germination. For most seeds the germination medium should be at **field capacity** for optimum germination. Germination may proceed in media of low moisture content and the initial stages may even proceed utilising water available through high humidity conditions, although such conditions are usually not adequate for complete germination. Germination is generally impeded by excess moisture mainly due to a restriction of oxygen availability.

2.8.1.2 Gaseous environment

Air is composed of about 20 % oxygen, 0.03 % carbon dioxide and 80 % nitrogen and the seed of most plant species germinate well in an environment providing this mixture of gases. If the gaseous environment is changed, it becomes clear that: for most species oxygen is required for germination; higher than normal concentrations of carbon dioxide impede germination; and nitrogen has no influence.

In dry storage the seed respiration rate is so low that it is difficult to measure. During germination it increases dramatically and since respiration is essentially an oxidation process, an adequate quantity of oxygen must be supplied. With the exceptions of the seed of some aquatic species, a decrease in oxygen concentration usually impedes germination.

2.8.1.3 Temperature

The rate at which germination proceeds is temperature dependent. Temperature affects: the rate of water absorption, the rate of diffusion of respiratory gases and the rate of chemical reactions involved in the metabolism of the seed. At low temperatures germination rates are low and increases in temperature will, up to certain limits, increase the rate of germination. Once the limit is reached, further increase in temperature will reduce or prevent germination. High temperatures reduce enzyme efficiency and eventually a temperature is reached at which cellular protein is **denatured** and the seed is killed.

Different species have different optimum temperatures for germination and this is the reason why different species are often tested at different temperatures in the seed laboratory.

2.8.1.4 Light

Light is important for the germination of some species. Very small seeds have only minimal amounts of stored food for early embryo growth, and it is therefore necessary for them to become autotrophic quickly. If they germinate too deeply in the soil, they might exhaust their stored reserves before penetrating the surface. The light requirement prevents this from happening and ensures germination occurs only in seeds lying near the soil surface. *Nicotiana tabacum*, *Poa* sp. and some cultivars of *Lactuca sativa* may have specific requirements for light.

Most crop seeds will germinate whether light is present or not. Whether essential or not, light is often given in the seed laboratory to prevent excessive etiolation of the seedling and to promote the formation of chlorophyll.

There are a few species whose germination is inhibited by light and must be germinated in the dark, e.g. *Phacelia tanacetifolia* and *Trifolium subterraneum*, but once germinated, seedlings can be provided with light to aid seedling evaluation.

2.8.2 Other factors

Even when provided with ideal environmental conditions for germination, not every seed that is produced will germinate. Certain factors, at various stages in the history of the seed, may lead to abnormality or death of the embryo.

2.8.2.1 Chemical deficiency

An example of chemical deficiency is the disorder called **marsh spot**. This occurs when pea plants are grown in manganese deficient soil. Seeds from these plants have a discoloured area at the centre of the cotyledons and the plumular bud may not develop when the seed is germinated.

2.8.2.2 Weather experienced during seed development

Wet weather during seed development can:

- cause seed to sprout on the mother plant before harvest;
- favour the development and spread of **microflora**, which can discolour the seed, can affect normal germination or even kill the seed.

Alternating wetting and drying can cause shattering of embryonic structures leading to seedlings of the Poaceae with split coleoptiles and seedlings of dicotyledons with detached cotyledons and broken plumules.

2.8.2.3 Immaturity

Immaturity of seed at harvest is a particular problem in forage crops where seeds do not ripen synchronously and large differences in maturity can exist even between seeds on the same plant. The presence of immature seeds with high moisture content in a seed lot encourages the growth of storage microflora whose activities increase the temperature of stored seed and lead to germination damage in even the mature seed.

2.8.2.4 Mechanical damage

Mechanical damage occurring during harvesting and processing is most common in dry seasons when low moisture content at harvest makes seeds brittle and susceptible to cracking and breaking during combining. Although broken seed is easily removed, damage to the embryo is not usually detected until the seed has germinated. The processes involved in cleaning, dressing with chemical seed treatments, bagging and transporting of seed can also cause mechanical damage. Affected seed of the Poaceae can exhibit split coleoptiles and produce shoots but no roots (especially in rye, triticale and to a lesser extent wheat); whereas mechanically damaged dicotyledonous seed produces abnormals exhibiting a range of symptoms due to embryo fractures.

2.8.2.5 Heat damage

Seed harvested at a high moisture content must be artificially dried to avoid a spontaneous build up of heat and damage due to microflora, insects and mites. **Heat damage** or **drying damage** may occur when excessive heat is used for drying. The symptoms of this in cereal germination tests are, in order of increasing severity: slower germination, delay in emergence of primary leaf, stunted growth, termination of the germination process after emergence of coleorhiza and seed death.

Spontaneous heating during storage can decrease the germination capacity of seed stored in large bulks. Areas of excessive moisture encourage the activities of seed **microflora** and **microfauna** whose respiration produces hot spots. Since moisture migrates from hot to cooler areas, this can set off further local heating and this can result in a chain reaction.

2.8.2.6 Effect of chemicals

Many seed lots are treated with **fungicides**, and in some cases **insecticides**, to protect the seed or the germinating seedling from fungi and insects. Seedlings with symptoms of phytotoxicity may develop and germination can be reduced if:

- the seed is treated with excess chemical;
- the seed moisture content is higher than recommended; or if the seed has sprouted; or
- the seed has sprouted or is mechanically damaged.

The seed may be accidentally contaminated by chemicals during growth and storage. If a herbicide/desiccant is applied to the growing crop whilst the seed has a high moisture content or if the harvested seed is contaminated with volatile sprout suppressants, seedlings showing abnormal symptoms may develop and germination can be reduced.

Chemicals in the germination medium caused by, for example, bleaches used in paper making, can suppress germination, cause the production of abnormal seedlings or kill the seed.

2.8.2.7 Insects and mites

Insects and mites may attack seeds during storage and, apart from contributing to an increase in temperature, they inflict damage. Insects, e.g. *Oryzaephilus surinamensis* (saw-tooth grain beetle) tend to feed indiscriminately, but mites, e.g. *Cicaris siro* (the flour mite) feed exclusively on the embryo.

2.8.2.8 Plant pathogens

Apart from microflora causing problems noted above, certain plant pathogens are seed-borne and can have a detrimental effect on germination, e.g. *Septoria nodorum* (wheat), *Alternaria* sp. (brassicas and clovers) and *Phoma betae* (beet).

2.8.2.9 Longevity

Like all other organisms, the seed has a finite longevity. Seed ageing depends on a number of different factors including storage conditions. The seed ages rapidly if subjected to severe conditions, e.g. high temperature and high humidities, and as a seed lot ages, increasing numbers of abnormal seedlings and dead seeds are found in germination tests.

2.9 Physiology of seed dormancy

During the life cycle of a plant, a great number of seeds may be produced. However, not all of these will germinate, even though they may be placed under conditions normally regarded as favourable for germination. Such seeds can be shown to be viable in biochemical tests such as the **tetrazolium** test and they can be induced to germinate by various methods and treatments. Seeds in this state are said to be **dormant**.

Dormancy may be defined as a state of suspended growth and reduced metabolism and is present to some extent in the seed of most plants. It is:

- a ‘resting’ phase where growth and metabolism appear to be suspended;
- a physical or physiological condition of viable seed that prevents germination even in the presence of otherwise favourable germination conditions;
- the mechanism that has evolved to prevent seed germination in environmental conditions unfavourable to the subsequent growth and development of the plant.

In addition, dormancy may enable germination to be spread erratically over a long period of time thereby preventing:

- the extinction of a population through an unexpected environmental threat, e.g., drought, flood or fire; and
- competition from other seed from the same population.

These characteristics are undesirable in an agricultural crop and ever since crop production began some 10,000 years ago, there has been a tendency to select crop plants with a minimum of seed dormancy, uniform germination and synchronous fruiting. However, a certain degree of dormancy is essential to prevent sprouting on the mother plant. Plants with a long history of domestication generally show less seed dormancy than wild or recently domesticated species.

The degree of dormancy in seed depends on an interaction between the environment, during crop maturation, and the genetic make-up of the seed, i.e. dormancy is a **genetic** characteristic and the degree of dormancy is influenced by the environment. In most years dormancy in crop seeds is transient in nature and only sufficient to prevent pre-harvest sprouting; however, in temperate regions after cold wet growing seasons, seed may be unable to germinate for several months unless dormancy is broken.

For a seed to be classified as dormant it must be imbibed. A common misconception is to consider dry seed in dry storage as being dormant; it is not, it is in a state of quiescence.

“*Some seeds are born dormant,*
Some seeds become dormant,
Some seeds have dormancy thrust upon them.”

This ‘verse’ by Jock Thomson[3] helps one remember the three different types of dormancy, i.e.:

- **innate** dormancy;
- **induced** or **secondary** dormancy;
- **enforced** dormancy.

These types of dormancy are now considered in relation to seed testing.

3 Past president of ISTA (1968–1971).

2.9.1 Innate dormancy

This is a genetic characteristic of the species and there are three types:

1. **Immaturity of the embryo** – post-harvest embryo development required before germination is possible;
2. **After-ripening required** – post-harvest biochemical/hormonal/physiological changes required before germination is possible;
3. **Specific environmental stimulus required**.

Embryo immaturity and an after-ripening requirement are present to some extent in the seeds of most species and these prevent germination on the mother plant. The length and period of this type of dormancy varies from species to species and also from seed to seed within a population. This is the type of dormancy most frequently encountered in the seed testing laboratory especially when testing has to be done before post-harvest embryo development and/or after-ripening is complete.

2.9.1.1 Immaturity of embryo

Seeds of some species are shed before they are morphologically mature. The embryo of holly (*Ilex aquifolium*) is an undifferentiated mass of cells when the seed is shed, but during post-harvest maturation the cells differentiate a well-defined structure. Cherry seed embryos (*Prunus cerasus*) increase in size, weight and metabolic activity after being shed from the tree, and similar changes take place in many genera, e.g. *Ranunculus*, *Plantago*, *Fraxinus*, *Viburnum* and several species of *Pinus*.

2.9.1.2 After-ripening requirements

Even though morphological growth of the embryo is complete, physiological immaturity of the seed may prevent germination. The embryo is dormant and germination will not occur until the seed has undergone a period of after-ripening.

Seeds with embryo dormancy usually require either a period of low temperature imbibition or a period of dry storage before germination is possible.

2.9.1.2.1 Low temperature imbibition

After dispersal from the mother plant, the dormant seed is subjected to winter conditions and dormancy is broken during this period; germination then occurs during the spring. It is the low temperatures experienced by the seed that 'breaks' dormancy. In the laboratory pre-conditioning moistened seed at temperatures between 5 and 10 ºC simulates winter conditions. This process is called pre-chilling and its duration is dependent on the species and the depth of dormancy. Whereas, a seven day pre-chilling period is usually all that is required to break dormancy in cereals, rose seed (*Rosa multiflora*) require a period of up to three months.

2.9.1.2.2 Dry storage

The removal of dormancy by periods of dry storage at room temperature is widespread among seeds and almost universal in cereals. For most cereals dry storage for one to two and a half months at temperatures between 10 °C and 20 °C will remove dormancy. In the laboratory pre-drying the seed at a temperature of up to 35 °C for periods up to one week often accelerates this natural process.

2.9.1.2.3 Physiological changes during after-ripening

During after-ripening a change occurs in the seed's hormone levels. Hormones are substances that assist in co-ordinating functions between different parts of the plant and it is generally accepted that innate dormancy is controlled by a balance between hormones that impose dormancy (**inhibitors**) and those that stimulate active growth (**promoters**). **Gibberellins** are promoters and have long been known to effectively break dormancy of, for example, buds of various woody plants, potato tubers and seeds such as cereals and tobacco.

The rise in gibberellin content is usually preceded by a fall in inhibitor content and it is probable that the temperature pretreatments used by seed analysts to overcome dormancy lead to an increase in the seed's internal levels of promoters and a decrease in the levels of inhibitors. The analyst can also break dormancy by external application of gibberellic acid, which presumably changes the relative concentrations of inhibitors/promoters.

2.9.1.3 Specific environmental stimulus required

Even when after-ripening is complete, not all seed will germinate; some environmental stimulus is required.

2.9.1.3.1 Mechanical

The seed coat structures are frequently critical to the dormancy of seeds, limiting the entry of water and oxygen and mechanically limiting the enlargement of the embryo. The seed coat (testa) of some leguminous seeds prevents water imbibition because of a hygro-scopically activated valve in the **hilum** (hard seed). Germination is not obtained even in moist conditions because of a limitation of moisture entry by closure of the hilum, brought about through a differential in moisture content between internal and external seed coat layers. When there is more moisture on the outside of the seed coat, the valve closes and water entry is prevented, whereas the valve opens and the seed dries further if there is less moisture on the outside. The lower the seed moisture, the more pronounced the impermeability of the seed coat and the deeper the dormancy. To break this dormancy the seed coat must be disrupted. This can happen naturally over a period of several years or can be accelerated using mechanical or chemical scarification.

2.9.1.3.2 Light

The germination of many seeds is enhanced by light and in some species germination will not occur until light is given, e.g., *Nicotiana tabacum*, *Poa* sp. and some cultivars of lettuce (*Lactuca sativa*). A light requirement may serve to bring about germination when the seed is near the soil surface or when the soil or surrounding vegetation has been disturbed and the subsequent competition consequently lessened.

The quality of light is important since in **photoblastic** seed, germination is controlled by a pigment **phytochrome** that can exist in two interchangeable forms. The red form (P_{FR}) stimulates germination and the far-red form (P_R) exists in dormant seed. The reactions of phytochrome are summarised in Figure 2.9.

Figure 2.9 The reactions of the photo-reversible pigment phytochrome, which is responsible for the photoperiodic control of seed germination.

Most crop seed will germinate whether light is present or not, but light, with low far-red content, is usually given in the seed laboratory to prevent excessive etiolation of the seedling and to promote the formation of chlorophyll.

2.9.1.3.3 Alternating temperatures

In nature there is a diurnal fluctuation in temperatures associated with day and night and in many species increased germination, or more uniform germination, can often be obtained by subjecting seed to **alternating temperatures** rather than any single temperature treatment. The physiological effect of alternating temperatures is thought to be associated with some physiochemical or structural change in the seed.

2.9.1.3.4 Potassium nitrate

Potassium nitrate is very effective in stimulating the germination of the dormant seed of many species when incorporated in the germination medium at a concentration of between 0.2 % and 0.3 %. It is particularly effective in breaking dormancy of grasses and does this by stimulating the pentose monophosphate shunt (a biochemical pathway that appears to be important in the early stages of germination).

2.9.1.3.5 Ethylene and carbon dioxide

Ethylene gas is a promotive plant hormone and in **synergistic** combination with carbon dioxide it can overcome the dormancy of some clover species, e.g. *Trifolium subterraneum* and *T. repens*. Increased levels of ethylene and carbon dioxide are achieved by sealing the germination replicates in polythene bags; the respiratory activity of the seed increases the carbon dioxide concentration and the natural degradation of polythene gives rise to an increase in the ethylene concentration. Ethylene alone can be used to break dormancy in *Helianthus annuus* and *Oryza sativa*.

2.9.1.3.6 Pre-washing

In many seeds water-soluble inhibitors in the seed coat prevent germination, in low moisture conditions. These inhibitors must be leached from the seed coat by sufficient water before germination is possible. In the seed laboratory inhibitors in the clusters of *Beta* are removed using a beet washer prior to germination.

2.9.2 Induced dormancy

Induced dormancy develops as a result of a particular environmental or climatic condition after the seed has been shed and will persist even after the inductive conditions have passed and the environment becomes favourable for germination. Once dormancy has been induced, in a non-dormant seed, the seed behaves as if it was innately dormant and dormancy breaking procedures must be used before germination is possible. Induced dormancy is not common and occurs when seeds are supplied with water in an otherwise unfavourable environment, e.g. restricted oxygen supply. It is known to occur in:

- *Avena fatua* – when oxygen is restricted;
- *Brassica nigra* – when carbon dioxide concentration is high;
- *Lactuca sativa* (some cultivars) – when imbibed at high temperatures.

2.9.3 Enforced dormancy

This describes a condition in which viable seed does not germinate because of some limitation of the environment. Unlike induced dormancy, it will not persist once the limitation is removed.

Some authorities object to the term ‘enforced dormancy’ because of a possible contradiction in terms, i.e. enforced dormancy results from incorrect test conditions and can be eliminated when test conditions are corrected, rather than by applying normal dormancy breaking procedures.

It is usually used to describe the situation of seeds deeply buried in soil. As soon as they are brought to the surface they germinate.

Water sensitivity in *Hordeum vulgare* is a form of enforced dormancy due to a relative excess of water in the germination environment. If the seeds are removed and dried before placing in a reduced quantity of water, they will germinate normally.

The seed analyst employs a large number of different dormancy breaking procedures that are used, alone or in certain combinations. The procedures used involve the use of pre-chilling, pre-drying, gibberellic acid application, light, alternating temperatures, nitrate application, increasing the concentration of carbon dioxide and ethylene and pre-washing. Knowledge of the physiology of dormancy explains the effectiveness of dormancy breaking treatments.

Section 3: Agriculture and seed testing

3.1 Agriculture

Man, after millennia of nomadic life as a hunter and gatherer, settled down about 10,000 years ago. He had learnt not only to collect seeds as food from nature's oversupply, but also to hoard seeds and to sow them on fields near his dwelling. This was the beginning of agriculture and fixed settlements and allowed the development of advanced civilisations.

Seeds are the staple food of the world and are the source of nutrition for more people than any other type of food. They offer a highly nutritious food to man and his animals, which can be easily stored and transported.

The family of Poaceae contributes more food seeds than any other plant family. Rice (*Oryza sativa*), wheat (*Triticum aestivum*) and maize (*Zea mays*) are the most important ones. Others include: oats (*Avena sativa*), barley (*Hordeum vulgare*), sorghum (*Sorghum* sp.), millet (*Panicum* sp., *Pennisetum* sp., *Setaria* sp.), rye (*Secale cereale*) and tef (*Eragrostis* sp.). Collectively the large-seeded grasses are called cereals. All civilisations of man are based on the cultivation of cereals, mainly because this offered the possibility of a constant food supply.

The main nutrients of the cereals are **carbohydrates** (starch and sugars) and **proteins**.

The second most important plant family contributing to world nutrition is the family of Fabaceae. It provides us with groundnut/peanut (*Arachis hypogaea*), soybean (*Glycine max*), bean (*Phaseolus vulgaris*), lentil (*Lens culinaris*), pea (*Pisum sativum*), chick-pea (*Cicer arietinum*) and many tropical and subtropical genera with edible seeds.

The main nutrients of the Fabaceae seeds are **proteins** and **fat**.

From the beginning of agriculture man has successfully tried to improve his crop plants and to adapt them to his requirements by selection, i.e. by sowing the seeds of only those individual plants that show the characteristics wanted. In this way, and later by controlled breeding and by means of genetic engineering, he has been able to select varieties with characteristics such as high yield, increased protein/oil/carbohydrate content, disease and herbicide resistance, standing ability, earlyness, etc. Today's high yielding, highly nutritious cultivars are the result of selection and breeding efforts and they have helped us to maintain food security in the face of a rapidly increasing world population.

3.2 Seed testing

Seed has been bartered and traded for centuries. At first, this trade was local but it has developed beyond borders and continents. Seed is harvested, processed, stored, packaged, sold and sometimes transported for long distances, before it is sown. To maximise margins, the user of seed, the farmer, is interested in achieving as high a yield as possible from the seed sown. One of the most important requirements in achieving this is high quality seed. The quality of seed can be adversely affected by a large number of factors.

Since seed is comparatively expensive, the need for **seed quality control** and obligatory **quality criteria** to protect farmers from poor quality seed, has increased. The **International Seed Testing Association** has elaborated such quality criteria on a scientific basis; standardised definitions to describe them; and developed methods to determine them. These are laid down in the **ISTA Rules**.

Seed quality[1] is a combination of many different characteristics, which are evaluated in seed testing laboratories. Among the most important ones are the two that were developed in the first seed testing station in Tharand, Germany by Professor Friedrick Nobbe (the father of seed testing) in 1869. These are the analytical purity and the germination tests. Other important seed lot characteristics include varietal purity, seed health and moisture content.

3.3 Objective of the analytical purity analysis

The objective of the analytical purity analysis is to determine the proportion of foreign components in a seed lot and to identify them. Generally an analytical purity test distinguishes between:

3.3.1 'Inert matter'

This may consist of:
- broken seeds;
- plant debris, e.g. empty glumes, pieces of leaves, flowers or stalks;
- non-plant organic material, e.g. ergot (*Claviceps purpurea*), sclerotia, bunt balls, insects and nematodes; and
- non-organic material, e.g. sand, soil and stones.

1 In this Handbook the term 'seed quality' refers to the quality of the seed lot.

3.3.2 'Other seeds'

'Other seeds' may include seeds of other crops or weed seeds: both are undesirable and the presence of noxious weeds, such as wild oats or *Elytrigia repens*, may have a detrimental effect on the fields where they are sown and the quality of the crop produced.

3.4 Objective of the germination test

The germination capacity is one of the most important quality attributes of seed and the objective of the germination test is to determine the maximum germination potential of a seed lot, which can then in turn be used to compare the quality of different lots and also estimate the field planting value. Since a seed lot consists of single seeds, each of which contributes to the quality of the lot, it is important that every seed tested in a germination test is checked and that every single seedling is examined with regard to its quality.

This is the subject of seedling evaluation and of this Handbook.

Section 4: Laboratory conditions for seedling evaluation

Biologically speaking, germination is a complex series of physiological processes, beginning with the seed taking up water and ending with the primary root piercing the seed coat. This is physiological germination. Germination in the seed testing laboratory begins with the primary root piercing the seed coat. It ends when the seedling has developed to the stage that it can be evaluated according to the ISTA Rules.

4.1 Laboratory equipment

Different kinds of equipment might be required in order to perform germination tests in a laboratory. The following indications are aimed at providing guidance for choosing adequate equipment and for using the equipment according to the ISTA Rules and the basic principles of quality assurance.

4.1.1 Containers

All kinds of plastic, glass, metal or pottery containers can be used, provided that there is no toxin production, and that they are clean and free from microorganisms (Figure 4.1). Innocuity and cleanliness of the containers should be checked together with the quality of the growing media used in them, using a biological test as described in Appendix 5 (A5.6).

4.1.2 Counting equipment

In addition to counting by hand with a spatula, two types of seed counters are frequently used: counting boards and vacuum counters. These tools are permissible as long as they do not influence the germination results or cause replicate results to be biased. Laboratories using counting equipment should have quality assurance procedures in place that demonstrate that its use results in replicates that are representative of the working sample.

Possible methods for evaluating counting equipment are described in 4.1.2.4.

4.1.2.1 Counting by hand with a spatula

For this method a spatula with a straight edge is required. A magnifying glass, may be needed for small seeded species.

The method is shown in Figure 4.2:

– Pour the pure seed on a smooth, leveled surface and mix it thoroughly using the spatula.
– Spread the seed into a long narrow line.
– Starting at one end of the line, the required number of seed is counted and removed in strict order as each seed reaches the end of the line. Seeds can be removed from the line in sub-groups of 5, 10 or 20 for ease of checking the number of seeds counted.

This method is very easy to use but can be difficult with spherical seed. The time needed and the accuracy of counting depends on the analyst.

Figure 4.1 Examples of two different containers used for germination tests.

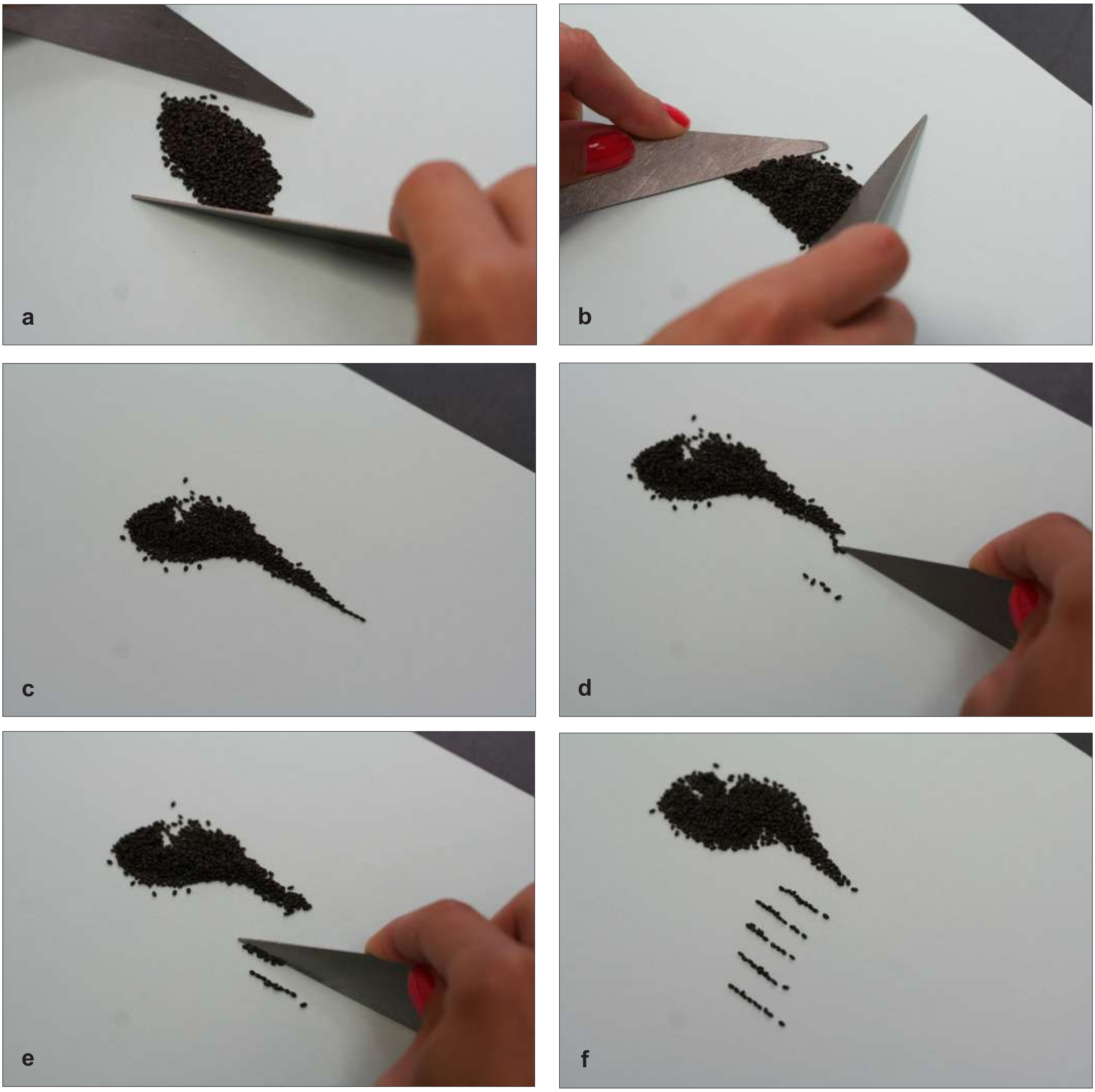


Figure 4.2 Counting by hand. Mixing the sample with spatula (**a** and **b**); drawing a line of seeds (**c**); counting sub-samples of 10 seeds from the line and keeping them separate for easy control (**d–f**).

4.1.2.2 Counting boards

Counting boards (Figures 4.3, 4.4) are often used for large seeds such as *Zea*, *Phaseolus* and *Pisum*. A counting board is approximately the size of the substrate on which the seeds are to be placed. The top consists of a board with 50 or 100 holes of the general size of the seeds to be counted. The diameter holes should be large enough that the largest seeds of the sample fit in and to avoid the selection of only small seeds if the holes are too small. However, they should not be too large to avoid collecting more than one seed in one hole. Below this board is another board which serves as a base; it may be solid to be slid backward and forward, or it may have corresponding holes which can be closed or opened by moving the top and the base against each other. In operation, the seeds are scattered over the board with the holes closed underneath. Excess seeds are removed after checking that all holes are filled and that there is only one seed in each hole. The holes are opened by sliding the movable board, and the seeds fall into place onto the substrate.

This equipment is easy to use, but care is needed to avoid damaging the seed.

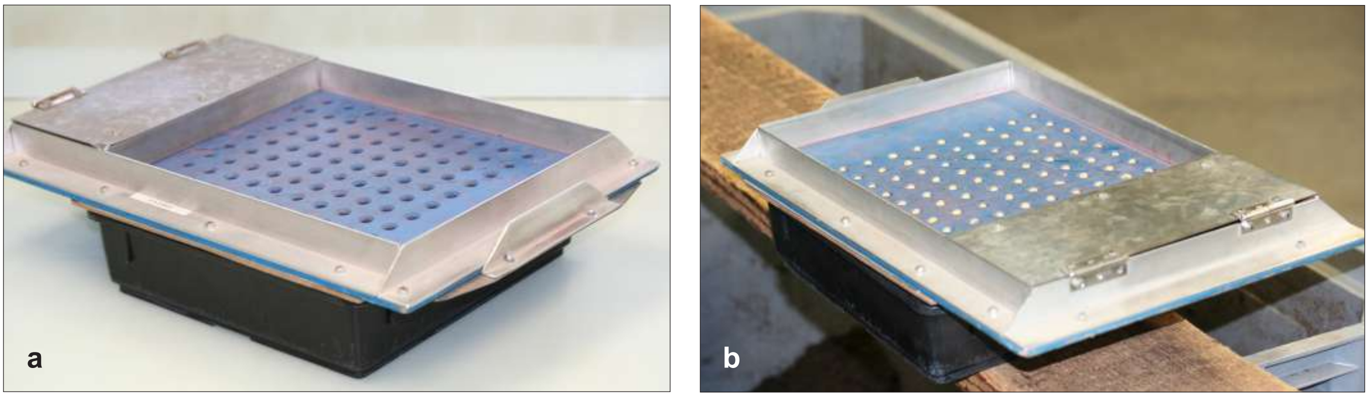


Figure 4.3 Counting board with (**a**) 100 holes; (**b**) placed above a plastic container filled with growing medium.

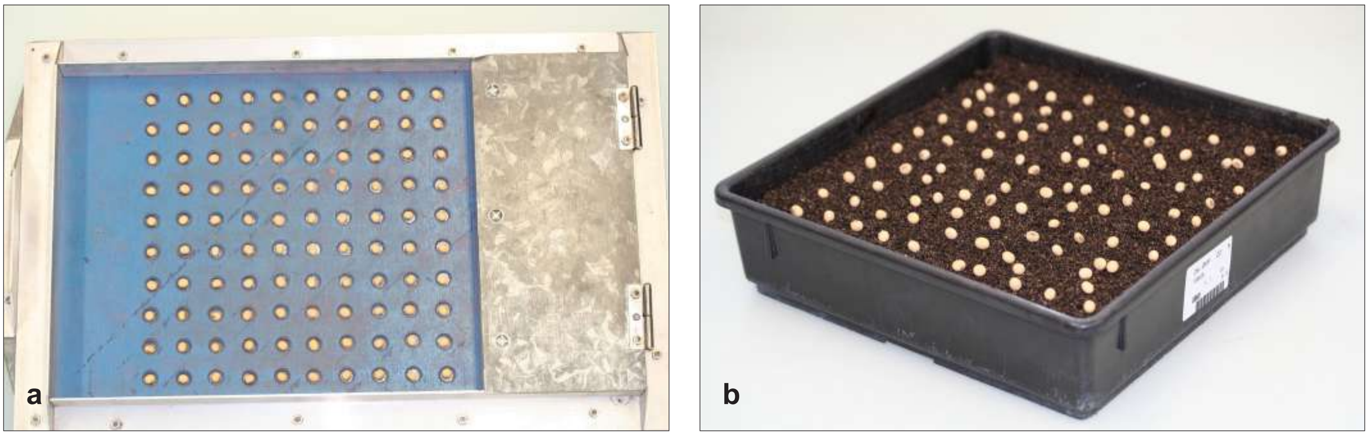


Figure 4.4 Counting board with (**a**) holes filled with seeds; (**b**) seeds placed on the substrate.

4.1.2.3 Vacuum counters

Vacuum counters (Figure 4.5) are often used for species with regularly shaped and relatively smooth seeds such as cereals or species of *Brassica* and *Trifolium*. A vacuum counter consists of three essential parts: a vacuum system including pipes which do not restrict air flow, a range of counting plates or heads to suit the range of seeds tested and the size of germination substrates, and a vacuum release valve. An ordinary household vacuum cleaner may be used as a vacuum system. The heads, containing 50 or 100 holes, should be slightly smaller than the substrate and should have an edge to prevent the seeds from rolling off. The diameter of the holes should correspond with the seed size and the vacuum applied.

In operation, the seeds are poured evenly over the counting head with the vacuum off. The vacuum is then applied, surplus seeds are removed and a check is made that all holes are filled and only one seed is on each hole. The head is then placed on the germination substrate and the vacuum released so that the seeds fall into place on the substrate.

The counting head is a very efficient tool for quick and precise planting of seeds for germination testing.

Care should be taken that there is no selection of seeds. With vacuum counters, some precautions should be observed to avoid biased replicates: the counting head must not be plunged into the seed while a vacuum is applied, because this procedure selects light seeds. For the same reason, the vacuum should not be applied when the seeds are being poured on to the counting head. If the vacuum is applied when seeds are being poured onto the counting head, it must be shown that this does not affect the germination results.

4.1.2.4 Methods for evaluating counting equipment

Seed counters must not influence the germination results or cause replicate results to be biased. If the counting equipment is also used for preparing submitted or working sample, and has already been checked according to the appropriate sampling procedures (ISTA Handbook on Seed Sampling), then there is no need to check this equipment again.

If this is not the case, the following method can be used to check the quality of counting equipment with regard to their ability to produce reliable working samples for germination tests. Checks must be made for each type of counting equipment and for a number of representative species of those analysed in the laboratory, in terms of size and shape of the seeds. The following method is only one suggestion; it does not exclude any other efficient method that could be used for the same purpose.

Section 4: Laboratory conditions for seedling evaluation

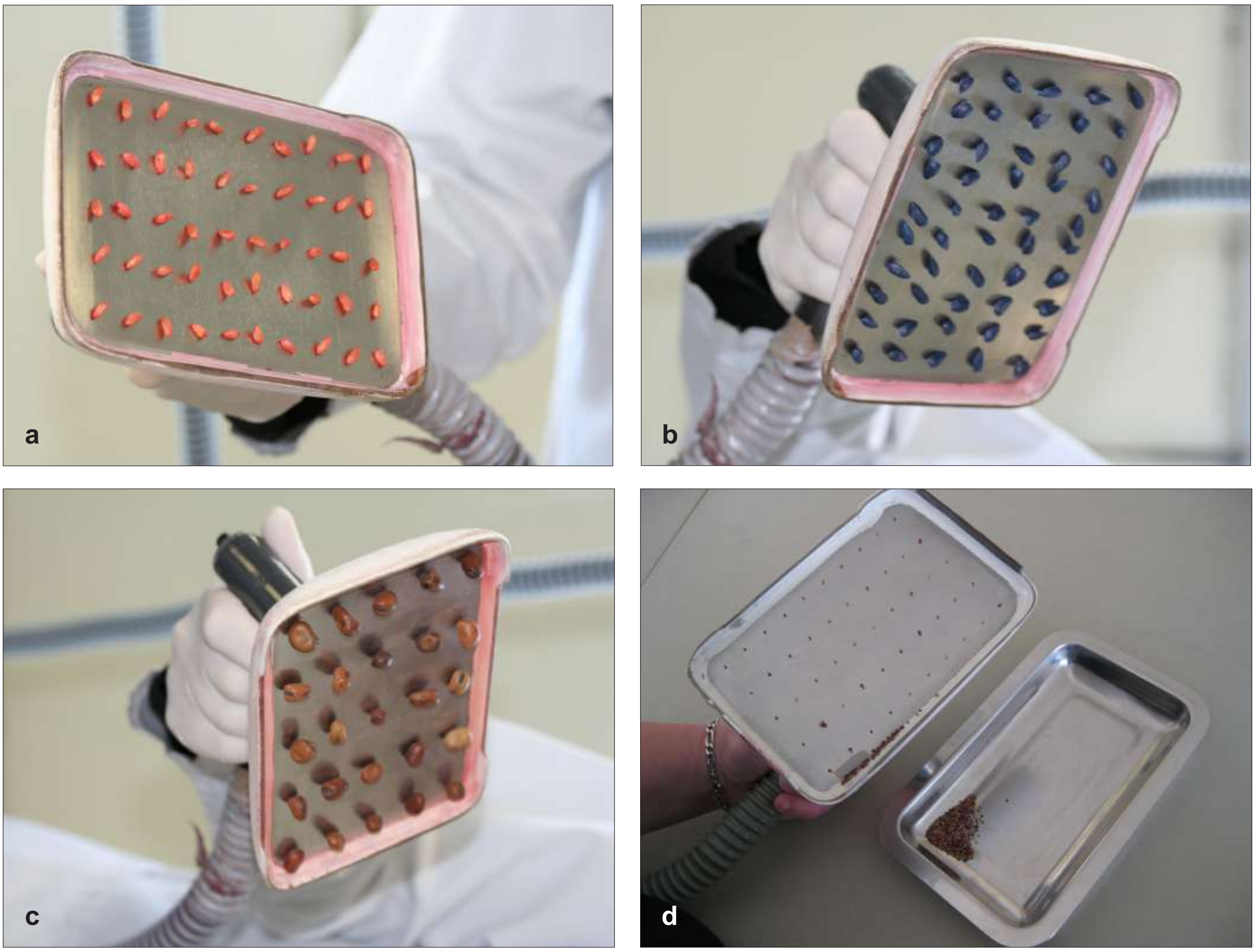


Figure 4.5 Use of vacuum counting heads to deposit seeds onto the substrate: **a** cereal; **b** sunflower; **c** lupine; **d** *Trifolium*.

Description of a suggested method

1. Ten different samples of pure seeds from the same species are used. The sample size should be the size of the whole working sample (2500 seeds) or half the working sample (1250 seeds) from the purity test, in order to perform three germination tests on 400 seeds.
2. 400 seeds are taken from the well-mixed pure seed sample.
3. Replicates of 100 seeds are used, but split replicates of 50 or even 25 seeds may be necessary, depending on the size of the seeds.
4. Seeds are germinated following the conditions prescribed in the ISTA Rules, and results of percentages of normal seedlings obtained from each replicate of 100 seeds are recorded and combined in tests on 400 seeds.
5. Germination results obtained for each test on 400 seeds are checked for their homogeneity by comparing the difference between the highest and the lowest germination percentages to the maximum tolerated range given in Table 5B, Part 1.
6. When the sample size is half the working sample from the purity test (approximately 1250 seeds), germination results obtained on the three different tests of 400 seeds from the same pure seed sample are compared using Table 5D, Part 1.
7. Counting equipment can be used for the germination test when:
   a) a maximum of two tests are out of tolerance (Table 5B, Part 1) from the total of the 30 tests (10 samples × 3 germination tests);
   b) a maximum of one combination of three tests (tests 1, 2 and 3) is out of tolerance (Table 5D, Part 1) from the total of the ten samples tested.

An example is given in Table 4A. In this example, all the germination tests are in tolerance regarding Table 5B, Part 1, but with sample 4, results from tests 1, 2 and 3 are out of tolerance (Table 5D, Part 1). The counting equipment can be used for this species.

Table 4A Example of method for evaluating counting equipment.

Sample	Germination test (4 replicates of 100 seeds)	Normal seedlings (%): result of each test	In or out of tolerance (ISTA Rules Table 5B)	Normal seedlings (%): average result of 3 tests	Tests 1 2 & 3 in or out of tolerance (ISTA Rules Table 5D)
1	Test 1	96	In	97	IN
	Test 2	98	In		
	Test 3	97	In		
2	Test 1	97	In	97	IN
	Test 2	96	In		
	Test 3	98	In		
3	Test 1	93	In	93	IN
	Test 2	94	In		
	Test 3	93	In		
4	Test 1	88	In	92	OUT
	Test 2	95	In		
	Test 3	94	In		
5	Test 1	85	In	84	IN
	Test 2	85	In		
	Test 3	83	In		
6	Test 1	97	In	97	IN
	Test 2	97	In		
	Test 3	96	In		
7	Test 1	95	In	95	IN
	Test 2	94	In		
	Test 3	97	In		
8	Test 1	96	In	96	IN
	Test 2	97	In		
	Test 3	96	In		
9	Test 1	98	In	98	IN
	Test 2	98	In		
	Test 3	98	In		
10	Test 1	98	In	98	IN
	Test 2	98	In		
	Test 3	98	In		

4.1.3 Germination apparatus

In order to provide germinating seeds and seedlings with the conditions of moisture, light and temperature required by the ISTA Rules, the laboratory must be equipped with apparatus providing uniform and controllable conditions such as Copenhagen tables (Jacobsen apparatus), incubators, or germination cabinets or rooms.

4.1.3.1 The Copenhagen table

The Copenhagen table or tank, or Jacobsen apparatus (Figure 4.6), usually consists of a water tank underneath a germination table, which is a stainless steel surface with slits or holes upon which filter paper substrates with seeds are placed. The substrates are kept moist by means of wicks which extend down through the slits or holes into the underlying water. The distance between the substrates and the water level determines the rate of water supply. To prevent drying out, the substrates are covered with bell jars provided with holes, allowing for ventilation without undue evaporation. The temperature is set either indirectly, by heating or cooling the water, or directly, by heating the table, and is usually automatically regulated. The apparatus may be used for all prescribed constant or alternating temperatures.

4.1.3.2 The germination incubator and the room germinator

The incubator is used for germinating seeds in darkness or light, or providing seeds with pretreatments to break dormancy, such as prechilling or preheating. The room germinator is a modification of the incubator, but is large enough to permit workers to enter and place the tests within it. Germination incubators and room germinators are well insulated and are equipped with both heating and cooling systems to ensure the maintenance of the required temperatures. The temperature must be evenly distributed to ensure that all samples placed in the incubator/room have a temperature within the prescribed temperature limits for the test (±2 °C) or pretreatment. If the incubator/room does not have a system capable of providing alternating temperatures, samples can be transferred from one incubator/room to another running at a different temperature to achieve the desired alternative temperature cycle. Tests must be supplied with sufficient water for germination and must not be allowed to dry out. This can be achieved through maintaining a high humidity by using 'wet' incubators or using humidifiers in germination rooms. Tests can also be performed in closed containers.

Figure 4.6 Copenhagen table (**a**) and germination tests (**b**) on filter paper. **c** At lower right, the bell jar and substrate with seeds have been removed to show the wick suspended from the slot into the underlying water.

Section 4: Laboratory conditions for seedling evaluation

Figure 4.7 Room germinator. **a** Humidity is maintained using humidifiers. **b** Humidity is maintained inside each moisture-proof container.

4.2 Growing media

Germination tests, according to the ISTA Rules, are conducted using a growing medium as substrate. The growing medium provides sufficient pore space for air and water, anchorage for the root system and contact with water that is required for plant growth. Growing media used for germination tests are paper, sand and organic growing media.

4.2.1 General specifications for growing media

The following general specifications apply for all growing media and should be controlled as part of a laboratory's quality control procedures.

Water retention: When the appropriate amount of water is added, the particles of the growing medium should have the capacity to hold sufficient water to provide continuous movement of water to the seeds and seedlings, but also provide sufficient pore space for aeration required for optimal germination and root growth. The water content of the growing medium should be adjusted to the maximal water holding capacity. When necessary the water retention can be adjusted to the needs of a particular species, and in such cases it should be expressed as a percentage of the maximum retention.

pH: The growing medium must have a pH value within the range 6.0–7.5 when checked within the substrate.

Conductivity: The growing medium must have a conductivity of no more than 40 millisiemens per metre.

Cleanliness and freedom from toxicity: The growing medium must be free from seeds, fungi, bacteria or toxic substances that could interfere with the germination of seeds, the growth of seedlings or the evaluation of tests.

Appendix 5 lists illustrative standard operating procedures that may be used by laboratories to confirm that the media which they use for germination tests comply with the specifications. These quality control tests can be performed by the seed testing laboratory or laboratories specialising in such tests.

4.2.2 Growing media characteristics

4.2.2.1 Paper growing media

The paper should be wood, cotton or other purified vegetable cellulose. The paper may take the form of filter papers, blotters or towels. The paper should be such that:

- the roots of seedlings grow on and not into the paper;
- it possesses sufficient strength to enable it to resist tearing when handled during germination tests.

4.2.2.2 Sand growing media

The sand should be reasonably uniform and free from very small and large particles. Round particles are preferable, and it is recommended that sand with sharp particles that may impair seedling development is avoided. It is recommended that at least 90 % of the particles should pass through a sieve having holes or meshes of 2.0 mm width.

4.2.2.3 Organic growing media

These are mixtures of organic compounds and mineral particles that are reasonably uniform and free from very small and large particles. The organic compounds can be fibres such as peat, coconut fibres or wood fibres, with a size less than 5 mm. The proportion of mineral particles such as sand, perlite and vermiculite should be between 15 and 30 % in terms of volume. It is recommended that 90 % of the organic growing media particles should pass through a sieve with holes or a mesh of 3.0 mm width.

4.2.3 Methods using paper

Top of paper (TP): The seeds are placed on top of one or more layers of moist paper that are placed: on the Copenhagen tank apparatus; into transparent boxes in an incubator or in a germination cabinet or room.

Between paper (BP): The seeds are placed between two layers of moist paper (for example, under a cover of filter paper, in folded envelopes or in rolled towels). These are placed: on the Copenhagen tank apparatus; into boxes in an incubator or in a germination cabinet or room.

Pleated paper (PP): The seeds are placed in a pleated accordion-like, moist paper strip. In some cases, the pleated paper has water added after planting the seeds. The pleated paper is used in different ways. It can be placed on one or several layers of filters or a flat strip of paper is wrapped around the pleats. The method used depends on the species tested. Completed strips are kept in boxes within an incubator or germination cabinet or room. This method can be used as an alternative where TP or BP methods are prescribed.

4.2.4 Methods using sand or organic growing media

Top of sand (TS), top of organic growing medium (TO): The seeds are pressed into the surface of the moist sand or the organic growing medium.

Sand (S) or organic growing medium (O): The seeds are placed on a level layer of moist sand or organic growing medium and covered with 10–20 mm of uncompressed substrate, depending on the size of seed. To ensure good aeration it is recommended that the bottom layer may be loosened by raking before sowing. Sand and organic growing media tests are placed in an incubator or in a germination cabinet or room.

Sand or organic growing media may be used instead of paper, even if not prescribed in the ISTA Rules:

- when the evaluation of a diseased sample proves impracticable because of the spread of infection between seeds and seedlings on paper substrate;
- when seedlings show phytotoxic symptoms; and
- for investigational purposes and to confirm evaluation of seedlings in cases of doubt.

4.2.5 Soil

Soil is not recommended as a primary substrate. However, it may be used as an alternative to organic growing media when seedlings show phytotoxic symptoms or if evaluation of seedlings is in doubt on paper or sand. If soil is used it must meet the growing media specifications given in 4.2.1.

4.2.6 Effect of growing media on seedling appearance

The substrate can have a significant effect on the appearance of the resultant seedlings:

Top of paper and pleated paper: seedlings are upright, and their essential parts are usually well developed.

Between paper: seedlings may become long and thin, and growth may be distorted in the restrictions imposed by paper envelope or rolled towels.

Organic growing media, sand and soil: seedling development is more advanced than those of the same age that have been grown in rolled towels.

4.3 Moisture

The ISTA Rules prescribe that the substrate must at all times contain sufficient moisture to meet the requirements of germination. Sufficient moisture depends on the species being tested. *Trifolium pratense* and *Pinus sylvestris* are sensitive to excessive moisture of the substrate. The same is true of species with very small seeds, such as *Begonia*, *Kalanchoe* and *Nicotiana*. If the germination substrate is too wet, such species produce weak, glassy looking seedlings, and the root tips of seedlings may become brown and decayed. Other species, such as *Pinus palustris*, require positively wet conditions for normal germination, and if sufficient water is not available, the primary root and hypocotyl curl and growth is hampered.

4.4 Light

Seeds of most species will germinate either in light or in darkness. However, except for a few species where light may be inhibitory to the germination (for example *Phacelia tanacetifolia* and *Trifolium subterraneum*), illumination of the substrate from an artificial source or by indirect daylight is generally recommended. This leads to better developed seedlings that are more easily evaluated.

The effect of light on the development and appearance of seedlings is greater than that of substrate. Light (artificial or natural diffuse light)[1] inhibits excessive elongation, promotes the formation of chlorophyll and gives seedlings a natural look. Grown in continuous darkness, seedlings remain pale and they become thin and etiolated. They become susceptible to saprophytic fungal attack, and it is difficult to distinguish albino[2] seedlings. Once germinated, growth in light facilitates the correct evaluation of the seedlings and is strongly recommended.

4.5 Temperature

The speed of germination and seedling development are dependent on temperature. The temperatures appropriate for optimal germination of each species are prescribed in Table 5A of the ISTA Rules. Temperatures should be as uniform as possible throughout the germination apparatus, cabinet or room, and the variation in temperature of the substrate from the prescribed temperature should not be more than ±2 °C.

Temperature plays an important role in the germination laboratory, and its measurement and control must be covered by a laboratory quality control system. Appendix 5 contains an illustrative standard operating procedure that can be used by laboratories as a guide to develop their own procedures and arrangements for temperature measurement and control.

4.6 Pretreatment of the seed

The seed testing objective of the germination test is to determine the maximum germination potential of a seed lot, which can then in turn be used to compare the quality of different lots and also estimate the field planting value.

To meet this objective, it is important that as many seeds as possible germinate and that the seedlings can be evaluated within the test period.

There are several reasons why seeds, though exposed to favourable germination conditions, will not germinate. The most important ones are seed dormancy (Sections 2.9 and 2.10) and hardseededness. These obstacles to germination may be removed by various pretreatments.

4.6.1 Breaking physiological dormancy

Many seeds are in a state of physiological dormancy, i.e. a blocked development of the embryo which prevents germination, even if the external conditions are favourable. The ISTA Rules permit various treatments that promote germination by breaking dormancy. The most frequently used are:

Pretreatments
- preheating
- prechilling
- prewashing
- removal of seed structures

Treatments during the germination test
- KNO_3
- GA_3
- sealed polythene envelopes
- alternating temperature
- light

Preheating: Before planting, seeds are exposed to temperatures of 30–35 °C, for up to seven days. For certain tropical and subtropical species, temperatures of 40–50 °C are recommended (for example, *Arachis hypogaea*: 40 °C; *Oryza sativa*: 50 °C).

Prechilling: Before exposing the seeds to the prescribed germination temperature they are kept on the moist substrate at a low temperature (usually between 5 °C and 10 °C) for an initial period of up to seven days. Tree and shrub seeds are prechilled at a temperature between 1 °C and 5 °C, for two weeks to a year, depending on the species.

Prewashing: Naturally occurring inhibitors in seed such as *Beta vulgaris* may be removed by washing the seed in running water at a temperature of 25 ±2 °C. After washing, the seed is dried back at a maximum temperature of 25 ±2 °C before planting on or in the germination substrate.

Removal of seed structures: For certain species of Poaceae, germination can be promoted by removal of outer structures such as involucres (rings of bracts or bristles surrounding the base of an inflorescence) of bristles and the lemma and palea.

Using potassium nitrate (KNO_3): KNO_3 is mainly used to break the dormancy of grasses and *Hordeum vulgare*. A 0.2 % solution, instead of water, is used to moisten the substrate. The KNO_3 solution should be stored in closed containers and kept in the dark, at room temperature and should be used within 3 months from preparation.

1 Care must be taken regarding the wavelength and intensity of the light, as both could have a detrimental effect on germination. High-intensity light could also have an effect on the temperature of the substrate.

2 A genetic abnormality leading to white or yellow seedlings.

Using gibberellic acid (GA_3): GA_3 is mainly used to break the dormancy of temperate cereals, and a 0.02 % to 0.1 % (200–1000 ppm) solution of GA_3, instead of water, is used to moisten the substrate. The GA_3 solution should be stored in closed containers, kept in the dark, at a maximum of 5 °C, and should be used within a week from preparation.

Sealed polythene envelopes: Seed of some *Trifolium* species are germinated in sealed polythene envelopes. Figure 4.8 illustrates a method that can be used to test *Trifolium repens* in sealed polythene envelopes.

Alternating temperatures: The seed is germinated using an alternating temperature regime, with the high-temperature phase having a duration of 8 hours and the low-temperature one, 16 hours.

Light: When light is used as a dormancy-breaking stimulus, it should be of sufficient intensity and suitable wavelength. Tests should be illuminated for at least 8 hours in every 24-hour cycle, and this should coincide with the high temperature phase if an alternating temperature regime is used.

4.6.2 Removing hardseededness

Hardseededness is the structural impermeability of the seed coat to water and gases. It is a widespread phenomenon in species of the Fabaceae and other plant families. For many species, no attempt is made to germinate such seeds remaining at the end of the test period, and they are reported as 'hard seeds'.

Where a fuller assessment is required, special measures must be taken. These measures may be applied **prior** to the start of the germination test, or if it is suspected that the treatment may adversely affect non-hard seeds, it should be carried out on the **hard seeds remaining** after the prescribed test period. In the latter case, the seeds are returned to the seedbed after treatment.

Three different methods can be used:

Soaking the seeds in water: The seeds are soaked in water for up to 24–48 hours. The germination test is started immediately after soaking.

Mechanical scarification: Careful piercing, chipping, filing or sand papering of the seed coat may make the seed permeable to water. Care must be taken to scarify the seed coat where damage to the embryo and resultant seedling is avoided. The most suitable site is immediately above the tips of the cotyledons and as far away from the base of the radicle as possible.

Acid scarification: Digestion of the seed coat in concentrated sulphuric acid is effective in some species such as *Brachiaria* sp. and *Macroptilium* sp. The seeds are soaked until the seed coat becomes pitted. Before the start of the germination test the seed must be thoroughly washed in running water.

4.6.3 Disinfection of the seed

To reduce the effect of seed-borne fungi and to promote normal germination the ISTA Rules permit fungicidal treatment of samples of *Arachis hypogaea* and *Beta vulgaris* only, before planting seed for germination. When a fungicide pre-treatment is used, the name of the chemical, the percentage of active ingredients and the method of treatment must be reported.

Fungicide treatments exist in two different forms: liquid or powder. Before applying the fungicide to the seed, it is necessary to know the amount of product that can be applied to the seed. Usually, this information can be found in commercial technical sheet related to the chemical product or in different guides edited at national level. This concentration depends on the seed species. The treatment must be spread homogeneously in the sample.

4.6.3.1 Preparation of the fungicide and the seeds

Depending on the application rate, seeds have to be counted or weighed and the quantity of fungicide has to be measured either in volume (liquid form) or in weight (powder form).

4.6.3.2 Application of the fungicide to the seeds

When the fungicide treatment is liquid, then the adapted volume of fungicide is added to the seeds in a closed container and gently agitated to allow an efficient seed treatment application without altering the germination due to a too long agitation period. The quantity of the solutions has to be sufficient for getting an even distribution of the product over all the seeds. A too low quantity will result in an uneven distribution of the product. Duration depends on the type of seeds. Seeds should be dried back at a maximum temperature of 25 ±2 °C before planting on or in the germination substrate.

When the fungicide treatment is in powder, then the adapted weight of fungicide is added to the seeds placed in a closed container or in a closed paper packet. The container or paper packet is gently stirred so that the fungicide product can be applied correctly to the seeds.

4.6.3.3 Application of the fungicide to the growing media

Another possible method used in laboratories consists in immersing paper substrate in a fungicide solution with a known concentration. After saturation or preparation of a specific volume of solution, the paper can be used for seed germination.

Figure 4.8 a For each replicate, two filter papers are moistened with water and then the seeds are planted on top. Replicates of 50 seeds are planted for this species so they are easier to evaluate.

Figure 4.8 b The planted seed are then covered with a moistened thin filter paper.

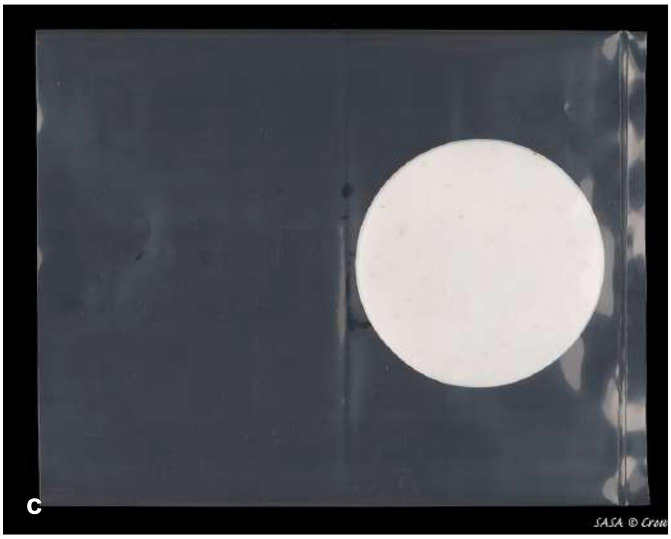


Figure 4.8 c The planted replicate is then put into a polybag. The bag size used in this example is 175 × 225 mm (the bag should be big enough to hold the filter papers without letting them touch the edges).

Figure 4.8 d The polybags are sealed using a bag sealer.

Figure 4.8 e The bag is sealed (the polybag melts so it sticks together – take care not to make holes).

Figure 4.8 f The sample is then placed in a germinator with test conditions according to the ISTA Rules.

Section 4: Laboratory conditions for seedling evaluation

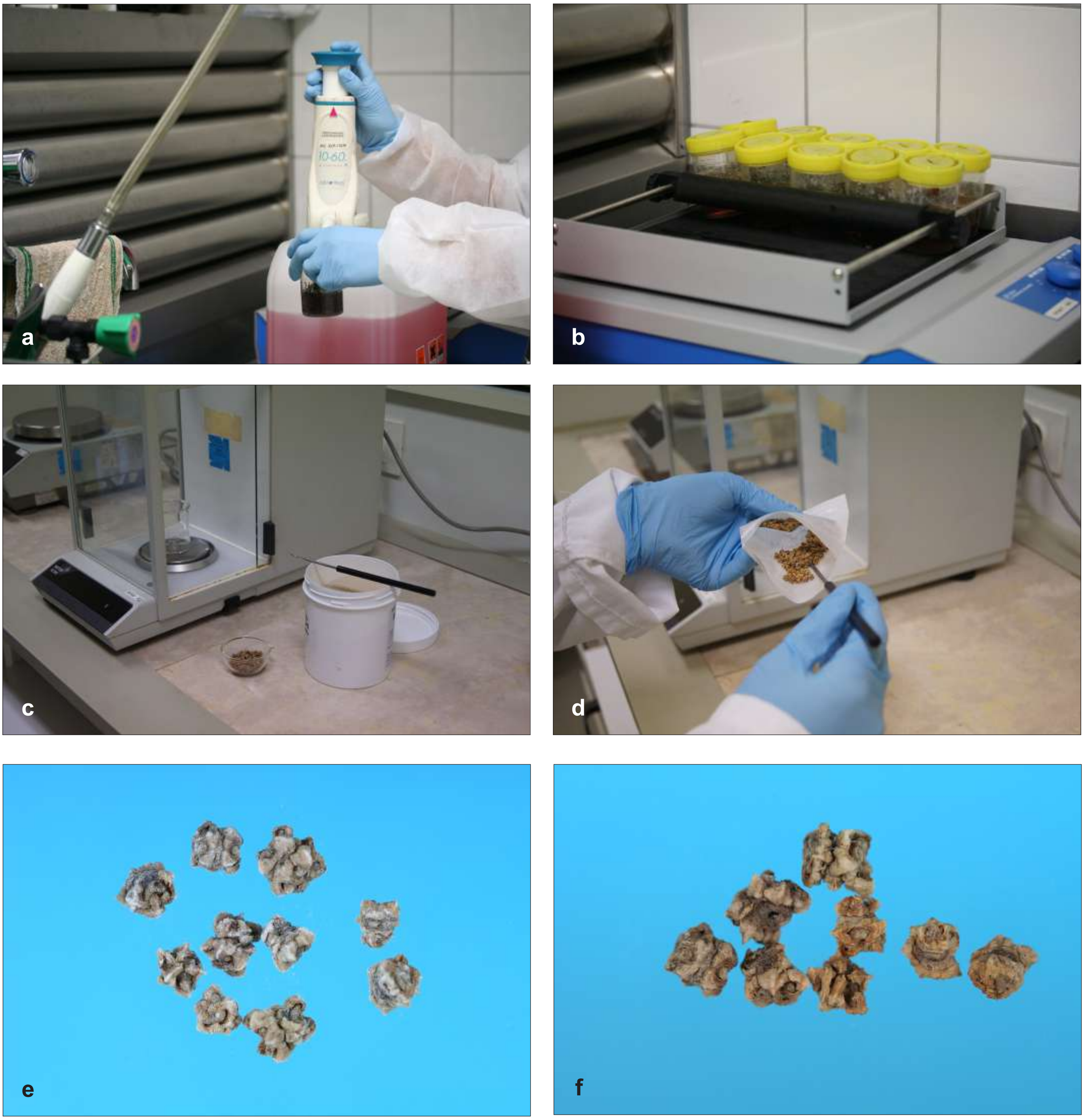


Figure 4.9 Addition of fungicide treatment to seeds (**a**). Gentle agitation of seeds with fungicide liquid treatment, using an orbital agitator (**b**). Seeds and chemical treatment (in powder form) are weighed before stirring in a beaker (**c**). Fungicide treatment can also be added to seeds directly into a paper packet before mixing (**d**). Seeds of *Beta vulgaris* before (**e**) and after (**f**) fungicide treatment.

Section 4: Laboratory conditions for seedling evaluation

4.6.4 Assessment of fresh seed

Guidance on the assessment of fresh seed at the end of a germination test is given in Figure 4.9.

Visual/physical attributes of categories of ungerminated seed

Hard seed: seeds that have not imbibed water and remain firm and hard.
Fresh seed: seeds that have imbibed water; they are clean and turgid and have the potential to germinate once dormancy is broken.
Dead seed: seeds that have imbibed water; they are soft and often discoloured and mouldy.

Note: Even with levels of fresh seed less than 5 %, the customer can request that the viability of these seeds is verified, even though this is not a requirement of the test.

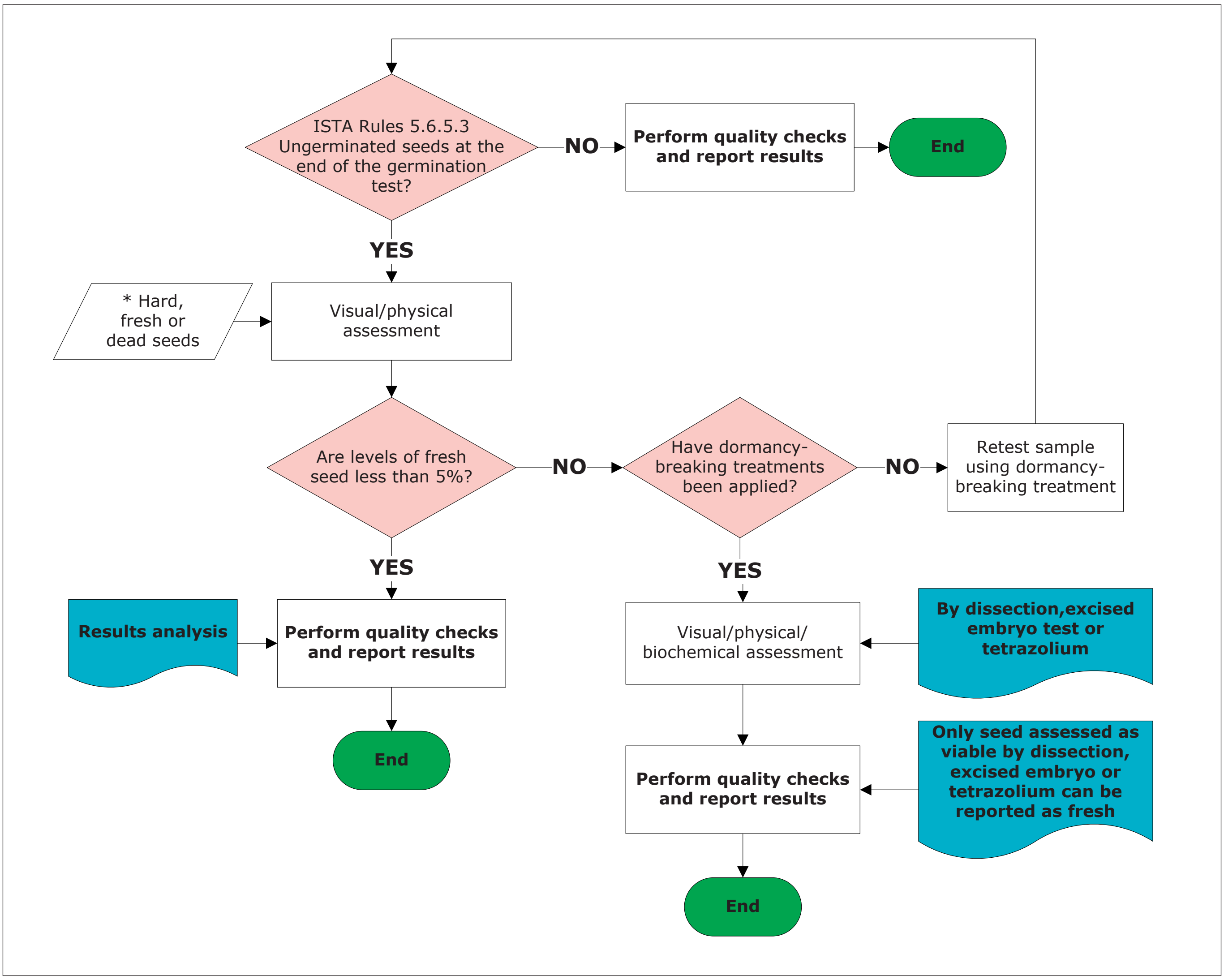


Figure 4.10 The assessment of fresh seed at the end of a germination test.

Section 5: Seedling structure

A seed in a strict botanical sense is derived from an ovule and consists of:
- the seed coat (testa);
- the embryo; and
- nutritive tissue.

Frequently, however, the unit tested in the laboratory and commonly termed 'seed', also contains accessory structures, such as the fruit wall (pericarp) or remains of floral structures (e.g. the cluster of beet or the glumes of forage grasses). In seed testing and in this Handbook no distinction is made between seed in the botanical sense and units retaining additional structures. All are called seeds and details of the formation and structure of different types of seeds and fruits are given in Section 1.

When an embryo has gone through the processes of germination and breaks the seed coat, it is called a 'seedling', which is the start of the plants' life cycle. Section 1 gives details of the plants' life cycle and the germination process. All seedlings have:
- a **root system**, which grows down into the soil;
- a **seedling axis** with the **terminal bud**, which grows up towards the light; and
- one, two or several **cotyledons** as lateral appendages to the seedling axis.

5.1 Root system

The main function of the root system is to grow down into the soil where:
- it anchors the plant in the soil;
- it absorbs water and dissolved nutrients from the soil; and
- it conducts these to the cotyledons and the terminal bud.

Roots may additionally be specialised to store water or food reserves.

In most seedling types the root systems look similar and develop in a similar way. At first, the primary root emerges from the seed coat, and eventually secondary roots develop on it. In a small number of species (mainly cereals) seedlings have a seminal root system. Instead of a single primary root a specific number of about equal sized seminal roots[1] appear nearly simultaneously.

5.1.1 Primary root

As soon as the embryonic root (radicle) pierces the seed coat at the beginning of germination, it is called the 'primary root'. It is usually white or translucent (red or yellow tinged primary roots occasionally occur), slender and straight and it rapidly elongates. Numerous root hairs[2] increase its absorbing surface.

5.1.2 Secondary roots

At a later stage of development secondary roots are formed. How soon this takes place, depends on the genetic and morphological make-up of the seedling.

In seed testing the term 'secondary roots' is used as a collective term for all types of roots other than the primary root. This includes:
- **lateral** roots, arising from the primary or any other root; and
- **adventitious** roots, arising from any structure other than a root (e.g. hypocotyl).

5.2 Seedling axis

The main function of the seedling axis is to grow up into the air and towards the light where:
- it conducts water and dissolved nutrients from the root to all aerial parts of the seedling;
- it bears the cotyledons and/or primary leaves, i.e. the first structures where photosynthesis takes place; and
- it transports carbohydrates, the product of photosynthesis, to all parts of the seedling.

The seedling axis consists of:
- the hypocotyl;
- the epicotyl; and
- the terminal bud.

5.2.1 Hypocotyl

The part of the seedling axis between the primary root and the point of attachment of the cotyledons is called the 'hypocotyl'. The transition of the primary root to the hypocotyl is often unclear, but may be recognised by the absence of root hairs on the hypocotyl. The conducting tissue of the hypocotyl forms a vital link between the primary root, below, and the cotyledons and the growing terminal bud, above, in the transfer of water and nutrients.

1 The few genera with a seminal root system are dealt with in the appropriate Seedling Group in Section 6.
2 Root hairs are not present on the primary roots of the conifers.

Seedlings with epigeal[3] germination usually show an elongated hypocotyl. Elongation of the hypocotyl draws the cotyledons out of the seed coat and above the soil. In seedlings with hypogeal germination the hypocotyl does not elongate; it usually can hardly be recognised.

5.2.2 Epicotyl

The part of the seedling axis between the point of attachment of the cotyledons and that of the primary leaves is known as the 'epicotyl'. In the majority of seedlings with epigeal germination the epicotyl only elongates at a later stage of development; therefore it cannot be recognised during the ordinary test period. Seedlings of Type F (e.g. *Phaseolus* and *Arachis*) that have epigeal germination with epicotyl elongation are exceptions. In seedlings with hypogeal germination, the epicotyl elongates and carries the seedling axis with the primary leaves above soil surface.

5.2.3 Stem

The axis of a young plant above the primary leaves is called the 'stem' and is not subdivided. At a later stage of development the stem will branch and lateral shoots with foliage leaves will be produced. This, however, takes place in the period well beyond that of an ordinary germination test.

5.2.4 Mesocotyl[4]

The 'mesocotyl' is part of the seedling axis specific to the monocotyledons and particularly to the Poaceae. It is found between the scutellum and the coleoptile and may elongate considerably, depending on species and germination conditions. Elongation of the mesocotyl helps to move the coleoptile with the leaf above the soil.

5.2.5 Terminal bud

The upper end of the seedling axis is called the 'terminal bud'. It contains the main growing point of the seedling (meristem) with leaf initials and more or less differentiated young leaves enveloping it. In seedlings with hypogeal germination the terminal bud is generally free and visible. In most seedlings with epigeal germination it is not visible, being hidden between the cotyledons, which are often closely joined at their base.

5.2.6 Cotyledon(s)

Depending on the systematic class the seedling may contain one, two or more cotyledons. The main functions of cotyledon(s) are:

- acting as a storage structure for nutrients;
- sustaining the young seedling by providing it with energy, through the absorption of nutrients from the endosperm;
- acting as the first photosynthetic structure of seedlings with epigeal germination;
- protecting the terminal bud.

Not all of these functions are usually realised in any one Seedling Type.

5.2.7 Dicotyledons

In seedlings with epigeal germination the two cotyledons show the characteristics of leaves. They are flat, expanded and green and represent the first photosynthetic structures of the young seedling (Seedling Type E, e.g. *Brassica*). After emerging from the seed coat they often increase their size considerably and keep photosynthesising far beyond the seedling stage (e.g. in *Cucurbita*). The cotyledons may be borne on stalks or be sessile on the seedling axis. Cotyledons are generally of much simpler shape than the primary or later foliage leaves.

The cotyledons of seedlings with hypogeal germination do not become photosynthetic remaining in the seed coat in the soil (Seedling Type G, e.g. *Pisum*). They are exclusively nutrient storage structures.

3 See Section 2 for a detailed description of epigeal and hypogeal germination.
4 Morphologically the mesocotyl is a compound structure, formed by addition of part of the cotyledon to the hypocotyl.

5.2.8 Monocotyledons

The cotyledon of monocotyledons is the seedling structure that has been most variably modified and transformed during evolution, often to such an extent, that the structure can hardly be recognised as a cotyledon.

In the comparatively few species with epigeal germination (Seedling Type A, e.g. *Allium*) the cotyledon becomes green and photosynthesises. In addition, the cotyledon tip remains in the seed coat to absorb nutrients from the endosperm.

Most monocotyledons show hypogeal germination and in many species there are two parts to the cotyledon with two different functions:

- The upper part where the cotyledon's tip serves as an structure to absorb nutrients from the endosperm; it remains in the seed coat.
- The lower part of the cotyledon emerges slightly from the seed coat. It has a sheath-like form and encloses the terminal bud (Seedling Types B and C, e.g. *Freesia* and *Asparagus*).

The cotyledon of the Poaceae has undergone further steps of extreme transformation. It consists of two apparently separated parts with two functions (Seedling Type D, e.g. *Zea*):

- The **scutellum** corresponds to the upper part of the cotyledon. It represents a much enlarged cotyledon tip and remains in close contact with the endosperm to absorb nutrients and to make them available to the growing embryo and young seedling.
- The **coleoptile** corresponds to the lower part of the cotyledon; it forms a closed sheath or tube with the terminal bud at its base. The primary leaf, produced by the terminal bud, grows through this tube and eventually emerges through its tip.

5.2.9 Cotyledons of the conifers

All conifer species presently tested in seed laboratories exhibit epigeal germination. Their cotyledons are green, long and narrow and their number varies with the genus from two (rarely) up to 18. They are borne as a whirl on top of the hypocotyl, the terminal bud lying in the centre (Seedling Type H, e.g. *Pinus*).

5.3 Primary leaf (leaves)

The first leaf, or pair of leaves, above the cotyledon(s), is (are) called the 'primary leaf (leaves)'. Depending on the species of the seedling, there is one primary leaf in genera with alternating leaf position or a pair of primary leaves, in genera with opposite leaf position.

There are only a comparatively small number of seedlings (mainly of the Seedling Types F and G, e.g. *Phaseolus* and *Pisum*), which develop their primary leaves within the test period. Most of the seedlings dealt with in seed testing do not produce their primary leaves until after the test period.

The shapes of primary leaves are generally between the very simple-shaped cotyledons and the more characteristic foliage leaves that develop at a later stage.

5.4 Secondary leaves (foliage leaves)

The foliage leaves are those parts of the plant commonly called 'leaves'. Photosynthesis is their major function and they are of no significance to the germination test since they do not develop during the test period.

Section 6: Seedling development under natural conditions

Under natural conditions the germinating seed lies in the soil. The root system grows away from the seed coat more or less perpendicularly into the soil. On the other hand, the seedling axis with its terminal bud rises through the soil to the light. How this occurs depends on the morphology of the seed and embryo with their specific control systems. To achieve the aim of reaching the light quickly, so that seedling photosynthesis can start, various parts of the embryo and young seedling, such as cotyledon, hypocotyl and epicotyl, elongate and/or bow and/or turn in the course of germination.

Seed testing deals with eight morphologically distinct types of seedlings.

6.1 Development of the seedlings of Type A

Seedling Type A comprises seedlings of **MONOCOTYLEDONS** with **EPIGEAL** germination. The seedling part that grows towards the light and becomes **GREEN** is the **COTYLEDON**.

Representative genus: ***Allium***

The embryo in the mature seed is embedded in the endosperm. The cylindrical embryo consists of a short radicle and a long, sometimes coiled cylindrical cotyledon with the plumule enclosed at its base.

At the start of germination the primary root pierces the seed coat; it quickly elongates and establishes the seedling in the soil. At the same time the cotyledon also elongates. Since it tends to grow upward, but is fixed between root and seed coat, it forms a bend or '**knee**' that aids its passage through the soil. The seed coat may emerge from the soil with the cotyledon, but frequently the seed coat together with the cotyledon tip is fixed in the soil. The tip of the cotyledon remains in the seed coat for some time and absorbs nutrients from the endosperm.

The terminal bud is enclosed by the sheath-like base of the cotyledon at the end of a much stunted seedling axis. Later the primary leaf emerges there through a fine opening just above the hypocotyl. The cotyledon remains green until the primary leaf has developed and takes over photosynthesis.

6.2 Development of the seedlings of Type B

Seedling Type B comprises seedlings of **MONOCOTYLEDONS** with **HYPOGEAL** germination. The seedling part that grows towards the light and turns **GREEN** is the **PRIMARY LEAF**.

Representative genus: ***Freesia***

The embryo in the mature seed is little developed: it mainly consists of a short, thick cotyledon. Neither the radicle nor the plumule, which lies at the base of the cotyledon, can be observed.

At the start of germination the primary root pierces the seed coat; it quickly elongates and establishes the seedling in the soil. There is little elongation of the cotyledon. Only the lowest, sheath-like part emerges from the seed coat and it remains in the soil. Most of the cotyledon remains enclosed in the seed coat and absorbs nutrients from the endosperm.

The terminal bud is enclosed by the sheath-like basal part of the cotyledon positioned at the end of a much stunted seedling axis. During germination it increases in size and opens. The primary leaf emerges through an opening in the cotyledon sheath and grows towards the light.

6.3 Development of the seedlings of Type C

Seedling Type C comprises seedlings of **MONOCOTYLEDONS** with **HYPOGEAL** germination. The seedling part that grows towards the light and turns **GREEN** is the **EPICOTYL** with the **TERMINAL BUD**.

Representative genus: ***Asparagus***

The embryo in the mature seed is little developed: it mainly consists of a short, thick cotyledon. Neither the radicle nor the plumule, which lies at the base of the cotyledon, can be observed.

At the start of germination the primary root pierces the seed coat; it quickly elongates and establishes the seedling in the soil. There is little elongation of the cotyledon. Only the lowest, sheath-like part emerges from the seed coat and it remains in the soil. Most of the cotyledon remains enclosed in the seed coat and absorbs nutrients from the endosperm.

During germination the sheath-like basal part of the cotyledon increases in size and opens. The terminal bud at the end of the elongating epicotyl emerges through the opening and grows towards the light.

6.4 Development of the seedlings of Type D

Seedling Type D comprises seedlings of **MONOCOTYLEDONS** with **HYPOGEAL** germination. The seedling part that grows towards the light and turns **GREEN** is the **PRIMARY LEAF**. It is usually enclosed in a transparent sheath called the **COLEOPTILE**.

Representative family: Poaceae

The embryo is situated at one end of the caryopsis[1]. In some genera it is comparatively well differentiated and both the radicle and the plumule are discernible. As with Seedling Types B and C, there are two parts to the cotyledon with different functions. The upper much enlarged part, the scutellum, remains in close contact with the endosperm and adsorbs nutrients from it. The lower, sheath-like part, the coleoptile encloses the terminal bud at its base. Together with the developing primary leaf, the coleoptile elongates, so that the leaf emerges from the soil under the cover of the coleoptile. The primary leaf emerges from the tip of the coleoptile during the development of the seedling.

6.5 Development of the seedlings of Type E

Seedling Type E comprises seedlings of **DICOTYLEDONS** with **EPIGEAL** germination. The seedling part that grows towards the light is the **HYPOCOTYL** with **TWO COTYLEDONS** attached that turn **GREEN**.

Representative genus: *Brassica*

Size, position and degree of differentiation of the embryo in the mature seed are very varied, depending on plant family or genus. However, a well differentiated embryo consists of the embryo axis with the two growing points, the radicle and the plumule, and two cotyledons attached to the embryo axis.

At the start of germination the primary root pierces the seed coat; it quickly elongates and establishes the seedling in the soil. At the same time the hypocotyl elongates. The shoot system is fixed between the primary root and the seed coat that is held in the soil. To reach the surface the hypocotyl forms an arch and this helps its passage through the soil. The seed coat may emerge from the soil with the cotyledons, but frequently the seed coat is retained in the soil, and the cotyledons are freed by means of further elongation of the hypocotyl.

In the light the hypocotyl straightens; the cotyledons expand and discard the seed coat, if it is still in place. The increase in size of the cotyledons may be considerable. The cotyledons quickly turn green and provide the seedling with energy through photosynthesis until the primary leaves replace them.

6.6 Development of the seedlings of Type F

Seedling Type F comprises seedlings of **DICOTYLEDONS** with **EPIGEAL** germination. The seedling part that grows towards the light and turns **GREEN** is the **HYPOCOTYL** with **TWO COTYLEDONS** and the **EPICOTYL** with the **PRIMARY LEAVES**.

Representative genus: *Phaseolus*

The embryo in the mature seed is well differentiated. There is a clear embryo axis with the radicle at one end and the plumule at the other. Primary leaves that are more or less fully formed enclose the meristem of the plumule. The cotyledons are attached to the embryo axis and enclose it. They usually completely fill up the seed coat.

At the start of germination the primary root pierces the seed coat; it quickly elongates and establishes the seedling in the soil. At the same time the hypocotyl elongates. The hypocotyl is fixed between the primary root and the seed coat, which is held in the soil. To reach the surface it forms a bend and this helps it push itself up through the soil. The seed coat may emerge from the soil with the cotyledons, but frequently the seed coat is retained in the soil, and the cotyledons are freed by means of further elongation of the hypocotyl.

In the light the hypocotyl straightens; the cotyledons quickly turn green, expand and discard the seed coat, if it is still in place. The increase in area of the cotyledons can be considerable depending on genus, but they can remain small and hardly turn green. In such cases they are discarded from the seedling at an early stage.

At an early seedling stage the epicotyl elongates and the terminal bud produces primary leaves, which quickly enlarge, turn green and take up photosynthesis.

1 Caryopsis: the one-seeded fruit or grain of Poaceae; the ovary wall is fused with the seed coat.

6.7 Development of the seedlings of Type G

Seedling Type G comprises seedlings of **DICOTYLEDONS** with **HYPOGEAL** germination. The seedling part that grows towards the light and turns **GREEN** is the **EPICOTYL** with the **PRIMARY LEAVES**.

Representative genus: *Pisum*

The embryo in the mature seed is well differentiated, with a clear embryo axis that has a radicle at one end and a plumule at the other. More or less fully formed primary leaves enclose the meristem of the plumule. The cotyledons are attached to the embryo axis and enclose it. They usually completely fill up the seed coat.

At the start of germination the primary root pierces the seed coat; it quickly elongates and establishes the seedling in the soil. At the same time the epicotyl elongates. A small bend is formed and this eases its passage through the soil. Since the hypocotyl does not elongate, the cotyledons with the seed coat remain in the soil.

In the light the epicotyl straightens. The primary leaf (or pair of leaves) turns green, expands and starts photosynthesising.

6.8 Development of the seedlings of Type H

Seedling Type H comprises seedlings of the **CONIFERS**; they show **EPIGEAL** germination. The seedling part that grows towards the light and turns **GREEN** is the **HYPOCOTYL** with the **COTYLEDONS**.

Representative genus: *Pinus*

The embryo in the mature seed is well differentiated; the radicle and the comparatively large cotyledons are clearly discernible.

At the start of germination the primary root pierces the seed coat; it quickly elongates and establishes the seedling in the soil. At the same time the hypocotyl elongates. The hypocotyl is fixed between the primary root and the seed coat, which is held in the soil. To reach the surface the hypocotyl forms a bend, which helps it push itself through the soil. The seed coat may emerge from the soil with the cotyledons, but frequently it is retained in the soil, and the cotyledons are freed by means of further elongation of the hypocotyl.

In the light the hypocotyl straightens; the cotyledons turn green, elongate and discard the seed coat, if it is still in place. They form a whorl in the centre of which the terminal bud and later the primary leaves are visible. The cotyledons photosynthesise and provide the seedling with energy for an extended period.

6.9 Seedling development in the laboratory

The development of the eight seedling types refers to germination in the field. The development of seedlings under laboratory conditions can be different, especially when paper substrates (TP, BP, and PP) are used. These differences, however, are unlikely to cause any difficulty in seedling evaluation but it is important to be aware of them.

- The primary root tends to grow downward into the soil. If the seeds lie on the paper, the primary root is forced to grow horizontally along the paper[2].
- In soil the shoot system of the seedlings of Types E and F are fixed between the primary root and the seed coat that is held in the soil. To reach the surface the hypocotyl forms an arch and this helps its passage through the soil. In the laboratory the shoot system is free to grow upward. It does so and lifts the seed coat, which is discarded later on. The arching of the seedling axis, which is a characteristic of germination under natural conditions, is not observed under laboratory conditions[3].
- The light provided for germination in the laboratory seldom corresponds with the natural daylight experienced by seedlings grown outdoors. In addition, the germination temperatures in the laboratory are usually higher than those found under natural conditions. Both these factors initiate strong elongation of the seedling axis in the laboratory. This results in seedling proportions that are different from those found in seedlings germinated outdoors.

2 Growth of the primary roots downward into the paper substrate is not desirable in seed testing, since they may easily break, when the seedling is removed from the paper.

3 In *Allium* the arch is innate occurring in the laboratory as well as the field and is known as the ‘knee’.

Section 7: Evaluation of seedlings of particular genera

Whether a seedling, grown in the laboratory under controlled and standardised conditions, is normal, i.e. would be capable of developing into a satisfactory plant, if grown under favourable conditions in the field, is decided by evaluating it. Each of the individual essential structures that have developed during the prescribed test period must be examined and evaluated. In addition, the seedling as a whole must be evaluated.

The seedlings of different genera have different morphologies and not all the essential structures described in *Section 6: Seedling development under natural conditions* are present in the seedlings of all species. In addition, some of the structures may not develop within the prescribed test period. A description of the seedling groups, and the criteria of their evaluation, according to **systematic families** is not possible. Whilst seedlings of most of the dicotyledon families germinate according to the same pattern, seedlings of species in the Fabaceae show different patterns. In the monocotyledons, most species exhibit hypogeal germination but some of the Liliaceae exhibit an epigeal germination pattern. For this reason seedlings or their genera[1] are arranged into groups that have similar morphologies and are evaluated using similar criteria. The types of seedlings are therefore divided into groups to which all genera are assigned. These groups will have reached a similar stage of development during the test period.

7.1 Criteria for assignment of genera to categories and groups

In this Handbook genera have been classified into two **categories**:

- **Agricultural and horticultural species**, represented by the letter **A**; and
- **Trees and shrubs**, represented by the letter **B**.

Categories **A** and **B** are then sub-divided into **groups** according to the following criteria:

• systematic class	1	monocotyledons
	2	dicotyledons
	3	conifers
• germination mode	1	epigeal germination
	2	hypogeal germination
• shoot development	1	without epicotyl elongation
	2	with epicotyl elongation
	3	no shoot elongation; shoot apex enclosed within a sheath (coleoptile)
	4	tuberous hypocotyl
• root system and its significance for evaluation	1	primary root essential
	2	secondary roots may compensate for the primary root
	3	several equal seminal roots

The letters and numbers are combined to form the **group number**. Thus, for example, **Seedling Group A-2-1-2-2** comprises seedlings:

- **A**-2-1-2-2 of agricultural or horticultural plants
- A-**2**-1-2-2 belonging to the dicotyledons
- A-2-**1**-2-2 with epigeal germination
- A-2-1-**2**-2 with epicotyl elongation
- A-2-1-2-**2** with secondary roots that are taken into account if the primary root is defective.

In this way any genus can be assigned to the group that covers its systematic and morphological characteristics. Moreover, additional groups could be formed using this system if required.

1 There are only few genera with species of which germinate according to different patterns.

Section 7: Evaluation of seedlings of particular genera

7.2 Seedling groups

Considering the above criteria all species covered in the ISTA Rules can be assigned to fourteen **Seedling Groups** that cover the relevant systematic and morphological characteristics.

7.2.1 Category A: Agricultural and horticultural species

Seedling type	Seedling group	Representative genera
Type A	A-1-1-1-1	*Allium*
Type B	A-1-2-1-1	*Freesia*
Type C	A-1-2-2-1	*Asparagus*
Type D	A-1-2-3-1	*Lolium*
	A-1-2-3-2	*Oryza*, *Sorghum*, *Zea*
	A-1-2-3-3	*Hordeum*, *Secale*, *Triticum*
Type E	A-2-1-1-1	*Brassica*, *Beta*, *Daucus*, *Dianthus*, *Helianthus*, *Lactuca*, *Trifolium*, *Zinnia*
	A-2-1-1-2	*Cucumis*, *Gossypium*
	A-2-1-4-3	*Cyclamen*
Type F	A-2-1-2-2	*Arachis*, *Phaseolus*
Type G	A-2-2-2-2	*Pisum*, *Vicia*

7.2.2 Category B: Trees and shrubs

Seedling type	Seedling group	Representative genera
Type E	B-2-1-1-1	*Robinia*
Type G	B-2-2-2-2	*Quercus*
Type H	B-3-1-1-1	*Abies*, *Pinus*

Section 8: Seedling evaluation

Physiological germination is a series of complex physiological processes and begins with the seed taking up water and ends with the primary root piercing the seed coat. To the seed tester, **laboratory germination** begins with the primary root piercing the seed coat and ends when the seedling has developed to the stage that it can be evaluated according to the **ISTA Rules**.

When the seedlings have reached a defined stage of development, they are individually evaluated with regard to the soundness of their essential parts and counted as either a **normal** or **abnormal** seedling. Sometimes two or more successive counts are required before all seeds have germinated and reached the stage of development required before evaluation is possible. Insufficiently developed, weak, unbalanced, deformed and damaged seedlings are left on the test until the final count. **In cases of doubt or when there are large numbers of such seedlings, the ISTA Rules allow the test to be extended.** Decayed seedlings and mouldy seeds are removed at interim counts in order to reduce the risk of secondary infection.

8.1 Normal seedlings

'Normal seedlings', as defined by the ISTA Rules:

> *show the potential for continued development into satisfactory plants when grown in good quality soil and under favourable conditions of moisture, temperature and light*.

Such potential for continued development is dependent on the soundness and correct functioning of the essential seedling parts during germination and early seedling stages.

Experience and comparative tests have shown, that to be classified as normal, a seedling must comply with one of the following categories:

- **Intact seedlings** – seedlings with all their essential structures well developed, complete, in proportion to each other and healthy.
- **Seedlings with slight defects or deficiencies** – seedlings showing certain slight defects to their essential structures. Such seedlings must have an otherwise satisfactory and balanced development, comparable to that of intact seedlings of the same test.
- **Seedlings with secondary infection** – seedlings which would have complied, but have been infected by fungi or bacteria from sources other than the parent seed.

Three categories of normal seedlings can be therefore differentiated:

- **intact** seedlings;
- **slightly defective** seedlings; and
- **secondarily infected** seedlings.

8.1.1 Intact seedlings

All the essential parts of an 'intact' seedling are:

- **well developed**;
- **complete**;
- **in proportion to each other**; and
- **healthy**.

An intact seedling, depending on the kind of seed being tested, possesses a **specific combination** of the following essential parts:

8.1.1.1 Intact root system

It may consist of

- a long and slender **primary root**, which is usually covered with numerous root hairs and ends in a fine tip (e.g. *Brassica*);

or it may consist of

- **secondary roots** (in addition to the primary root) which are produced within the official test period in certain genera (e.g. *Zea*, *Cucurbita*);

or it may consist of

- **several seminal roots** instead of a primary root. This only occurs in a few genera (e.g.*Triticum*, *Cyclamen*).

8.1.1.2 Intact seedling axis

It may consist of

- a straight, elongated **hypocotyl** in seedlings with **epigeal** germination (e.g. *Brassica*, *Pinus*);

or it may consist of

- an elongated **epicotyl** in seedlings with **hypogeal** germination (e.g. *Asparagus*, *Pisum*);

or it may consist of

- both, an elongated **hypocotyl** and an elongated **epicotyl** in certain seedlings with **epigeal** germination (e.g. *Phaseolus*);

or it may consist of

- an elongated **mesocotyl** in certain grass and cereal seedlings (e.g. *Sorghum*).

8.1.1.3 Specific number of cotyledons

Required is:

- **one cotyledon** in monocotyledons, and rare exceptions of dicotyledons (e.g. *Cyclamen*); it may be green and leaf like (e.g. *Allium*, *Cyclamen*) or be partly or completely enclosed in the seed coat (e.g. *Asparagus*, Poaceae);

or required are:

- **two cotyledons** in dicotyledons that may be green and expanded in seedlings with **epigeal** germination (e.g. *Brassica*), or hemispherical and fleshy and enclosed in the seed coat in seedlings with **hypogeal** germination (e.g. *Pisum*);

or required are:

- **several cotyledons** in conifers (2 to 18 depending on genera), which are long and narrow and form a green spiral (whorl) on top of the seedling axis.

8.1.1.4 Specific number of intact primary leaves

Required in so far as they have developed during the seed testing period, prescribed in the ISTA Rules:

- **one primary leaf** in species with alternating leaf position (e.g. *Pisum*);

or:

- **two primary leaves** in species with opposite leaf positions (e.g. *Phaseolus*).

8.1.1.5 Intact terminal bud

This may be:

- **little developed**, lying between the cotyledons and hardly visible in most seedlings with **epigeal** germination (e.g. *Brassica*);

or it may be:

- **well developed** and free, being formed by a number of scale leaves or the primary leaves in most seedlings with **hypogeal** germination (e.g. *Pisum*).

8.1.1.6 Intact coleoptile in seedlings of Poaceae

The first green leaf grows up within it and eventually emerges from it.

8.1.2 Seedlings regarded as slightly defective

Experience has shown that certain defects or deficiencies on particular essential parts of a seedling can be regarded as slight and be tolerated, because they would not hamper development into a satisfactory plant, provided the seedling is otherwise normal.

The following are regarded as **acceptable defects**:

- Small, limited discoloured or necrotic spots on the primary root or seedling axis;
- A defective primary root, provided that sufficient strong secondary roots are present[1];
- The presence of only *one* (but strong and intact) seminal root in the group of cereals within the family of Poaceae (Seedling Group A-2-1-4-3) and of two seminal roots in *Cyclamen* and a few other genera in Seedling Group A-2-1-1-3.
- Superficial[2] or healed cracks or splits on the seedling axis (hypocotyl, epicotyl, mesocotyl) or in the cotyledons;
- Loose twists[3] of the seedling axis or coleoptile;
- In compliance with the 50 % rule (see 8.4.1), small spots, necroses, decay or missing parts of cotyledons or primary leaves;
- One cotyledon or primary leaf instead of two or an additional (third) cotyledon or primary leaf;
- The existence of a split in the coleoptile of grasses and cereals which runs down from the tip; but not further than one third of the coleoptile length[4]; and
- In the family of Poaceae, retarded growth of the green leaf within the coleoptile provided it reaches at least half the length of the coleoptile.

8.1.3 Seedlings with secondary infection

A seedling that is decayed by fungi or bacteria is classified as normal, if it is evident that the parent seed is not the source of infection (secondary infection), and all the essential parts of the seedling are otherwise normal.

1 This applies for the genera of certain families only, such as Cucurbitaceae and Malvaceae (e.g. *Gossypium*), as well as to the group of large-seeded genera within the family of Fabaceae and to certain genera within the family of Poaceae (e.g. *Zea*, *Oryza*). Seedlings allowed secondary roots instead of an intact primary root are dealt with in Seedling Groups A-1-2-3-2, A-2-1-1-2, A-2-1-2-2, A-2-2-2-2, B-2-2-2-2 in Sections 13, 16, 18, 19 and 21 of this Handbook.

2 Cracks and splits not affecting the conducting tissues.

3 One to two (at the most) turns along the whole length of the seedling part.

4 Specific rules for the evaluation of splits in the coleoptile and damage to the primary leaf in *Zea mays* are given in Section 13.

8.2 Abnormal seedlings

'Abnormal seedlings', as defined by the ISTA Rules:

> *do not show the potential for continued development into satisfactory plants when grown in good quality soil and under favourable conditions of moisture, temperature and light*.

8.2.1 Causes of abnormality[5]

There are many reasons why seeds produce abnormal seedlings that lack the potential to develop into satisfactory plants. The most frequent ones are:

8.2.1.1 Mechanical injury of the embryo

Damage to the embryo of the seed is usually the result of external causes such as:
- mechanical handling during harvesting and processing;
- drought and desiccation;
- the rapid intake of water in very dry seeds; and
- insect and mite damage.

When the damage results in seedlings with any of the essential structures missing or in seedlings where balanced development does not occur, these seedlings are classified as abnormal.

Possible types of abnormalities resulting from mechanical damage include:
- split, stunted or missing primary root;
- deep cracks and splits[6] in the seedling axis or in the cotyledons;
- split coleoptiles in temperate cereals where the split occurs in places other than the tip or where it extends from the tip to a distance more than one third of the length of the coleoptile;
- broken or separated cotyledons or parts of the axis; and
- broken or otherwise damaged coleoptile.

8.2.1.2 Heat damage to the embryo

Seeds harvested at a high moisture content, must be dried artificially to prolong seed viability during storage and to avoid spontaneous heating and damage due to micro-organisms, insects and mites in store. Heat damage may occur, if excessive heat is used for drying or if spontaneous heating occurs during storage. In some circumstances heat damage can also occur on the mother plant during excessively hot weather prior to harvest.

Defects and symptoms of heat damage are:
- slow germination;
- retarded growth of the leaf within the coleoptile (in cereals);
- stunted growth of the seedling; and
- failure to germinate.[7]

8.2.1.3 Chemical damage to the embryo

If herbicides have been applied to the growing crop, or if the seed is accidentally contaminated with volatile chemicals in store (e.g. potato sprout suppressant), or if the seed is treated with excessive amounts of fungicidal and/or insecticidal seed treatment chemical, seedlings showing phytotoxic symptoms[8] and/or negative geotropism may develop.

8.2.1.4 Deficiencies in the physiological make-up of the seed or embryo

These may be due to external factors experienced by the seed during its formation, development and storage. Examples of these external influences include:
- Manganese deficiency of the mother plant during maturation of *Pisum sativum* seeds may cause so-called 'marsh spot', a physiological disorder exhibiting discoloured areas at the centre of the cotyledons and the absence of an apical meristem;
- Wet weather during seed maturation favours the development and spread of fungi and other micro-organisms on the seed;
- Wet weather may also lead to 'sprouting' (germination on the mother plant), which often results in damaged seedlings;
- Frosty weather during seed maturation on the mother plant;
- The genetic make-up inherited from the parent plants;

5 Usually it is not possible to determine the cause of individual abnormal seedlings without a thorough knowledge of the 'history' of a seed lot. For everyday seed testing it is sufficient to decide whether a seedling is normal or abnormal. However, certain types of abnormal seedlings in a test may give valuable hints as to germination conditions or as to the handling of the seed. Numerous broken *Trifolium* seedlings indicate mechanical damage during the processing of the seed lot; thickened and shortened roots and coleoptiles of cereals are usually the result of over-treatment with fungicides and insecticides or treatment at too high a seed moisture content.

6 Affecting the conducting tissue.

7 In temperate cereals with heat damage, the seed imbibes water but does not germinate: the seed remains firm and is difficult to differentiate from dormant seed without the aid of a tetrazolium test. The seed and embryo may be discoloured.

8 Phytotoxic symptoms may also occur in circumstances when seed treatments are applied to seed with high moisture contents.

- Premature harvesting and insufficiently mature seeds, respectively, may lead to various seedling defects;
- Natural or accelerated ageing of the seed due to unfavourable storage conditions may lead to internal disturbances in germination or early seedling development; and
- Physiological disturbances may also be the result of genetic abnormalities or of the natural ageing process.

Characteristic abnormalities as a consequence of disturbances of this type may include (among many others):
- retarded growth of the seedling as a whole, or of individual parts of it;
- short, stubby or spindly primary or seminal roots;
- short and thick or otherwise deformed seedling axis or coleoptile;
- curled, discoloured or necrotic cotyledons (or primary leaves);
- negative geotropism (e.g. roots growing upward);
- yellow or white albino seedlings with chlorophyll deficiency; and
- spindly or glassy seedlings.

8.2.1.5 Primary infection and disease of the seedling

Attack of fungi or bacteria usually results in decay of individual seedling parts or of the whole seedling (which eventually leads to the death of the seedling). Injured or weak seedlings are especially susceptible.

8.2.1.6 Unknown causes

Information on the types of abnormals and their causes may be of value to the seed user. However, it is often not possible to assign individual abnormal seedlings to a specific cause, without a detailed knowledge of the history of the seed lot. For normal seedling evaluation work this is not essential. It is sufficient to know whether or not a seedling is classed as normal or abnormal, except where a particular type of abnormality is due to germination conditions. In such cases allowances must be made for the germination conditions and the sample should be retested, if necessary.

8.2.2 Apparent abnormalities

If seeds are germinated in the laboratory under non-optimal conditions, the seedlings may show apparent abnormalities that are not due to damage or physiological disturbances of the embryo but to the germination conditions.

The following are examples of the causes of such apparent abnormalities:
- In the restricted conditions of blotter envelopes, bent and twisted or even broken seedlings may be found;
- If the seeds are not planted in the correct orientation (radicle-part of the seed downward) in rolled towels, the seedlings may have their roots initially growing upward and the coleoptiles or seedling axes growing downward;
- If the seedbed is too wet seedlings may show short retarded roots or decayed root tips;
- Chemicals in the germination medium, e.g. bleaches used in paper making, may suppress germination and/or cause abnormalities (mainly of the root).

It is important to recognise such abnormalities as apparent abnormalities. **In such cases allowances must be made for the germination conditions and the sample should be retested, if necessary.**

8.3 Stage of development for seedling evaluation and evaluation guidelines

As a general rule, seedlings must not be removed from the test, before all their essential structures have developed to such an extent, that they can be evaluated reliably and without doubt. This implies that for the majority of seedlings in a test (depending on the Seedling Type being tested):
- the cotyledons have freed themselves from the seed coat (mainly in Seedling Type E, Group A-2-1-1-1, e.g. *Lactuca* and *Brassica*);

or
- the primary leaves have expanded in sand tests, or protrude from between the cotyledons in rolled-towel-tests (in Seedling Type F, Group A-2-1-2-2, e.g. *Phaseolus*);

or
- the primary leaf has emerged from the coleoptile (in Seedling Type D, Group A-1-2-3-3, e.g. *Triticum* and Group A-1-2-3-2, e.g. *Zea*).

If at the end of the test period, including the authorised extension to the test period, there are just a few seedlings left[9], which have not reached the appropriate stage of development, they may be evaluated to the best of the analyst's knowledge and experience. The appearance of other seedlings in the test is used for guidance. If there are more than 5 % underdeveloped, and therefore doubtful seedlings, the sample must be retested and appropriate investigations should be made to determine the cause of such underdeveloped seedlings.

9 As a guideline, the percentage of doubtful seedlings evaluated should be no more than 5 %.

There are two exceptions to the general rule:
- some species of dicotyledons with epigeal germination (Seedling Type E); and
- some dicotyledonous tree and shrub species.

8.3.1 Seedling Type E

In many species of epigeal dicotyledons (e.g. *Daucus* and *Beta*) most of the seedlings will not have freed their cotyledons from the seed coats by the end of the test period. At the end of the test, including the authorised extension to the test period, at least the **seedling neck** with the base of the cotyledons should be clearly visible. If there is doubt about the soundness of the cotyledons, the seed coat should be carefully removed in order to examine and evaluate the cotyledons and the terminal bud. If the seed coat cannot be removed without damage to the seedling, the seedling should be considered abnormal.

8.3.2 Dicotyledonous trees and shrubs

The stage of evaluation of dicotyledonous tree seedlings is a further exception to the above rule. Germination and seedling development is often very slow and such seedlings are considered normal, when ***the sum of primary root and hypocotyl length is four times (or more) the seed length*** – provided the other criteria of normality are met.

8.4 Guidelines

Experience, and the results of investigations have led to the development of some specific guidelines for the evaluation of certain types of seedlings, irrespective of their Seedling Type or Group. There are guidelines for the evaluation of:
- damaged cotyledons and primary leaves – **the 50 % rule**;
- seedlings with necrosis of the cotyledons;
- seedlings with splits and cracks;
- seedlings with loops and spirals;
- seedlings where secondary roots are taken into consideration when the primary root is damaged or missing;
- seedlings exhibiting negative geotropism;
- multigerm seeds; and
- samples where a large number of doubtful seedlings are produced.

8.4.1 Damaged cotyledons and primary leaves – the 50 % rule

This rule is used in the evaluation of cotyledons and primary leaves. Seedlings are considered normal as long as half or more of the total cotyledon tissue is functional, but abnormal when more than half of the tissue is missing, necrotic, discoloured or decayed. An intact seedling of the same test shall be taken as a standard.

Figure 8.1 Seedlings of *Brassica* illustrating the 50 % rule. The seedling on the left is taken as the standard; the seedling in the middle is normal; and the seedling on the right is abnormal.

The 50 % rule[10] is also applied in cases where defective primary leaves are to be evaluated, in species such as *Phaseolus*.

Figure 8.2 The *Phaseolus* seedling on the left is the standard and using the 50 % rule the other three seedlings are abnormal.

10 In this case it applies to damaged or deformed primary leaves only, not for small, but normally shaped ones.

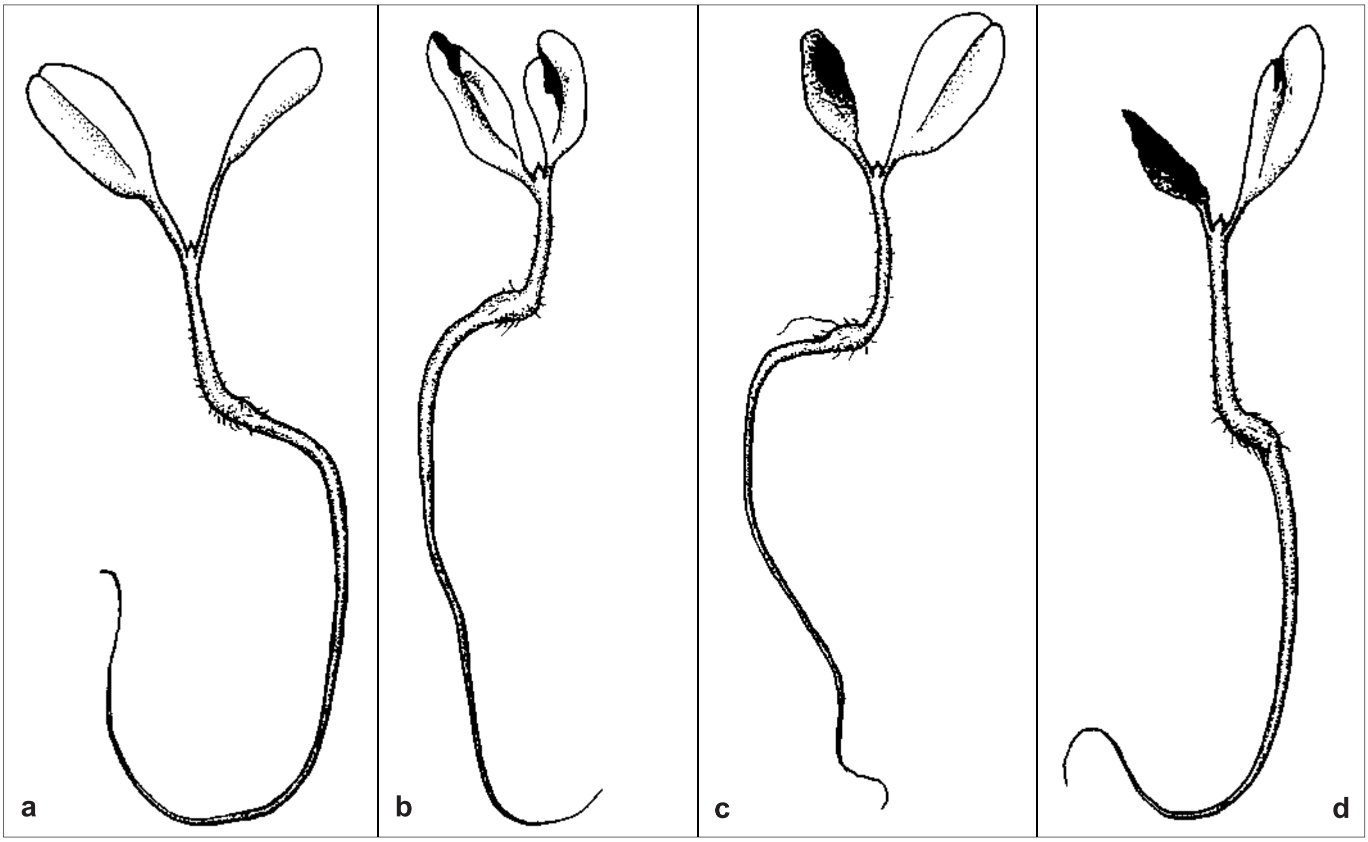
Figure 8.3 A *Helianthus* seedling with green normal terminal bud but with albino tissue surrounding the conductive tissue at the base of the cotyledons. On the basis of observations and experience on the performance of these seedlings they are considered as abnormal.

The 50 % rule is not valid, if the tissues around the terminal bud or the terminal bud itself are necrotic or decayed. Such seedlings are abnormal irrespective of the condition of the cotyledons.

8.4.2 Seedlings with necrosis of the cotyledons

Necrosis can occur as a result of conditions experienced during seed formation and maturation on the mother plant. It can also be the result of natural ageing of a seed lot or poor storage conditions. Necrosis is **not** the result of a seed borne disease or infection but it is thought to be the result of a physiological condition of the affected seed. Necrosis is **not** transmitted from seedling to seedling within a germination test.

The symptoms of necrosis range from a slight discolouration to areas of deep lesions that distort surrounding tissues and disrupt the conductive tissue. In general the 50 % rule is applied when evaluating cotyledons. Where the necrosis is close to the terminal bud or affects the transport of sap, the seedling is considered abnormal irrespective of the area affected by necrosis. **It must be emphasised that if the terminal bud is necrotic the seedling is abnormal irrespective of the extent of necrosis elsewhere.**



Normal **Abnormal** **Normal** **Abnormal**

Figure 8.4 Drawings of seedlings of *Lactuca* illustrating the evaluation of seedlings with necrosis of the cotyledons: **a** Normal seedling; **b** Abnormal seedling – deep lesions distorting surrounding tissues; **c** Normal seedling – satisfies 50 % rule and necrosis not affecting terminal bud; or distorting the conductive tissue; and **d** Abnormal seedling – less than 50 % and necrosis too close to terminal bud.

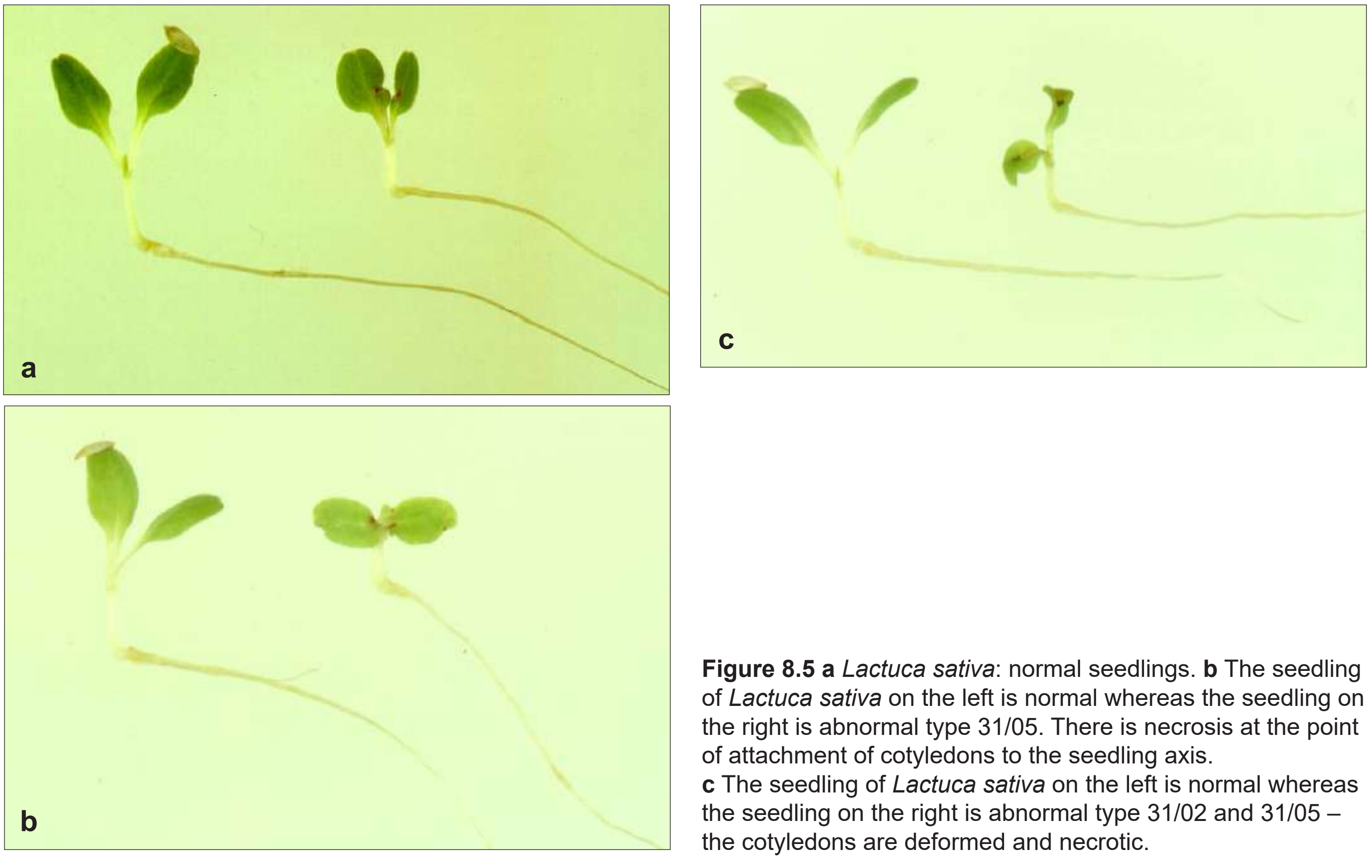


Figure 8.5 a *Lactuca sativa*: normal seedlings. b The seedling of *Lactuca sativa* on the left is normal whereas the seedling on the right is abnormal type 31/05. There is necrosis at the point of attachment of cotyledons to the seedling axis.
c The seedling of *Lactuca sativa* on the left is normal whereas the seedling on the right is abnormal type 31/02 and 31/05 – the cotyledons are deformed and necrotic.

The seedlings of *Lactuca* in Figure 8.5a, b and c illustrate some of the complications involved in the evaluation of necrotic seedlings. The cotyledons of the seedling are critically deformed due to the necrosis (Figure 8.5b, on the right) whereas the conductive tissue of the seedling (Figure 8.5c, on the right) has been disrupted by the necrosis. Both seedlings are evaluated as abnormal.

8.4.3 Seedlings with splits and cracks

Sometimes the essential parts of seedlings may be split or cracked as a result of a number of different phenomena including: weathering; mechanical damage during harvest and processing; and imbibition damage during the early stages of germination.

8.4.3.1 Cracked and broken (fractures)

In the case of deeply cracked or broken tissue, seedlings are considered normal if healed tissue has developed over the broken area and the affected tissue is not growing in a different direction to the rest of the seedling. Healed breaks are considered normal provided the conductive tissue is not disrupted.

Please note: This guidance on cracks and fractures also applies to the root system of seedlings.

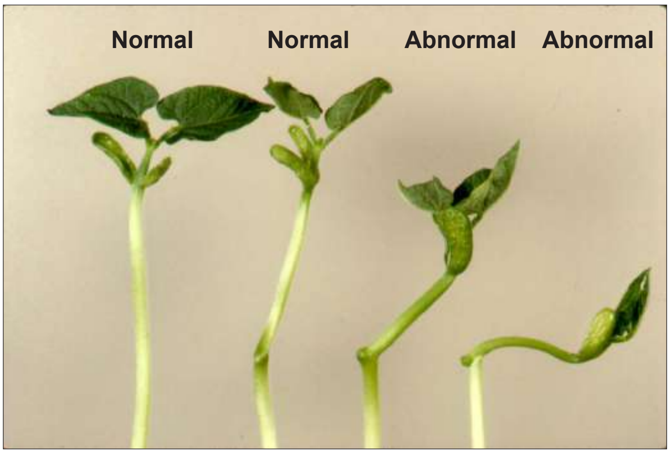


Figure 8.6 The evaluation of seedlings with deeply cracked and broken hypocotyls. The illustration is of *Phaseolus* but the guidance is applicable to all genera.

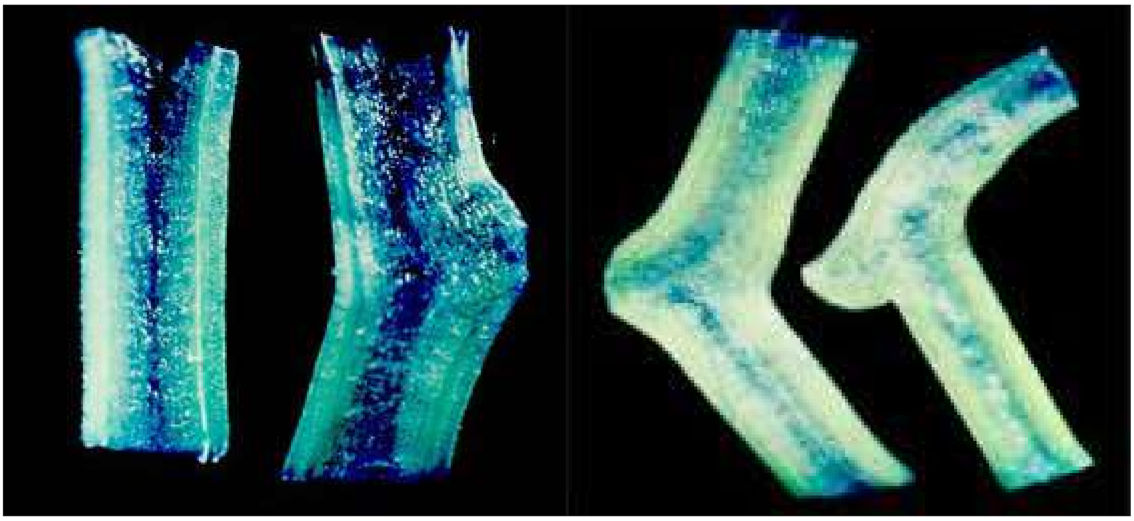


Figure 8.7 Longitudinal sections through the hypocotyls of the seedlings shown in Figure 8.6. The sections were stained with methylene blue to reveal the conductive tissue.

Sometimes adventitious roots may develop at the point of the break of a hypocotyl or epicotyl. This is generally considered an artefact of laboratory testing due to the high humidity provided for germination tests. Provided the conductive tissue is not disrupted the seedling can be considered normal.

Normal | **Abnormal**

Figure 8.8 *Phaseolus* seedlings showing the development of adventitious roots from cracks in the hypocotyls. The seedling on the left is considered normal since there is no disruption to the conductive tissue. The seedling on the right is considered abnormal as there is severe injury and disruption to conductive tissue. This phenomenon can also occur in the laboratory testing of legumes and Cucubitaceae.

8.4.3.2 Splits

A split is considered a hindrance to the development of the seedling when it reaches the central cylinder or it affects the transport of sap. To verify the depth of a split a transverse cut is made:

- If the split does not reach the central cylinder, the seedling is considered **normal**;
- If the split reaches the outer layers of the central cylinder:
  - it is **normal** if healing has occurred provided there is no deformation of the epicotyl or hypocotyl;
  - it is **abnormal** if no healing has occurred;
- If the split reaches or crosses the central cylinder it is **abnormal**, irrespective of whether healing has taken place or not.

Please note: This guidance on splits also applies to the root system of seedlings.

Figure 8.9 The evaluation of splits in hypocotyls or epicotyls.

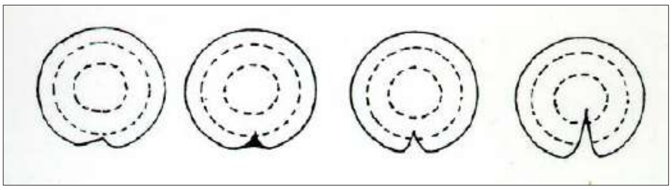


Figure 8.10 Drawings of transverse sections of the seedling hypocotyls shown in Figure 8.9.

8.4.4 Seedlings with loops and spirals (twists)

A loop or spiral[11] in the hypocotyl or epicotyl is considered a hindrance to the development of a seedling. However, these may be caused by the laboratory testing procedures and it is important to distinguish such apparent abnormalities from naturally occurring defects. In a loop the axis of rotation is more or less perpendicular to the seedling whereas in a spiral the rotational axis is parallel to the seedling.

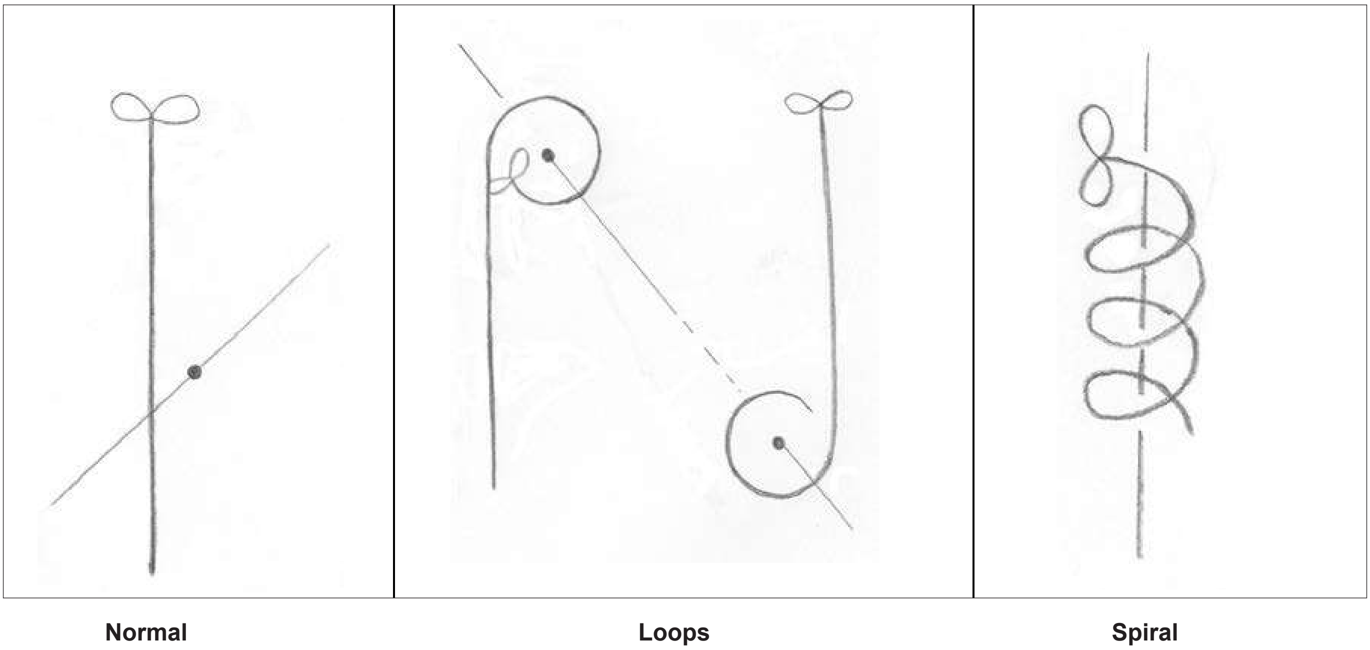


Figure 8.11 Drawings illustrating the differences between loops and spirals.

11 In the ISTA Rules, reference is made to loops, twists and spirals. The terms 'twist' and 'spiral' are synonymous. In this Handbook, 'spiral' is used.

8.4.4.1 Loops

Guidance has been developed for the evaluation of loops. If the loop is forming:
- An open U: the seedling is considered normal;
- A closed U:
  – without primary root defect, the seedling is considered normal;
  – with primary root defect, the seedling is considered abnormal;
- A complete loop: seedling is considered abnormal.

Note: For Seedling Group A-2-1-2-2, if there is no damage causing the loop on the hypocotyls (on the upper or lower part), then the seedling is considered normal. See photos in Section 18.

8.4.4.2 Spirals

Guidance has been developed for the evaluation of spirals. If it is:
- A loose spiral: the seedling is considered normal;
- A tight spiral: the seedling is considered abnormal.

More than one spiral can occur over the whole length of the hypocotyl/epicotyl. Provided it is loose, and there are less than three full turns they can be considered normal.

Normal **Normal** **Normal** **Abnormal**

Figure 8.12 Seedlings illustrating the evaluation of a loop in the hypocotyl or epicotyl. Care must be taken in the evaluation of such seedlings since germination procedures such as rolled paper towelling may induce such seedlings.

Normals **Normal** **Abnormal** **Abnormal**

Figure 8.13 Seedlings illustrating the evaluation of a spiral in the hypocotyls, epicotyl or mesocotyl.

8.4.5 Seedlings where secondary roots are taken into consideration when primary root is damaged or missing

In specific genera of Fabaceae, especially large-seeded genera (e.g. *Phaseolus*, *Pisum*, *Vicia*) and Poaceae (e.g. *Zea*) and in all genera of the Cucurbitaceae (e.g. *Cucumis*, *Cucurbita*, *Citrullus*) and Malvaceae (e.g. *Gossypium* and *Hibiscus*) a seedling with a defective or missing primary root can be considered normal provided there are sufficient, well developed secondary roots. Figure 8.14 illustrates the use of this rule in the evaluation of some *Pisum* seedlings.

Care must be taken when applying this rule since it is not applicable in many genera (Figure 8.15). A full list of genera to which it applies is given in this Handbook.

Normal Normal Abnormal Abnormal

Figure 8.14 Seedlings of *Pisum* illustrating the evaluation of root systems where the presence of secondary root is taken into consideration.

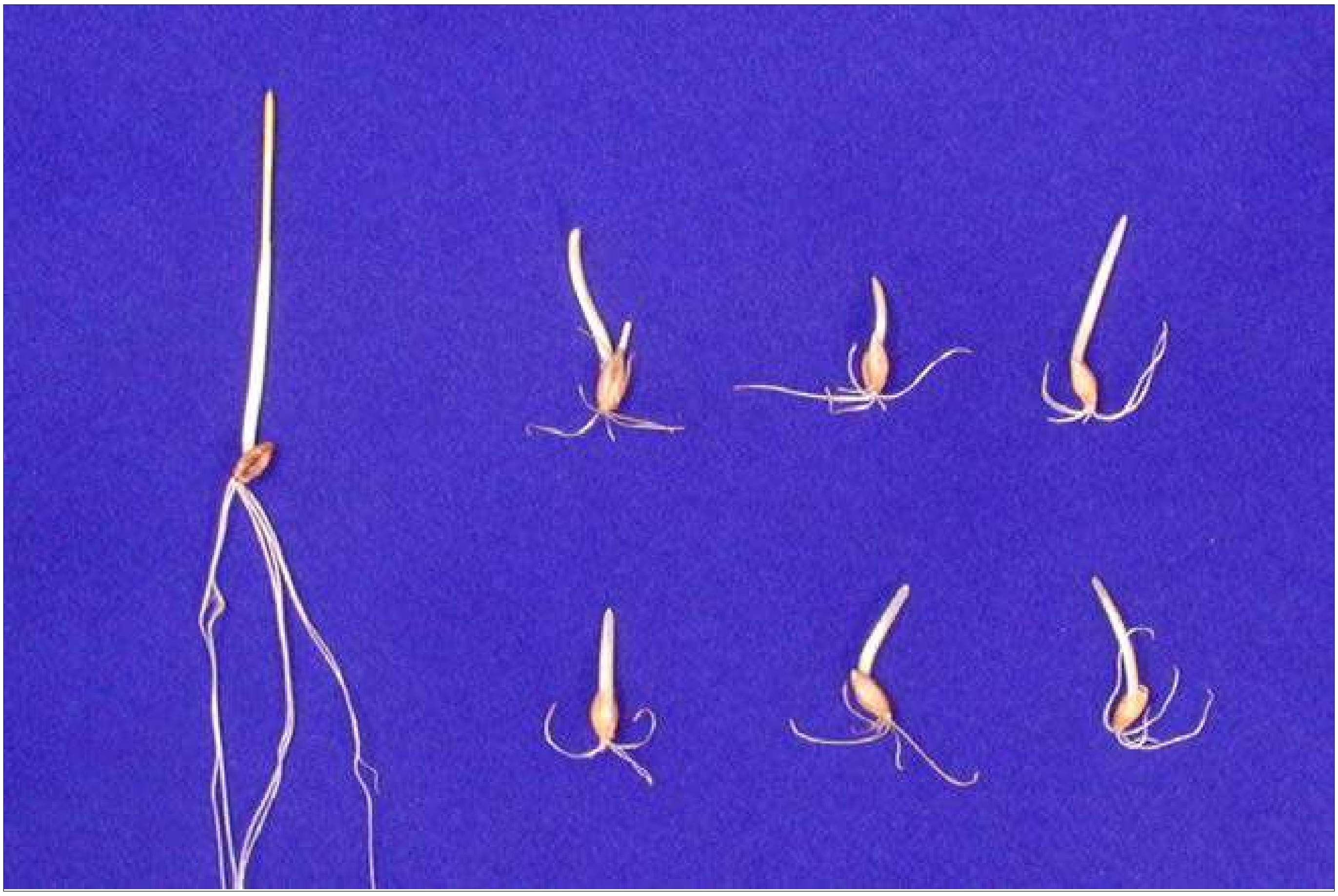
Normal Normal Abnormal Abnormal

Figure 8.15 Seedlings of *Helianthus* illustrating the evaluation of root systems where the presence of secondary roots **is not** taken into consideration. The seedling second on the left has slight defects to the primary root but this is not considered great enough to warrant an abnormal classification.

8.4.6 Negative geotropism

Sometimes the primary roots of seedlings lose contact with the substrate and grow upward. It may appear that such roots have lost their natural tendency to grow downward into the soil (positive geotropism). This phenomenon may also be the result of adverse germination conditions (e.g. too much moisture or toxic substances in the substrate) or physiological damage (e.g. the application of desiccants such as glyphosate (*Round-up*[12]) to the mother crop).

If there are many such seedlings in a test, the sample should be retested in compost or soil. In the compost/soil test, seedlings that are negatively geotropic with primary roots growing upwards must be classed as abnormal. In seedlings with seminal roots, the seedling can be classed as normal as long as one seminal root is growing downwards.



Normal **Abnormal**

Figure 8.16 Barley seedlings with roots exhibiting negative geotropism due to the application of pre-harvest glyphosate treatment to the mother crop.

12 © Monsanto.

8.4.7 Evaluation of multigerm seed units

The evaluation of seed units that produce more than one seedling is as follows:

8.4.7.1 Compound seed units

Seed-like structures containing more than one true seed (e.g. multiple florets in certain grasses, unseparated shizocarps of Apiaceae, clusters of *Beta*, fruits of *Tetragonia* or *Tectona grandis*) are tested as single seeds and classed as normal when at least one normal seedling has been produced. In a cluster of *Beta vulgaris* producing more than one seedling, the seed unit is considered normal as long as one of the seedlings produced is normal.

8.4.7.2 True seeds containing more than one embryo

In the seeds of most species twins or even triplets are possible, but exceptional and rare. They are counted as one and classed as normal if at least one of the twins (or triplets) is normal. Fused twins or triplets are regarded as abnormal.

Figure 8.17 *Beta vulgaris* (abnormal type 11/03): the primary root is retarded.

Figure 8.18 Seedlings of Poaceae from which more than one seedling has been produced. As long as one of the seedlings is normal, the seed unit can be considered normal.

8.4.8 Samples where a large number of doubtful seedlings are produced

Retests in sand, compost or soil

When seed samples, germinated in paper substrates, produce a number of doubtful seedlings, that are difficult to assess, a retest in sand, compost or soil should be conducted to check the original evaluation.

Sand, and especially compost and soil are more natural substrates than paper. They often provide a more reliable and realistic evaluation of a sample, for the following reasons:

- Sand, compost and soil act as a barrier to the rapid spread of pathogenic and saprophytic fungi within a germination test;
- Compost and soil absorb phytotoxic substances, such as residues of chemical seed treatments and natural substances released from the seed. The toxic effect on the seedlings (e.g. seedlings with stubby, stunted or retarded primary roots) can be partly or completely neutralised in compost and soil thereby allowing the seedlings to grow normally. **If the phytotoxicity remains after a retest in compost or soil, seedlings thus affected must be considered abnormal**;
- If necessary, certain doubtful seedlings may be grown and observed over a longer period in compost and soil.

Seedling evaluation according to groups

Category A: Agricultural and horticultural species

This section deals with the evaluation of seedlings of the individual groups germinated on artificial substrates.

In order to show the group-specific properties and to facilitate a correct seedling evaluation, each group is dealt with under the following aspects:

- The group number is briefly explained;
- The specific morphology of the seedling type is described;
- A genus, which may serve as a representative of the group, is named;
- The development of the seedling during the prescribed test period is described;
- A survey of normal and abnormal seedlings typical of the respective group is given;
- Where necessary, supplementary remarks are added; and
- Illustrations of normal and abnormal seedlings of the representative genus and – where available – of additional genera belonging to the group conclude the group description.
- Figures: in general, the seedling on the left is a normal seedling that was obtained in the same test as the abnormal seedlings to the right of it. Normal or abnormal is indicated under each seedling.

At the end of the Handbook (Appendix 2) there is an index of seedling groups for the all genera covered by the ISTA Rules. The group number given to each indicates the criteria by which seed of that genus must be evaluated by in a germination test. An index is also given of different types of seedling abnormalities.

Section 9:
Seedling Type A – Seedling Group A-1-1-1-1

A-1-1-1-1

Monocotyledons
with epigeal germination
without epicotyl elongation

The primary root is essential

Representative genus: *Allium*

The seedling part that grows towards the light and becomes green is the cotyledon.

The shoot system consists of a hardly discernible hypocotyl with the terminal bud enclosed by the lower part of the long green, cylindrical cotyledon. There is no epicotyl elongation. The tip of the cotyledon remains within the seed coat to absorb food reserves from the endosperm.

The root system consists of the primary root, usually with root hairs, which is essential. Secondary roots are not taken into account in seedling evaluation.

Development of the seedling during the test

In the mature seed the embryo is embedded in endosperm. The cylindrical embryo consists of a short radicle and a long, sometimes coiled cylindrical cotyledon with the plumule enclosed at its base.

At the start of germination, the primary root pierces the seed coat and elongates without producing lateral roots. During the full test period, seedlings may develop one or two adventitious roots at the transition zone between primary root and cotyledon.

The green part of the seedling consists of the cylindrical cotyledon with its tip normally remaining embedded in the endosperm. The cotyledon shows a sharp bend, called the ‘knee’ towards its upper end. At the base of the cotyledon there is a fine opening, through which the primary leaf emerges; but this seldom occurs during the test period.

Differentiation of normal and abnormal seedlings

Normal seedlings	
Seedling as a whole	
all essential structures	are **normal** as detailed in the following:
root system	
the primary root	is **intact** *or* shows **acceptable defects** – • discoloured or necrotic spots • healed cracks or splits • superficial cracks or splits[1]
shoot system	
the cotyledons	are **intact** – with a definite ‘knee’ *or* show **acceptable defects** – • discoloured or necrotic spots • loose twists

1 Superficial cracks or splits: not affecting the conducting tissues.

Abnormal seedlings	
Seedling as a whole	
the seedling	is **abnormal** if it • is deformed • is fractured • releases the cotyledon before the primary root from the seed coat • consists of fused twin seedlings • is yellow or white • is spindly • is glassy • is decayed as a result of primary infection
one or more of the essential structures	are **abnormal**, as detailed in the following:
root system	
the primary root	is **defective** if it • is stunted or stubby • is retarded • is missing • is broken • is split from the tip • is trapped in the seed coat • shows negative geotropism • is constricted • is spindly • is glassy • is decayed as a result of primary infection
shoot system	
the cotyledons	are **defective** if they • are short and thick • are broken • are bent over or forming a loop • are forming a spiral • do not show a definite 'knee' • are constricted • are spindly • are glassy • are decayed as a result of primary infection

Supplementary remarks

None

Normal **Abnormal** **Abnormal**

Figure 9.1 *Allium cepa* (abnormal type 11/01): the primary root is stunted.

Normal **Abnormal** **Abnormal** **Abnormal**

Figure 9.3 *Allium cepa* (abnormal type 32/04): the cotyledon is without a knee.

Normal **Abnormal** **Abnormal** **Abnormal**

Figure 9.2 *Allium cepa* (abnormal type 32/01 and 32/02): the cotyledon is short and thick and forming a loop.

Normal **Abnormal** **Abnormal**

Figure 9.4 *Allium cepa* (abnormal type 11/04): the primary root is missing and even though secondary roots are present, the seedling is abnormal.

Section 10: Seedling Type B – Seedling Group A-1-2-1-1

A-1-2-1-1

Monocotyledons
with hypogeal germination
without epicotyl elongation

The primary root is essential

Representative genus: *Freesia*

The seedling part that grows towards the light and becomes green is the primary leaf.

The shoot system shows no appreciable elongation. The terminal bud, enclosed in the basal, sheath-like part of the cotyledon, forms flat, sword-like leaves. The upper part of the cotyledon remains in the seed to absorb food reserves from the endosperm.

The root system consists of the primary root, usually with root hairs. Secondary roots are not taken into account in seedling evaluation, if the primary root is defective.

Development of the seedling during the test

The cylindrical embryo in the seed is embedded in hard, semi-transparent, non-starchy endosperm.

At the start of germination, the primary root pierces the seed and elongates. It is followed by the sheath-like basal part of the cotyledon, which encloses the terminal bud. The upper part of the cotyledon remains in the seed to absorb food reserves. The terminal bud produces a flat, sword-like primary leaf, which emerges from the basal part of the cotyledon.

Differentiation of normal and abnormal seedlings

Normal seedlings	
Seedling as a whole	
all essential structures	are **normal** as detailed in the following:
root system	
the primary root	is **intact** *or* shows **acceptable defects** – • discoloured or necrotic spots • healed cracks or splits • superficial cracks or splits[1]
shoot system	
basal cotyledon part of the cotyledon	is **intact**
the primary leaf	is **intact** *or* shows **acceptable defects** – • discoloured or necrotic spots • slight damage or deformation

1 Superficial cracks or splits: not affecting the conducting tissues.

Abnormal seedlings	
Seedling as a whole	
the seedling	is **abnormal** if it • is deformed • is fractured (e.g. the seed is broken off) • consists of fused twin seedlings • is yellow or white • is spindly • is glassy • is decayed as a result of primary infection
one or more of the essential structures	are **abnormal**, as detailed in the following:
root system	
the primary root	is **defective** if it • is stunted or stubby • is retarded • is missing • is broken • is split from the tip • shows negative geotropism • is constricted • is spindly • is glassy • is decayed as a result of primary infection
shoot system	
the basal cotyledon part	is **defective** if it • is deformed • is decayed as a result of primary infection
the primary leaf	is **defective** if it • is deformed • is missing • is discoloured or necrotic • is decayed as a result of primary infection

Supplementary remarks

None

Normal Normal Normal Normal

Figure 10.1 *Freesia refracta*: normal seedling development.

Normal Abnormal Abnormal

Figure 10.2 *Freesia refracta* (abnormal type 11/04): the primary root is missing.

Section 11: Seedling Type C – Seedling Group A-1-2-2-1

A-1-2-2-1

Monocotyledons
with hypogeal germination
without epicotyl elongation

The primary root is essential

Representative genus: *Asparagus*

The seedling part that grows towards the light and becomes green is the epicotyl with the terminal bud.

The shoot system consists of an elongated epicotyl, which bears several small scale-leaves, the top ones forming the terminal bud.

The root system consists of the primary root and sometimes, depending on the species tested, secondary roots. Secondary roots are not taken into account in seedling evaluation.

Development of the seedling during the test

The cylindrical embryo in the seed is embedded in hard, semi-transparent, non-starchy endosperm.

At the start of germination, the primary root pierces the seed coat and is followed by a very short hypocotyl and the basal part of the cotyledon. The upper part of the cotyledon remains in the seed coat to absorb food reserves from the endosperm. Soon after the primary root emerges several secondary roots may develop[1]. The epicotyl emerges from the sheath-like basal part of the cotyledon and elongates. It bears several small scale-leaves, the top ones forming the terminal bud.

Differentiation of normal and abnormal seedlings

Normal seedlings	
Seedling as a whole	
all essential structures	are **normal** as detailed in the following:
root system	
the primary root	is **intact** *or* shows **acceptable defects** – • discoloured or necrotic spots • healed cracks or splits • superficial cracks or splits[2]
shoot system	
the hypocotyl and basal cotyledon part	are **intact**
the cotyledon	is **intact** – with a definite ‘knee’ *or* shows **acceptable defects** – • discoloured or necrotic spots • loose twists
the terminal bud	is **intact**

1 See supplementary remarks.
2 Superficial cracks or splits: not affecting the conducting tissues.

Abnormal seedlings	
Seedling as a whole	
the seedling	is **abnormal** if it • is deformed • is fractured (e.g. the seed is broken off) • consists of fused twin seedlings • is yellow or white • is spindly • is glassy • is decayed as a result of primary infection
one or more of the essential structures	are **abnormal**, as detailed in the following:
root system	
the primary root	is **defective** if it • is stunted or stubby • is retarded or missing • is broken or split from the tip • shows negative geotropism • is constricted • is spindly • is glassy • is decayed as a result of primary infection
shoot system	
the hypocotyl and basal cotyledon part	are **defective** if they • are deformed • are decayed as a result of primary infection
the epicotyl	is **defective** if it • is short and/or thick • is deeply cracked[3] or broken • is missing • is spindly • is glassy • is decayed as a result of primary infection
the terminal bud	is **defective** if it • is deformed • is missing • is decayed as a result of primary infection

Supplementary remarks

The most frequent *Asparagus* species dealt with in seed testing are:

- *A. officinalis* (an agricultural crop); and
- *A. setaceus* and *A. densiflorus* (two ornamentals crops).

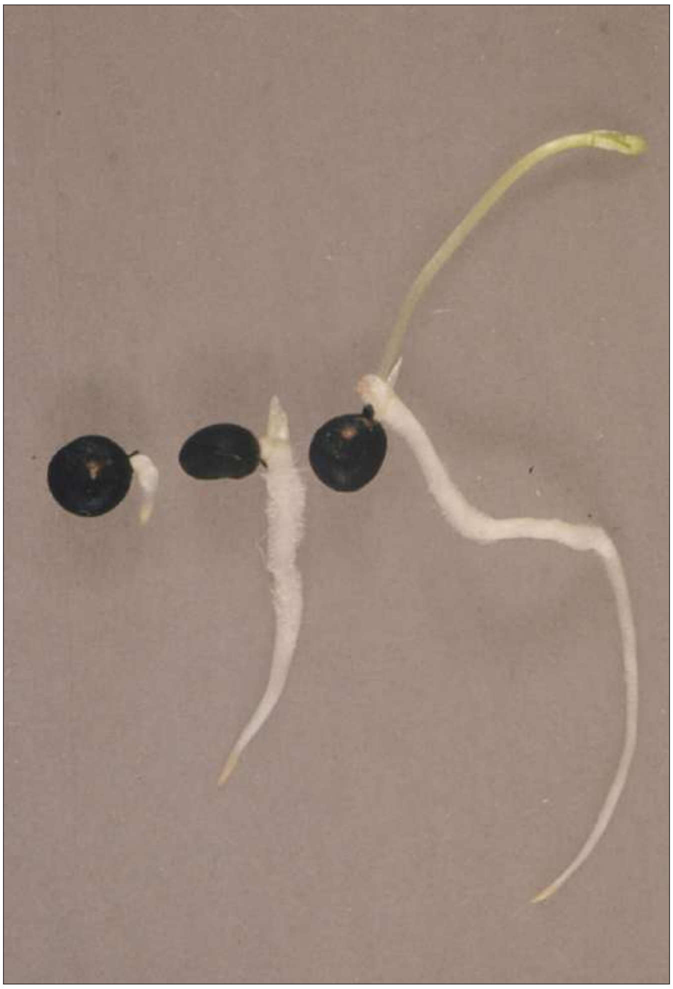
They differ in their root systems. *A. officinalis* has a slender primary root whereas *A. setaceus* has a very stout primary root. In both these species secondary roots do not normally develop within the test period.

On the other hand, in *A. densiflorus*, soon after the primary root emerges, several secondary roots are produced. One of them serves as a water storage organ even at an early stage of seedling development. At first, it has a club-like appearance and at a later stage it looks like an icicle. Seedlings of *A. densiflorus* without this type of secondary root are considered abnormal.

3 Deeply cracked: crack affecting the conducting tissues.

Normal **Normal** **Normal**

Figure 11.1 *Asparagus densiflorus*: normal seedling development.

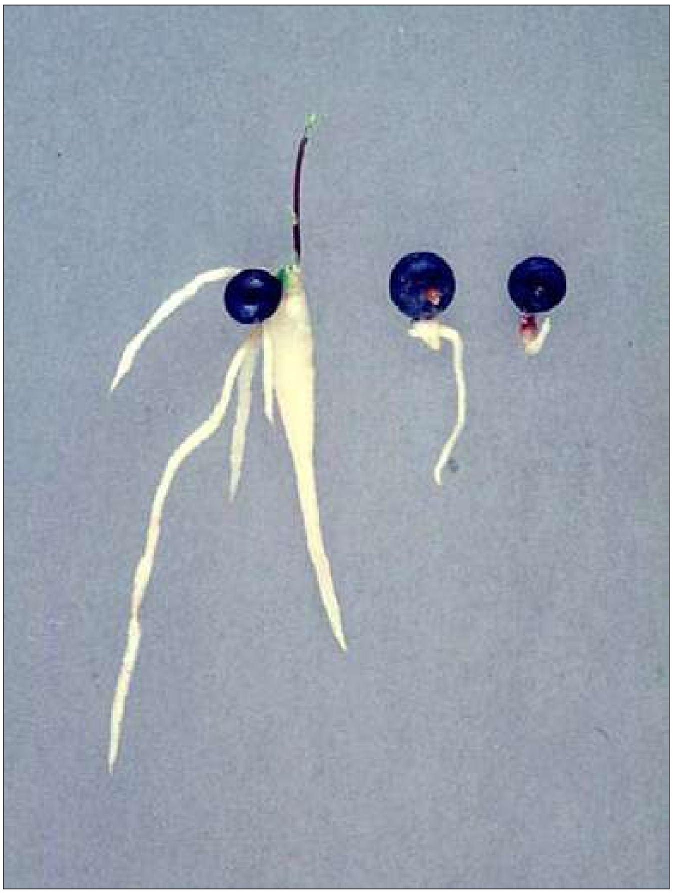


Normal **Normal** **Normal**

Figure 11.2 *Asparagus officinalis*: normal seedling development.

Normal **Normal** **Normal** **Normal**

Figure 11.3 *Asparagus setaceus*: normal seedling development.



Normal Abnormal Abnormal

Figure 11.4 *Asparagus densiflorus* (abnormal type 21/05 and 22/03): epicotyl and terminal bud are missing.

Normal Normal Normal

Figure 11.5 *Asparagus officinalis* (abnormal type 22/03): the terminal bud is missing. Even though two auxiliary shoots are present, the seedling is abnormal.

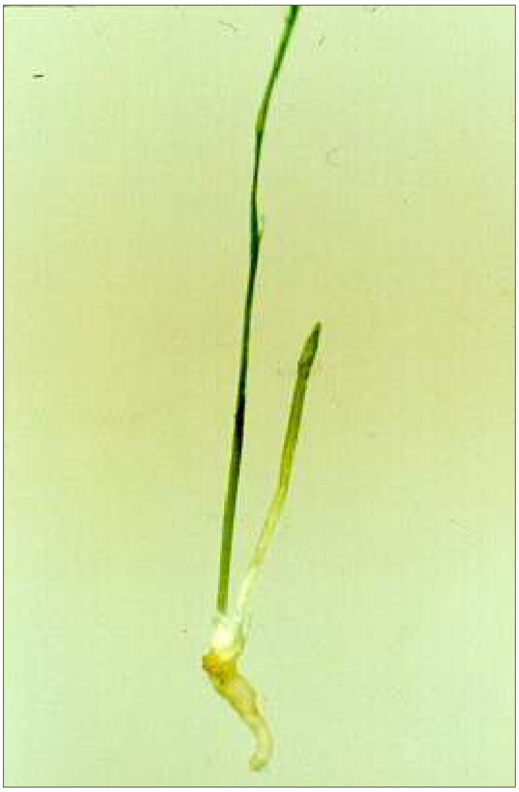


Figure 11.6 *Asparagus officinalis* (abnormal type 11/01): the primary root is stunted.

Section 12: Seedling Type D – Seedling Group A-1-2-3-1

A-1-2-3-1

Monocotyledons with hypogeal germination

The primary root is essential

Representative genus: *Lolium*

The seedling part that grows towards the light and turns green is the primary leaf. It is usually enclosed in a transparent sheath called the coleoptile.

The first foliage leaves develop within a protective sheath: the coleoptile. The coleoptile is regarded as the basal part of the cotyledon, which is considerably modified, compared with the cotyledon of the dicotyledons. The upper part of the cotyledon is called scutellum. It remains within the seed coat in contact with the endosperm.

The root system consists of a primary root, usually covered with root hairs. Secondary roots may develop during the test period. When the primary root is not sufficiently developed, secondary roots may be taken into account in seedling evaluation.

Development of the seedling during the test

The fruit of Poaceae is a caryopsis. In a great number of species of this group the caryopsis remains tightly enclosed with lemma and palea (for example in *Lolium*). In a number of other species the caryopsis is only loosely enclosed with lemma and palea, so that there may be both enclosed and naked caryopsis in a sample of seed (for example in *Arrhenatherum*). The embryo is situated at one end of the caryopsis. It consists of the embryo axis with the scutellum attached near to its centre. The embryo axis consists of the radicle at its lower end and the plumule at its upper end. Both ends are protected by sheath-like structures: the radicle by the coleorrhiza and the plumule by the coleoptile. The part of the seedling axis between the attachment of the scutellum and the coleoptile is termed the mesocotyl.

At the start of germination, the coleorrhiza first breaks through the seed coat and the primary root radicle pushes through the coleorrhiza immediately afterwards. The appearance of the primary root is followed by elongation of the coleoptile with the first leaf developing inside. Later in the test, the first leaf emerges from the coleoptile near its tip. Secondary roots may develop during the test period. The scutellum remains in close contact with the endosperm and serves to provide the growing seedling with nutrients. The mesocotyl may elongate considerably depending on the species tested and in response to test conditions.

Differentiation of normal and abnormal seedlings

Normal seedlings	
Seedling as a whole	
all essential structures	are **normal** as detailed in the following:
root system	
the primary root	is **intact** *and* the length of the primary root should be at least 50 % of the shoot length where secondary roots are absent *or* shows **acceptable defects** – • the length of the primary root can be less than 50 % of the shoot length provided: – it is at least 30 % of the shoot length; and – secondary roots are present; and – the combined length of the primary root and secondary roots are at least 60 % of the shoot length • discoloured or necrotic spots • healed cracks or splits • superficial cracks or splits[1]

1 Superficial cracks or splits: not affecting the conducting tissues.

Normal seedlings	
shoot system	
the mesocotyl (if developed)	is **intact** *or* shows **acceptable defects** – • discoloured or necrotic spots • healed cracks or splits • superficial cracks or splits[2]
the coleoptile	is **intact** *or* shows **acceptable defects** – • discoloured or necrotic spots • loose twists • a split for one third or less from the tip
Coleoptile elongation of grass seedlings is often limited under test conditions. Therefore, seedlings with comparatively short coleoptiles are considered normal, if otherwise normal.	
the primary leaf	is **intact** and emerging through the coleoptile near the tip *or* shows **acceptable defects** – • discoloured or necrotic spots • slightly retarded[3] growth

Abnormal seedlings	
Seedling as a whole	
the seedling	is **abnormal** if it • is deformed • is fractured • consists of fused twin seedlings • is yellow or white • is spindly • is glassy • is decayed as a result of primary infection • has a scutellum detached from the endosperm
one or more of the essential structures	are **abnormal**, as detailed in the following:
root system	
the primary root	is **defective** if it • is stunted or stubby • is retarded[4] • is missing • is broken • is split from the tip • is trapped in the fruit coat • shows negative geotropism • is constricted • is spindly • is glassy • is decayed as a result of primary infection

2 Superficial cracks or splits: not affecting the conducting tissues.
3 Slightly retarded: not reaching to the tip of the coleoptile, but at least half the length of the coleoptile.
4 The primary root is considered retarded if:
• in the absence of secondary roots, the primary root is less than 50% of the shoot length; or
• when secondary roots are present, it is less than 30% of the shoot length.

Abnormal seedlings	
shoot system	
the mesocotyl (if developed)	is **defective** if it • is deeply cracked or broken • is forming a loop • is tightly twisted • is decayed as a result of primary infection
the coleoptile	is **defective** if it • is deformed (for example short and thick) • is broken • is missing • shows a damaged tip or has no tip • is bent over • is forming a loop or spiral • is tightly twisted • is split from the tip for more than one third of its length • is split other than from the tip • is spindly • is decayed as a result of primary infection
the primary leaf	is **defective** if it • extends less than half the length of the coleoptile • is missing • is shredded or otherwise deformed • is yellow or white • is decayed as a result of primary infection

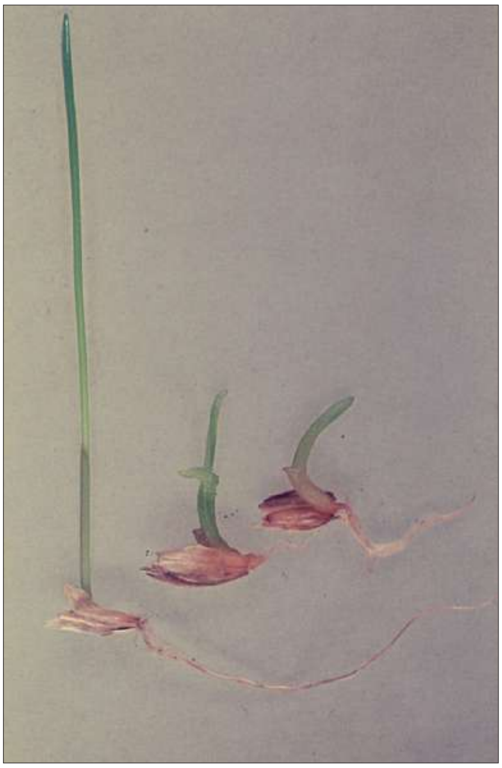
Supplementary remarks

Detached endosperm

Seedlings where the scutellum has become detached from the endosperm are considered abnormal.

Evaluation of the root system

In the absence of secondary roots, the primary root should be at least 50 % of the shoot length to be considered normal. Where secondary roots are present, the combined length of the primary and secondary roots should be at least 60 % of the shoot length with the minimum length of primary root being 30 % that of the shoot.



Normal **Abnormal** **Abnormal**

Figure 12.1 *Lolium* spp. (abnormal type 00/01): the seedling is deformed.

Normal **Abnormal** **Abnormal**

Figure 12.2 *Lolium* spp. (abnormal type 00/06): the seedling is yellow or white.

Normal **Abnormal** **Abnormal**

Figure 12.3 *Lolium* spp. (abnormal type 11/04): the primary root is missing.

Normal **Abnormal** **Abnormal**

Figure 12.4 *Lolium* spp. (abnormal type 41/08): the coleoptile is split from the tip for more than ⅓ of its length.

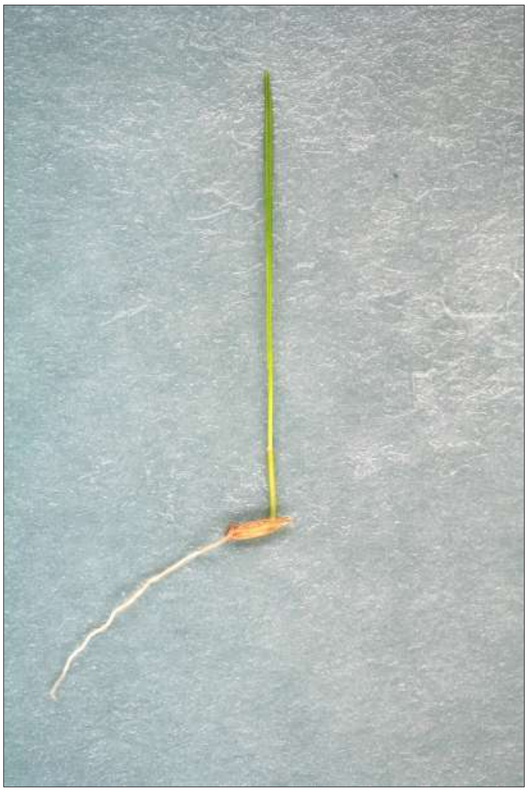


Figure 12.5 *Lolium* spp. (normal seedling): the length of the primary root is more than 50 % of the shoot length.

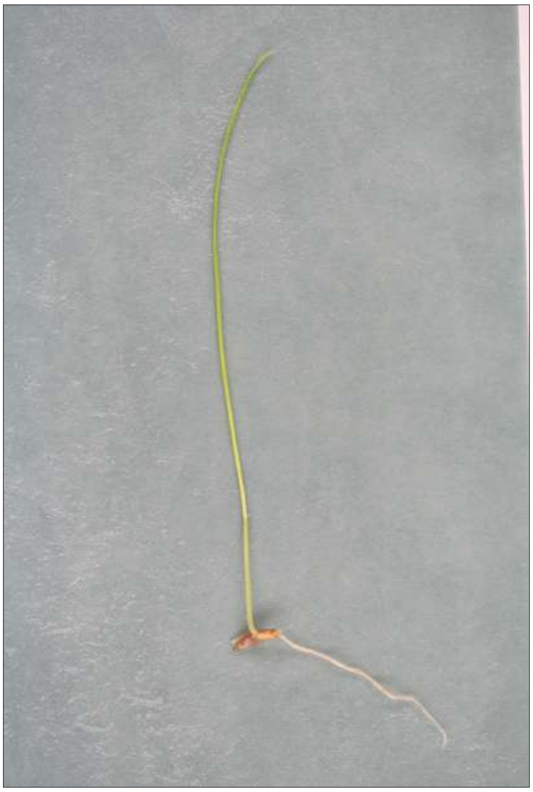


Figure 12.6 *Lolium* spp. (abnormal type 11/03): the length of the primary root is less than 50 % of the shoot length and secondary roots are missing.

Figure 12.7 *Lolium* spp. (normal seedling): the combined length of the primary root and secondary roots exceeds 60 % of the shoot length, with the primary root being at least 30 % the length of the shoot.

Figure 12.8 *Lolium* spp. (abnormal type 11/03): the combined length of the primary root and secondary roots are less than 60% of the shoot length.

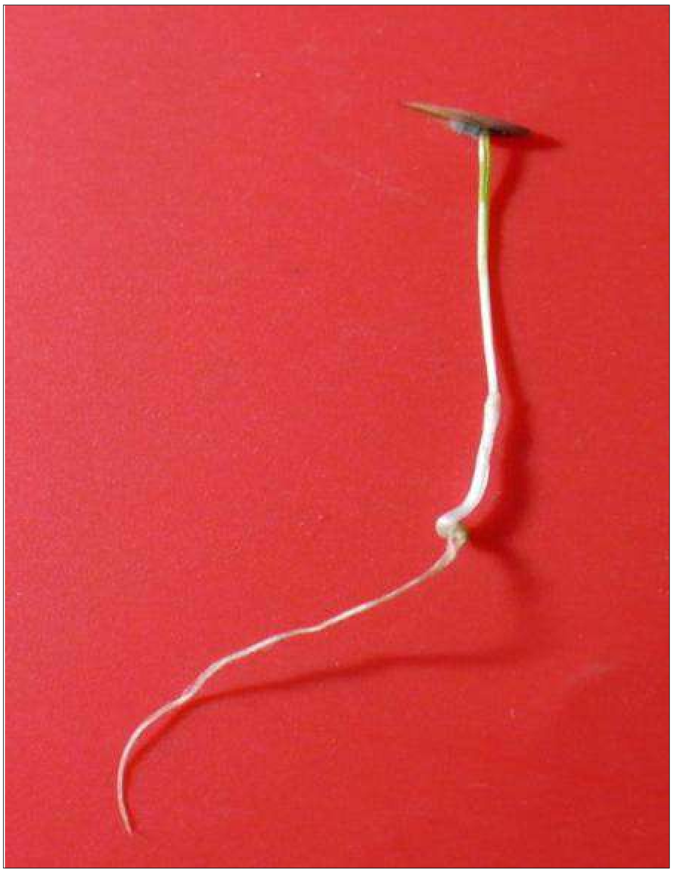


Figure 12.9 *Lolium* spp. (abnormal type 00/12): the seedling has a detached endosperm.

Section 13: Seedling Type D – Seedling Group A-1-2-3-2

A-1-2-3-2

Monocotyledons with hypogeal germination

The primary root may be replaced by secondary roots

Representative genus: *Zea*

Additional illustrated genera: *Oryza*, *Sorghum*

The seedling part that grows towards the light and turns green is the primary leaf. It is usually enclosed in a transparent sheath called the coleoptile.

The first foliage leaves develop within a protective sheath: the coleoptile. The coleoptile is regarded as the basal part of the cotyledon, which is considerably modified, compared with the cotyledon of the dicotyledons. The upper part of the cotyledon is called scutellum. It remains within the seed coat in contact with the endosperm.

The root system consists of a primary root, usually covered with root hairs and often numerous secondary roots, which are taken into account in seedling evaluation if the primary root is defective.

Development of the seedling during the test

The mature grain of the species of this group consists of a single caryopsis, which is comparatively large. In some species the caryopsis is tightly enclosed with the lemma and palea (e.g. in *Oryza*) in the harvested seed unit, whereas in other species the seed unit is a naked caryopsis (e.g. in *Sorghum* and *Zea*). The embryo is situated at one end of the caryopsis. It consists of the embryo axis with the scutellum attached near to its centre. The embryo axis consists of the radicle at its lower end and the plumule at its upper end. Both ends are protected by sheath-like structures: the radicle by the coleorrhiza and the plumule by the coleoptile. The part of the seedling axis between the attachment of the scutellum and the coleoptile is termed the mesocotyl.

At the start of germination the coleorrhiza first breaks through the seed coat and the primary root radicle pushes through the coleorrhiza immediately afterwards. Abundant secondary roots are usually produced during the test period. The appearance of the primary root is followed by elongation of the coleoptile with the first leaf developing inside. Later in the test the first leaf emerges from the coleoptile near its tip. The scutellum remains in close contact with the endosperm and serves to provide the growing seedling with nutrients. The mesocotyl may elongate considerably depending on the species (e.g. *Sorghum*) tested and in response to test conditions (e.g. in darkness).

Differentiation of normal and abnormal seedlings

Normal seedlings	
Seedling as a whole	
all essential structures	are **normal** as detailed in the following:
root system	
the primary root	is **intact** *or* shows **acceptable defects** – • discoloured or necrotic spots • healed cracks or splits • superficial cracks or splits[1]
Seedlings with a defective primary root are classed as normal, if sufficient normal secondary roots have developed.	

1 Superficial cracks or splits: not affecting the conducting tissues.

Normal seedlings	
shoot system	
the mesocotyl (if developed)	is **intact** *or* shows **acceptable defects** – • discoloured or necrotic spots • superficial cracks or splits[2] • loose twists
the coleoptile	is **intact** *or* shows **acceptable defects** – • discoloured or necrotic spots • loose twists • a split of one third or less from the tip
the primary leaf	is **intact**, *emerging* from the coleoptile near the tip *or* shows **acceptable defects** – • discoloured or necrotic spots • slightly retarded[3] growth
For *Oryza* and *Zea* see also supplementary remarks.	

Abnormal seedlings	
Seedling as a whole	
the seedling	is **abnormal** if it • is deformed • is fractured • consists of fused twin seedlings • is yellow or white • is spindly • is glassy • is decayed as a result of primary infection • has a scutellum detached from the endosperm
one or more of the essential structures	are **abnormal**, as detailed in the following:
root system	
the primary root	is **defective** if it • is stunted or stubby • is retarded or missing • is broken or split from the tip • shows negative geotropism • is constricted • is spindly • is glassy • is decayed as a result of primary infection
Seedlings with a defective primary root are classed as normal, if sufficient normal secondary roots have developed.	
shoot system	
the mesocotyl (if developed)	is **defective** if it • is cracked or broken • is forming a loop or spiral • is tightly twisted • is decayed as a result of primary infection
the coleoptile	is **defective** if it • is deformed (e.g. short and thick) • is broken or missing • has a damaged or missing tip • is strongly bent over • is forming a loop or spiral • is tightly twisted • is split for more than one third from the tip • is split other than from the tip • is spindly • is decayed as a result of primary infection

2 Superficial cracks or splits: not affecting the conducting tissues.
3 Slightly retarded: not reaching to the tip, but at least half the length of the coleoptile.

Abnormal seedlings	
shoot system	
the primary leaf	is **defective** if it • extends less than half the length of the coleoptile • is missing • is shredded or otherwise deformed • is yellow or white • is decayed as a result of primary infection

Supplementary remarks

Oryza sativa

With respect to seedling morphology and seedling development *Oryza sativa* differs somewhat from most of the other species of Poaceae common in seed testing. At the start of germination the first structure to show is the coleoptile, which is followed by the primary root some time later. The final length of the coleoptile is comparatively short, though it may vary somewhat, depending on variety and test conditions. When the first leaf penetrates the coleoptile it opens by a slit near the end and is gradually split further down by the emerging leaf. Yet, the basic part of the coleoptile remains closed and sheath-like, otherwise the seedling is considered abnormal. The first leaf consists of a leaf sheath only and remains tightly rolled. Only the second leaf, coming up through the first one, possesses a real leaf blade. The root system consists of a primary root and numerous secondary roots including lateral and adventitious roots.

Zea mays

By the time seedlings have reached the correct stage of development for evaluation, i.e. when the first foliage leaf has emerged from the coleoptile, the coleoptile often shows a long natural split due to enlargement of the leaves within the coleoptile.

Specific rules have been developed to evaluate coleoptile and first leaf damage in *Zea* and these are illustrated in Figure 13.1:

If the first leaf has emerged at time of evaluation, the seedling is abnormal if the coleoptile has any of the following defects together with damage to the primary leaf:

- coleoptile split for more than one-third of the length from the tip;
- coleoptile strongly bent over;
- coleoptile tip damaged or missing;
- coleoptile split at any location below the tip.

If the first leaf has not emerged at time of evaluation:

- tip of coleoptile damaged or missing;
- coleoptile split for more than one-third of the length from the tip;
- leaf protruding below the tip of the coleoptile.

Generally, the first leaf has emerged at time of evaluation in sand, compost or soil tests; but the first leaf has not emerged at time of evaluation in rolled towels tests.

If the first leaf is shredded or otherwise damaged, but the development of the following leaf (leaves) is satisfactory, the seedling is evaluated as normal, if otherwise normal. To assess the seedling correctly, it might be necessary to prolong the test until the second or third leaves have developed.

Zea mays and *Sorghum* spp.

Trapped coleoptile: A seedling with its coleoptile trapped under the lemma or the seed coat is considered normal, if development is otherwise normal. If the growth of such a seedling is stunted, it must be evaluated as abnormal.

Detached endosperm: Seedlings where the scutellum has become detached from the endosperm are considered abnormal.

ISTA codes for type of abnormal seedlings with a split in the coleoptile: In the case of *Zea mays*, the seedling is considered abnormal when the defect of the coleoptile is associated with leaf damage. To simplify the coding, the code of abnormal used, is the one describing the coleoptile defect, e.g. split from the top for more than a third of length (type 41/08) associated with a damaged leaf (42/03) will be considered as type of abnormal 41/08.

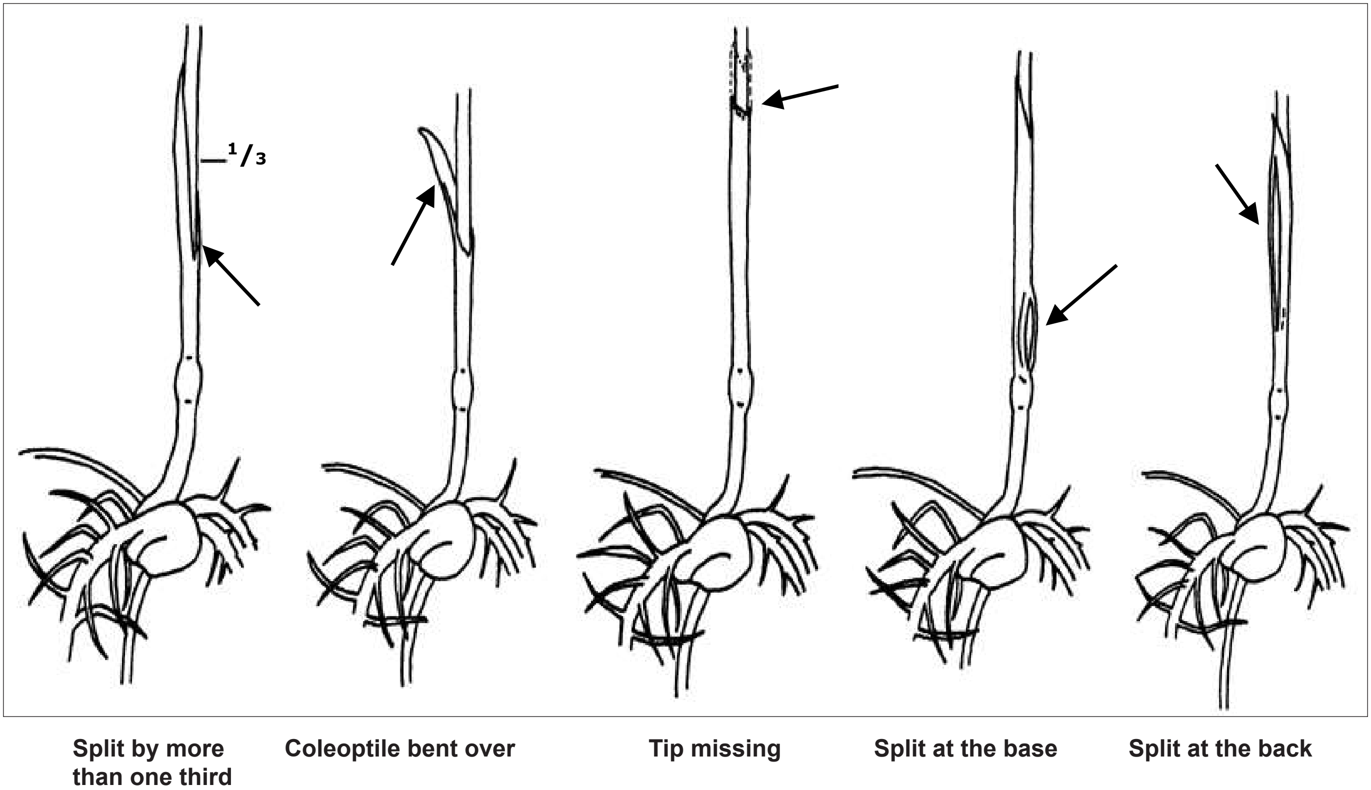


Figure 13.1 a Illustration of the coleoptile defects. Seedlings with these defects are normal if the first leaf is intact or only slightly damaged, as defined in Figure 13.1b. Seedlings with these defects are abnormal if first leaf is damaged, as defined in Figure 13.1b.

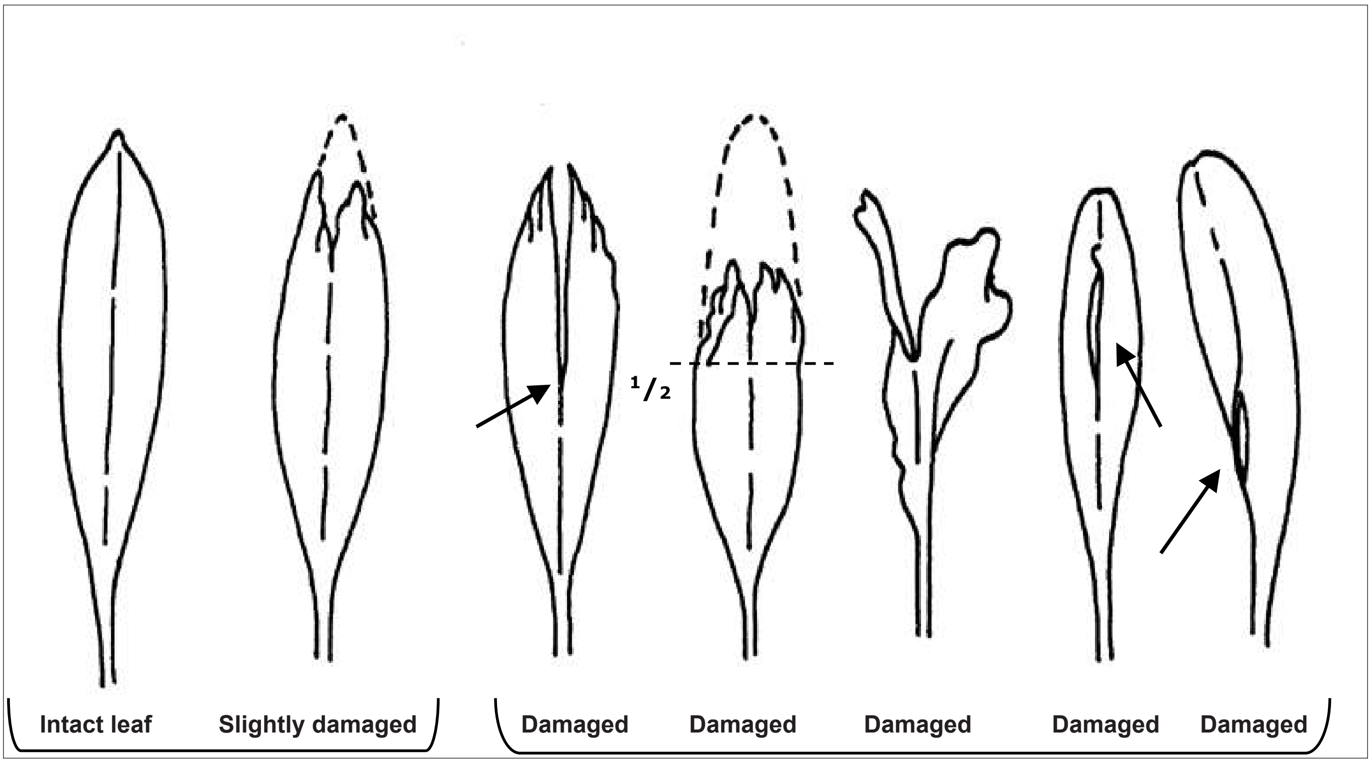


Figure 13.1 b Illustration of intact, slightly damaged and damaged first leaf, for evaluation of seedlings with coleoptile defects.

Normal **Abnormal** **Abnormal**

Figure 13.2 *Zea mays* (abnormal type 41/08): the coleoptile is split from the tip for more than a third of length and has damage to the first leaf.

Normal **Abnormals**

Figure 13.3 *Zea mays* (abnormal type 00/01): the seedling is deformed.

Normal **Abnormal** **Abnormal**

Figure 13.4 *Zea mays* (abnormal type 41/06): the coleoptile is forming a spiral.

Normal

Figure 13.5 *Zea mays* (normal): the coleoptile with split at the back and intact primary leaf.

Section 13: Type D – Group A-1-2-3-2 (Zea)

Figure 13.6 *Zea mays* (normal): a coleoptile with the tip missing and the primary leaf intact.

Figure 13.7 *Zea mays* (normal): a coleoptile with the tip missing and slight damage on the primary leaf.

Figure 13.8 *Zea mays* (normal): the coleoptile is split for more than one third and the primary leaf is slightly damaged for less than half of the length of the normal size.

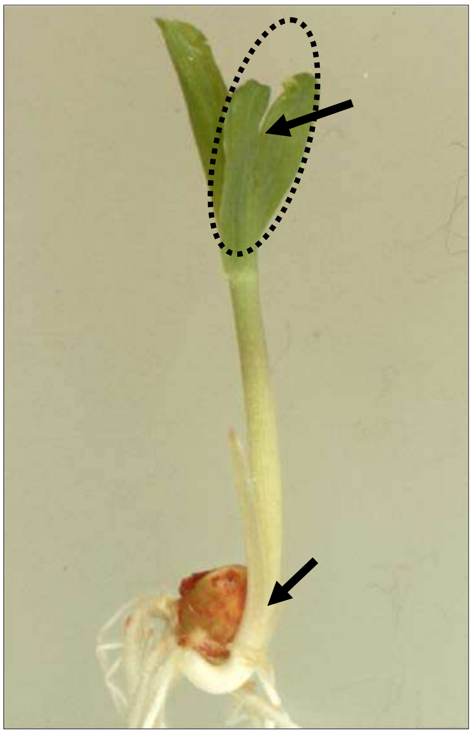


Figure 13.9 *Zea mays* (abnormal type 41/08): the coleoptile is split for more than one third and the leaf is damaged for more than half of the length of the normal size.

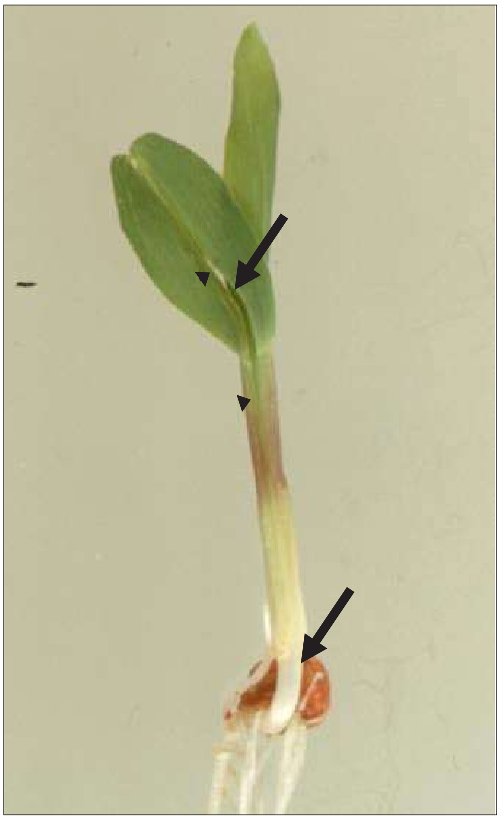


Figure 13.10 *Zea mays* (abnormal type 41/08): the coleoptile is split for more than one third and the leaf is split at the base.

Figure 13.11 *Zea mays* (abnormal type 41/08): the coleoptile is split for more than one third and the leaf is damaged.

Figure 13.12 *Zea mays* (abnormal type 41/05): the coleoptile is bent over and the leaf is split.

Figure 13.13 *Zea mays* (abnormal type 00/12): the seedling has a detached endosperm.

Figure 13.14 *Oryza sativa*: normal seedling development.

Figure 13.15 *Oryza sativa* (abnormal type 00/09): the seedling is decayed as a result of primary infection.

Figure 13.16 *Oryza sativa* (abnormal type 11/04): the primary root is missing and secondary roots are insufficiently developed.

Normal Abnormal Abnormal

Figure 13.17 *Sorghum* spp. (abnormal type 41/04): the coleoptile is damaged and has no tip.

Normal Abnormal Abnormal

Figure 13.18 *Sorghum* spp. (abnormal type 21/12): the mesocotyl and seed are decayed as a result of primary infection.

Figure 13.19 *Sorghum* spp. (abnormal type 00/12): the seedling has a detached endosperm.

Section 14: Seedling Type D – Seedling Group A-1-2-3-3

A-1-2-3-3

Monocotyledons with hypogeal germination

There are several seminal roots instead of a primary root

Representative genus: *Triticum*

Additional illustrated genera: *Hordeum*, *Secale*

The seedling part that grows towards the light and turns green is the primary leaf. It is usually enclosed in a transparent sheath called the coleoptile.

The first leaves develop within a protective sheath: the coleoptile. The coleoptile is regarded as the basal part of the cotyledon, which is considerably modified compared with the cotyledons of the dicotyledons. The upper part of the cotyledon is called the scutellum; it remains within the seed coat in contact with the endosperm.

The root system is composed of several seminal roots, usually with root hairs, with no distinction in size or evaluation.

Development of the seedling during the test

The caryopsis of the species in this group (temperate cereals) is comparatively large. Some of the species have their caryopsis tightly enclosed within the lemma and palea (e.g. *Hordeum*), whereas in others the caryopsis is naked (e.g. *Secale* and *Triticum*). The embryo is situated at one end of the caryopsis. It consists of the embryo axis with the scutellum attached near to its centre. The embryo axis has a radicle and a specific number of seminal root initials at its lower end, and a plumule at its upper end. Both ends are protected by sheath-like structures: the radicle by the coleorrhiza and the plumule by the coleoptile. The part of the seedling axis between the attachment of the scutellum and the coleoptile is called the mesocotyl.

At the start of germination, the coleorrhiza is first to break through the seed coat and the primary root pushes through the coleorrhiza followed almost simultaneously by the other seminal roots. The elongation of the coleoptile follows the appearance of the seminal roots with the primary leaf developing inside. Later the primary leaf may emerge from the coleoptile near its tip. The mesocotyl may elongate considerably depending on the species tested and as a response to test conditions.

The scutellum remains in close contact with the endosperm and serves to provide the growing seedling with nutrients from the endosperm.

Differentiation of normal and abnormal seedlings

Normal seedlings	
Seedling as a whole	
all essential structures	are **normal** as detailed in the following:
root system	
at least one seminal root	is **intact** *or* shows **acceptable defects** – • discoloured or necrotic spots • healed cracks or splits • superficial cracks or splits[1]
shoot system	
the mesocotyl (if developed)	is **intact** *or* shows **acceptable defects** – • discoloured or necrotic spots • healed cracks or splits • superficial cracks or splits[1]

1 Superficial cracks or splits: not affecting the conducting tissues.

Normal seedlings	
shoot system	
the coleoptile	is **intact** *or* shows **acceptable defects** – • discoloured or necrotic spots • loose twists • a split of one third or less from the tip • healed cracks or splits • superficial cracks or splits[2]
the primary leaf	is **intact**, emerging from the coleoptile near the tip, or reaching at least half the length of the coleoptile *or* shows **acceptable defects** – • discoloured or necrotic spots • slightly damaged

Abnormal seedlings	
Seedling as a whole	
the seedling	is **abnormal** if it • is deformed • is fractured • consists of fused twin seedlings • is yellow or white • is spindly • is glassy • is decayed as a result of primary infection
one or more of the essential structures	are **abnormal**, as detailed in the following:
root system	
seminal roots	are **defective** or **insufficient** if they • are stunted • are stubby • are retarded • are missing • show negative geotropism • are glassy • are decayed as a result of primary infection
A seedling is considered normal if there is one intact seminal root.	
shoot system	
the mesocotyl (if developed)	is **defective** if it • is deeply cracked or broken • is decayed as a result of primary infection
the coleoptile	is **defective** if it • is stubby (e.g. due to phytotoxic effect) • is broken • is missing • is damaged or missing from the tip for more than one third of its length • is bent over or forming a loop • is forming a spiral • is tightly twisted • is split from the tip for more than one third of its length • is split other than from the tip • is spindly • is decayed as a result of primary infection
the primary leaf	is **defective** if it • extends less than half the length of the coleoptile • is missing • is shredded or otherwise deformed • is yellow or white • is decayed as a result of primary infection

2 Superficial cracks or splits: not affecting the conducting tissues.

Supplementary remarks

Seedling stage

In sand and soil/compost tests, evaluation should not take place before the primary leaf has emerged from the coleoptile in most of the seedlings. Seedlings, which at the end of the test period have not reached this stage of development, are considered normal, if they are otherwise normal. Special attention should be paid to the leaf within the coleoptile: the seedling must be evaluated as abnormal, if the leaf does not reach at least half the length of the coleoptile. In paper towelling tests the primary leaf rarely emerges from the coleoptile prior to evaluation. As with sand and soil/compost tests, special attention should be paid to the leaf within the coleoptile: the seedling must be evaluated as abnormal, if the leaf does not reach at least half the length of the coleoptile.

Split coleoptiles

Seedlings with a split coleoptile are considered normal when the split runs down from the tip to one third or less of the coleoptile length. If the split extends to more than one third or occurs anywhere other than from the tip, the seedling must be classified as abnormal. When the length of the split is assessed, care should be taken not to enlarge the split by handling.

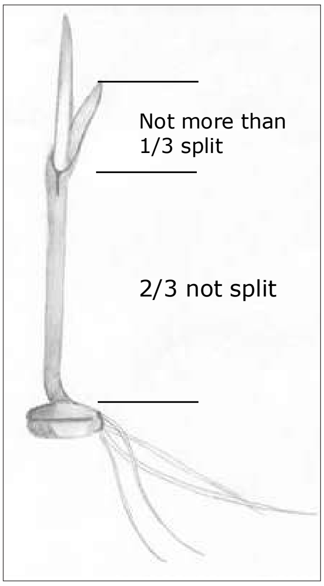


Figure 14.1 For the seedling to be evaluated as normal, a split from the tip of the coleoptile must not exceed one third of the coleoptile length (the ⅓ rule).

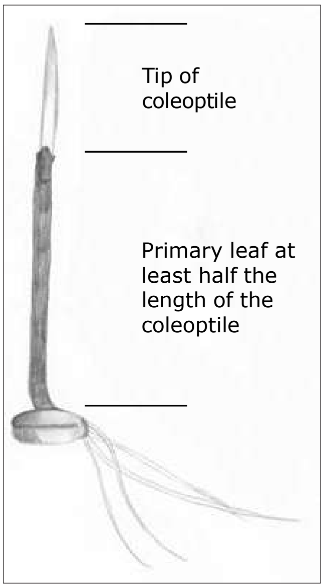


Figure 14.2 For the seedling to be evaluated as normal, the primary leaf must extend at least half the length of the coleoptile (the ½ rule).

Trapped coleoptile

A seedling with its coleoptile trapped under the lemma or the seed coat is considered normal, if development is otherwise normal. If the growth of such a seedling is stunted, it must be evaluated as abnormal.

Detached endosperm

Seedlings where the scutellum has become detached from the endosperm are considered abnormal.

Phytotoxic symptoms

Seed samples of chemically treated cereals, germinated on an artificial substrate such as paper, can sometimes produce seedlings with phytotoxic symptoms, such as stubby and swollen coleoptiles and stubby seminal roots. If there are 5 % or more of such seedlings in a test, the sample must be retested in soil or compost.

Soil/compost may allow a more realistic evaluation of the sample, as the soil/compost will absorb some of the chemical and the phytotoxic symptoms will diminish or even completely disappear. All seedlings with phytotoxic symptoms in soil/compost tests must be evaluated as abnormal.

Negative geotropism

Use of herbicides such as glyphosate in crops may result in the seed harvested from such crops displaying negative geotropism in germination tests conducted using media such as paper and sand. If there are 5 % or more of such seedlings in a test, the sample must be retested in soil/compost. Soil/compost may allow a more realistic evaluation of the sample, as the soil/compost will absorb glyphosate on the surface of the seed and symptoms will diminish or even completely disappear. All seedlings without one good seminal root growing downwards in compost tests must be evaluated as abnormal. Such abnormal seedlings have poor root hair development and as such have poor anchorage in soil/compost tests.

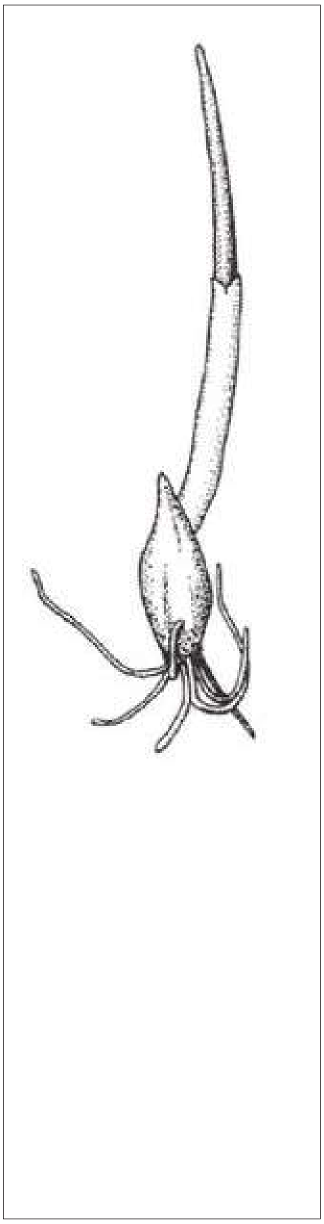


Figure 14.3 Seedling with negative geotropism evaluated as abnormal – all of the **seminal roots are short and splayed**.

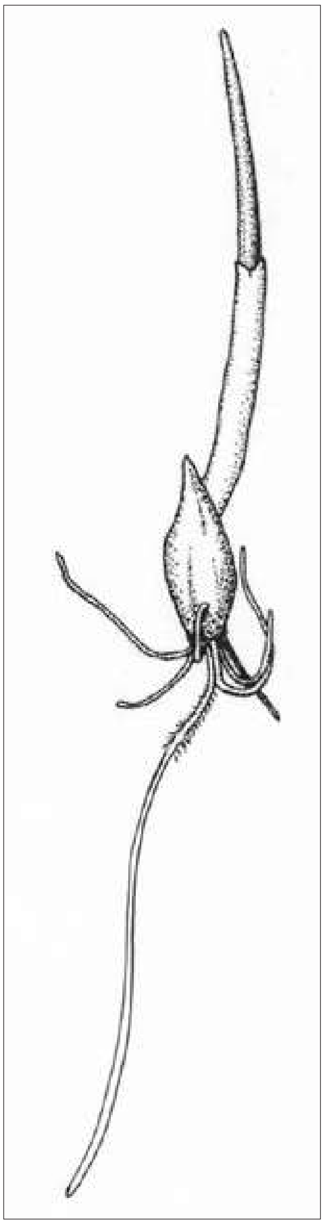


Figure 14.4 Seedling with negative geotropism evaluated as normal – one of the **seminal roots is of normal length**, has root hairs and is growing downwards.

Normal **Abnormals**

Figure 14.5 *Triticum aestivum* (abnormal type 00/10): phytotoxic seedlings with stubby and thickened seminal roots and coleptile.

Figure 14.6 *Triticum aestivum* (abnormal type 12/02): the seminal roots are stubby.

Figure 14.7 *Triticum aestivum* (abnormal type 12/04): the seminal roots are missing.

Figure 14.8 *Triticum aestivum* (abnormal type 12/07): the seminal roots are decayed as a result of a primary infection.

Normal Abnormal Abnormal

Figure 14.9 *Triticum aestivum* (abnormal type 41/04): the coleoptile tips are damaged with more than one third missing.

Normal Abnormal Abnormal Abnormal

Figure 14.10 *Triticum aestivum* (abnormal type 41/05): the coleoptile is forming a loop.

Normal Abnormal Abnormal

Figure 14.11 *Triticum aestivum* (abnormal type 42/01): the primary leaf extends for less than half the length of the coleoptile.

Figure 14.12 *Hordeum vulgare* (normal seedlings): loosely twisted coleoptile.

Normal **Abnormal** **Abnormal**

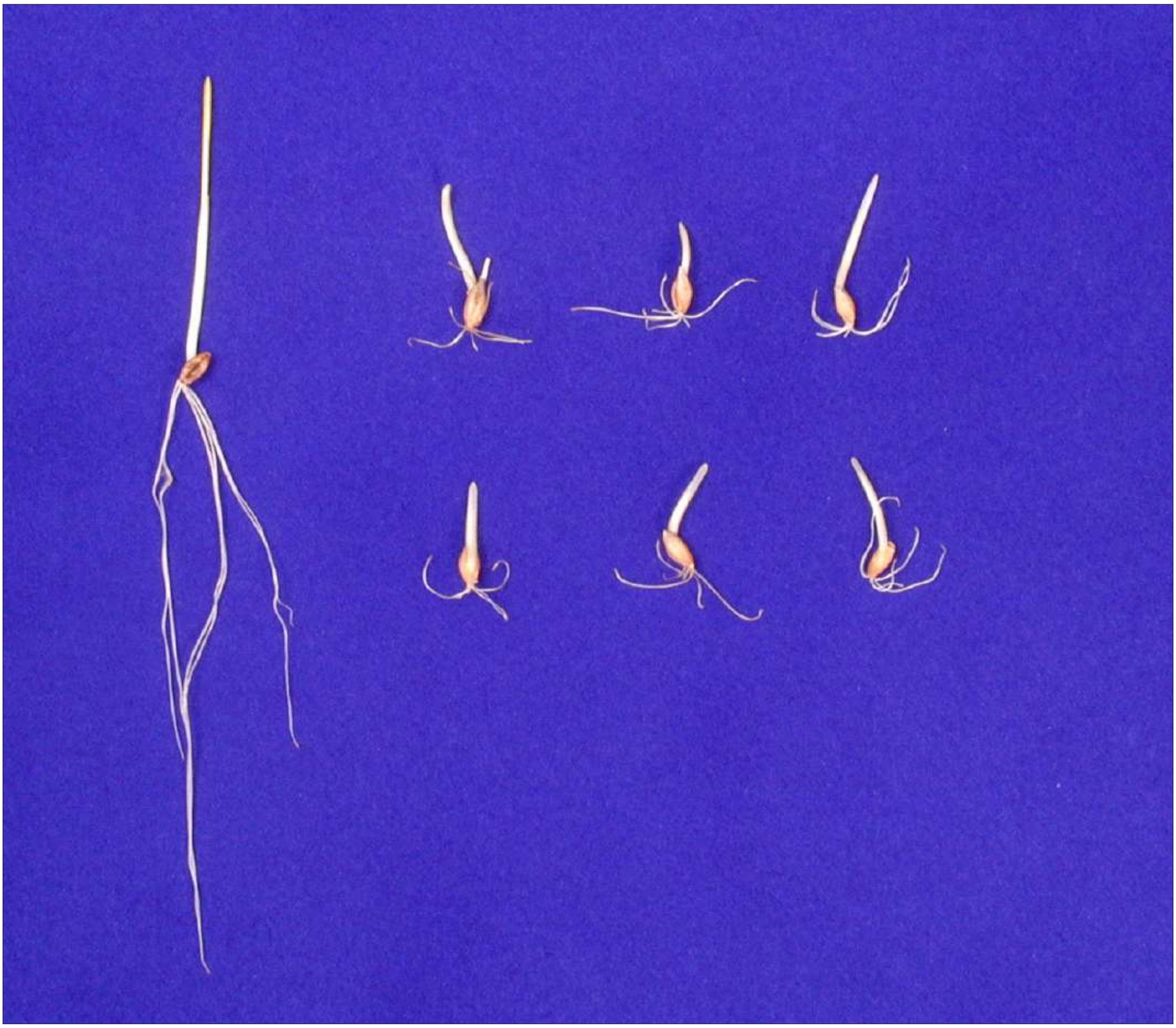
Figure 14.13 *Hordeum vulgare* (abnormal type 41/08): the coleoptile is split for more than one third the length from the tip and is split from other than the tip.

Normal **Abnormal** **Abnormal**

Figure 14.14 *Secale cereale* (abnormal type 41/08 and 42/03): the coleoptile is split for more than one third the length from the tip and the primary leaves are shredded.

Normal **Abnormal** **Abnormal**

Figure 14.15 *Secale cereale* (abnormal type 41/08 and 42/04): the coleoptile is split for more than one third the length from the tip and the leaf is protruding from the base of the coleoptile.



Normal **Abnormals**

Figure 14.16 *Hordeum vulgare* (abnormal type 11/08): seedlings are showing negative geotropism.

Section 15: Seedling Type E – Seedling Group A-2-1-1-1

A-2-1-1-1

Dicotyledons with epigeal germination without epicotyl elongation

The primary root is essential

Representative genus: *Brassica*

Additional illustrated genera: *Apium*, *Beta*, *Daucus*, *Dianthus*, *Helianthus*, *Lactuca*, *Trifolium*

The seedling part that grows towards the light is the hypocotyl with two cotyledons attached that turn green.

The shoot system consists of the elongated hypocotyl and two cotyledons with the terminal bud lying between them. There is no epicotyl elongation within the test period; epicotyl and terminal bud are not usually discernible.

The root system consists of the primary root, usually with root hairs, which must be well developed. Secondary roots may occasionally develop during the test period, but they are not taken into account in seedling evaluation.

Development of the seedling during the test

The embryos in the seeds of the genera of this group are of various sizes and shapes. They can be embedded in endosperm (e.g. *Daucus*) or in perisperm (e.g. *Beta* and *Dianthus*), or the food reserves can be contained in the cotyledons (e.g. *Brassica* and *Helianthus*).

At the start of germination, the primary root pierces the seed coat and elongates rapidly. Most of the species of this group do not usually develop secondary roots within the test period. The hypocotyl elongates and raises the cotyledons, which rapidly expand, become green and start photosynthesis. The epicotyl does not develop within the prescribed test period, and the terminal bud is hardly visible between the cotyledons.

Differentiation of normal and abnormal seedlings

Normal seedlings	
Seedling as a whole	
all essential structures	are **normal** as detailed in the following:
root system	
the primary root	is **intact** *or* shows **acceptable defects** – • discoloured or necrotic spots • healed cracks or splits • superficial cracks or splits[1]
shoot system	
the hypocotyl	is **intact** *or* shows **acceptable defects** – • discoloured or necrotic spots • healed cracks or splits • superficial cracks or splits[1] • loose twists
the terminal bud and surrounding tissues	(usually not visible) are **intact**
the cotyledons	are **intact** *or* show **acceptable defects** – • **up to 50 %** of tissue not functioning normally • only **one** (intact) cotyledon • three cotyledons

1 Superficial cracks or splits: not affecting the conducting tissues.

Abnormal seedlings	
Seedling as a whole	
the seedling	is **abnormal** if it • is deformed • is fractured • the cotyledons emerge before the primary root from the seed coat • consists of fused twin seedlings • is yellow or white • is spindly • is glassy • is decayed as a result of primary infection
one or more of the essential structures	are **abnormal**, as detailed in the following:
root system	
the primary root	is **defective** if it • is stunted or stubby • is retarded • is missing • is broken • is split from the tip • is trapped in the seed coat* • shows negative geotropism • is constricted • is spindly • is glassy • is decayed as a result of primary infection
A seedling is classed as abnormal if the primary root is defective, even if secondary roots have developed. * A seedling with its primary root trapped in the seed coat is considered normal, if by the end of the test the root tip has found its way out of the seed coat.	
shoot system	
the hypocotyl	is **defective** if it • is too short and/or thick • is deeply cracked or broken[2] • is split right through • is missing • is bent over or forming a loop • is tightly twisted or forming a spiral • is constricted • is spindly • is glassy • is decayed as a result of primary infection
the terminal bud or surrounding tissues	(usually not visible) are **defective**
the cotyledons	are **defective** if they • are **defective to such an extent, that less than 50 %** of the original tissue (or estimated tissue) is functioning normally • are swollen or curled • are deformed • are broken or otherwise damaged • are separate or missing • are discoloured or necrotic • are glassy • are decayed as a result of primary infection
Damage or decay of the cotyledons at the point of attachment to the seedling axis or near the terminal bud render a seedling abnormal – irrespective of the 50 % rule.	

2 Deeply cracked: crack affecting the conducting tissue.

Supplementary remarks

***Brassica* spp.**

It must be stressed that the seedlings should not be evaluated, before all essential parts have developed, particularly before the cotyledons have freed themselves from the seed coat, and can be evaluated properly. Otherwise abnormals may be easily overlooked.

The evaluation of the cotyledons is of special importance in *Brassica* species, not only with regard to necrotic and decayed areas, but also with regard to chlorophyll deficiency (white or yellow areas). In order to make this latter type of abnormals visible, the seedlings must be grown in full light – at least for some 24 hours before evaluation takes place. In general the 50 % rule is valid, but if the basal part of the cotyledons (the area around the petiole and the point of attachment of the cotyledons to the hypocotyl) is affected by discolouration, necrosis or decay, the seedling should be considered abnormal since the nutrition pathways to the seedling axis are blocked.

It must be pointed out, that a normal primary root is essential in all *Brassica* species and other Brassicaceae (e.g. *Raphanus*), even if a seedling with a defective or missing primary root produces a number of secondary roots. If there are 5 % or more seedlings with defective primary roots (e.g. stunted) in a sample tested using paper media, it is recommended that the sample should be retested in sand, soil or compost to check the result.

Lactuca sativa

So called physiological necroses may cause problems with regard to a correct evaluation of *Lactuca* seedlings. Physiological necroses include a series of various symptoms of abnormality on cotyledons and hypocotyls often associated with aged seed. The symptoms range from brown spots or areas on the cotyledons to completely discoloured or necrotic or decayed cotyledons, and from slightly shortened to extremely short and/or thick, bent or glassy hypocotyls.

With regard to cotyledon evaluation the 50 % rule is applied. However, discoloured or necrotic areas near the terminal bud and the attachment of the cotyledons to the seedling axis render a seedling abnormal – irrespective of the 50 % rule. For correct evaluation of the cotyledons, the seedlings should not be evaluated before the cotyledons have freed themselves from the seed coat. In order to promote this process and to facilitate evaluation, it is recommended light is given and bell jars are removed (where these are used in TP tests), a few hours prior to assessment. If the seed coat sticks to the cotyledons due to necrosis or decay and cannot be removed without damage to the seedling, the seedling must be considered abnormal.

Beta vulgaris

Beta vulgaris germination tests are sometimes difficult to evaluate and the results among different stations can show larger than normal variation. The main reason for this is difficulties in the evaluation and classification of seedlings that are infected with fungi (particularly *Phoma betae*). In the evaluation of seedlings it is vital that one clearly distinguishes between primary infection from secondary infection:

- Primary infection is present, if the parent seed is the source of infection.
- Secondary infection is present, if the source of fungus is from outside the parent seed, e.g. from other seedlings or from the structures of the cluster around the seed.

ISTA has adopted the following method to evaluate infected *Beta* seed, which has proved to give reliable results. Using this method each seedling is evaluated at two different stages of development:

1. at an early stage, to find out, whether primary or secondary infection is present; and
2. at a later stage, to evaluate seedling structures.

Procedure

The clusters are pre-washed and dried back according to the recommendations in the ISTA Rules before being planted in pleated filter paper, which is the best substrate for this method.

First count (on the 3rd or 4th day):

At this early stage the seedlings show only part of their primary roots and are too small for a final evaluation. However, at this early stage of seedling development it is easy to differentiate between primary and secondary infection:

- Any seedling with a primary root emerging healthy (white) from the cluster is considered normal and is marked with a sign (e.g. with a water-soluble pencil), irrespective of whether other parts of the root may be infected (brown).
- Any seedling with a primary root emerging diseased (brown or black) from the cluster is considered abnormal, no matter whether other parts of the root (e.g. tip) may be healthy (white); it is marked with a different sign.
- The diseased seedlings must be removed from the substrate to prevent spread of the infection, but the cluster concerned must remain in the test, since more seedlings might emerge from the cluster. Roots, which are clearly abnormal for reasons other than infection, are marked with an ‘A’.

Second count (usually on the 7th day):

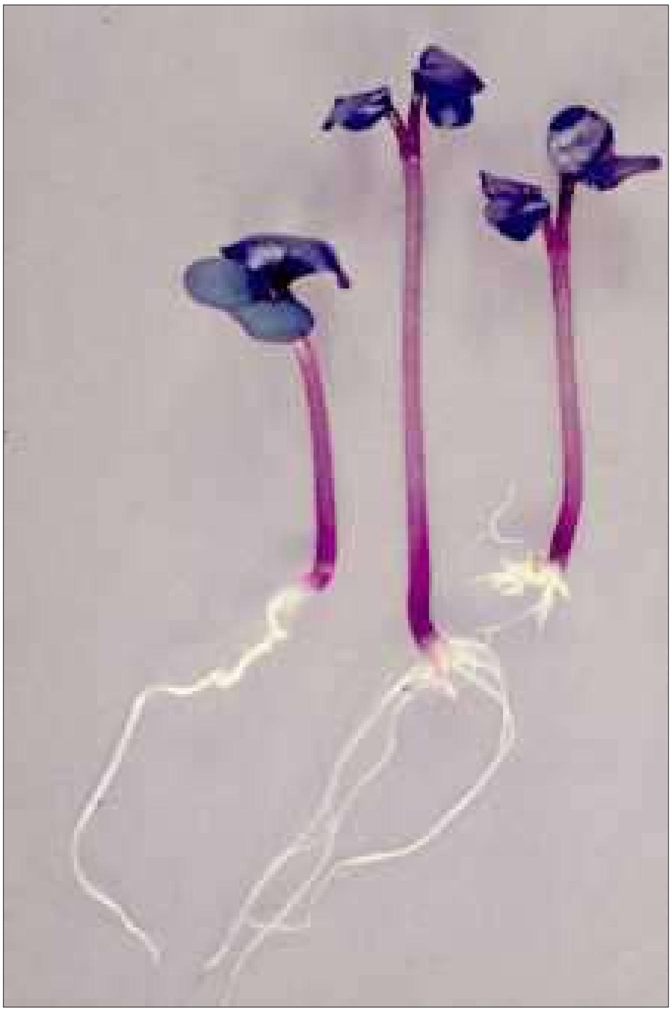
Most of the seedlings have now developed all their essential parts needed for the final evaluation:

- If a seedling is normal with regard to its essential parts and if its root was marked as healthy at the previous count, it is considered normal, and the cluster is removed and recorded. This is the case even if it is completely decayed at the time of the second count and the infection seems to have come from within the cluster.
- Clearly abnormal and decayed seedlings are again removed from the test, but their clusters have to remain in the substrate until the final count.

The same procedure is applied for the following and the final counts.

If in addition to germination, the monogermity[3] has to be determined, the procedure has to be adapted, but the principle remains the same.

Another means of avoiding problems associated with infection is the disinfection of the seed sample. ISTA Rules allow the application of a fungicide treatment to samples of *Arachis hypogaea* and *Beta vulgaris* prior to planting the seed for germination, when the seed lot is known not to have received such a treatment. When a fungicidal pre-treatment is used, the name of the chemical, the percentage of active ingredients and the method of treatment must be reported on the ISTA International Seed Analysis Certificate.



Normal Abnormal Abnormal

Figure 15.1 *Brassica* spp. (abnormal type 11/01): the primary root is stunted, even though secondary roots are present, the seedling is abnormal.

Normal Abnormal Abnormal Abnormal

Figure 15.2 *Brassica* spp. (abnormal type 11/07): the primary root is trapped in the seed coat.

Normal Abnormal Abnormal Abnormal

Figure 15.3 *Brassica* spp. (abnormal types 00/01): the seedling is deformed. The primary root is missing, the hypocotyl is too short and the cotyledons are necrotic.

3 Monogermity: a measure of the number of clusters that produce one normal seedling.

Normal Abnormal Abnormal

Figure 15.4 *Lactuca sativa* (abnormal type 31/05): the cotyledons are deformed and necrotic near the terminal bud (physiological necrosis).

Normal Abnormal Abnormal

Figure 15.5 *Lactuca sativa* (abnormal type 31/05): although less than 50 % of the cotyledons are deformed, it is abnormal due to physiological necrosis.

Normal Abnormals

Figure 15.6 *Lactuca sativa* (abnormal type 31/05): more than 50 % of the cotyledon area is discoloured; the seedling is abnormal (physiological necrosis).

Normal Abnormals

Figure 15.7 *Lactuca sativa* (abnormal type 21/01 and 22/04): the hypocotyl is too short and/or thick and the tissue surrounding the terminal bud is necrotic, the seed coat is stuck to the necrotic or decayed cotyledons.

Normal Abnormal Abnormal Abnormal

Figure 15.8 *Lactuca sativa* (abnormal type 21/06): the hypocotyl is bent over or forming a loop.

Normal Normal

Figure 15.9 *Beta vulgaris* (secondary infection): the seedling is emerging healthy from the cluster. The seedling is normal.

Normal Abnormals

Figure 15.10 *Beta vulgaris* (abnormal type 00/09, primary infection): the seedling is emerging diseased from the cluster. The seedling is abnormal.

Normal **Abnormal** **Abnormal** **Abnormal**

Figure 15.11 *Trifolium* spp. (abnormal type 00/02): the seedling is fractured.

Normal **Abnormal** **Abnormal** **Abnormal**

Figure 15.12 *Trifolium* spp. (abnormal type 00/08): the seedling is glassy.

Normal **Abnormal** **Abnormal** **Abnormal**

Figure 15.13 *Trifolium* spp. (abnormal type 11/07): the primary root is trapped in the seed coat.

Figure 15.14 *Trifolium* spp. (abnormal type 11/06): the primary root is split from the tip.

Figure 15.15 *Trifolium* spp. (abnormal types 21/03 and 11/01): the hypocotyl is deeply cracked or broken and two seedlings have stunted roots.

Figure 15.16 *Trifolium* spp. (abnormal type 21/07): the hypocotyl is forming a spiral.

Normal **Abnormal** **Abnormal** **Abnormal**

Figure 15.17 *Daucus carota* (abnormal types 00/01): the seedling is deformed (the primary root is missing 11/04 and the hypocotyl is short and/or thick 21/01).

Normal **Abnormal** **Abnormal** **Abnormal**

Figure 15.18 *Apium graveolens* (abnormal type 21/06): the hypocotyl is forming a loop.

Normal **Abnormal** **Abnormal** **Abnormal**

Figure 15.19 *Daucus carota* (abnormal type 21/12): the hypocotyl is decayed as a result of primary infection.

Figure 15.20 *Helianthus annuus* (abnormal type 11/01): the primary root is stunted; even though secondary roots are present, the seedling is abnormal.

Figure 15.21 *Helianthus annuus* (abnormal type 21/06): the hypocotyl is forming a loop.

Figure 15.22 *Helianthus annuus* (abnormal type 21/03): the hypocotyl is deeply cracked or broken.

Normal **Abnormals**

Figure 15.23 *Helianthus annuus* (abnormal type 21/09): the hypocotyl is constricted.

Normal **Abnormal** **Abnormal**

Figure 15.24 *Helianthus annuus* (abnormal type 21/01 and 21/07): the hypocotyl is short and/or thick; the hypocotyl is forming a spiral.

Normal **Abnormal** **Abnormal** **Abnormal**

Figure 15.25 *Helianthus annuus* (abnormal type 31/07, 21/12 and 11/12): decay as a result of primary infection.

Normal Abnormal Abnormal Abnormal

Figure 15.26 *Dianthus* spp. (abnormal type 11/03): the primary root is retarded.

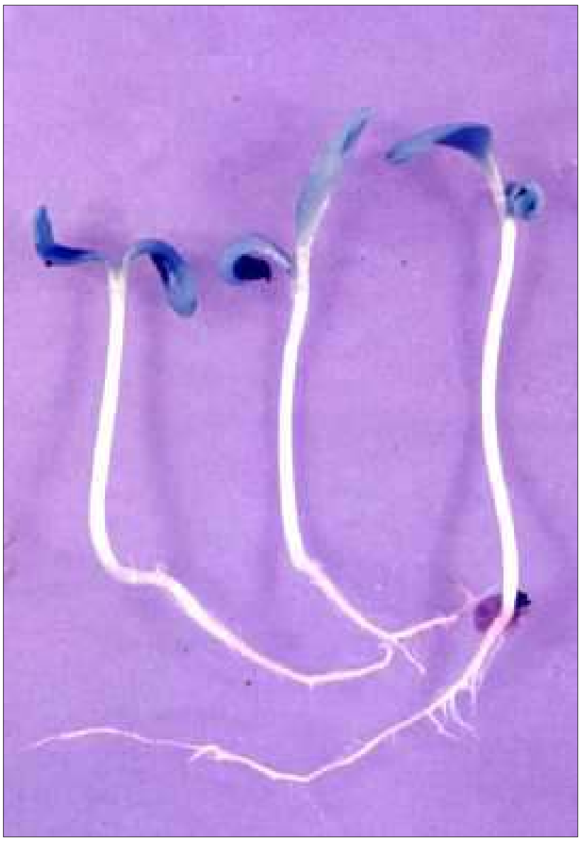


Figure 15.27 *Dianthus* spp.: there is one intact cotyledon and the seedlings are normal.

Figure 15.28 *Dianthus* spp.: there are three cotyledons and the seedlings are normal.

Section 16: Seedling Type E – Seedling Group A-2-1-1-2

A-2-1-1-2

**Dicotyledons
with epigeal germination
without epicotyl elongation**

The primary root may be replaced by secondary roots

Representative genus: *Cucumis*

Additional illustrated genus: *Gossypium*

The seedling part that grows towards the light is the hypocotyl with two cotyledons attached that turn green.

The shoot system consists of the elongated hypocotyl and two cotyledons with the terminal bud lying between them. There is no epicotyl elongation within the test period and the epicotyl and the terminal bud are not usually discernible. The root system consists of the primary root, usually with root hairs, and often secondary roots. Secondary roots are taken into account in seedling evaluation if the primary root is defective.

Development of the seedling during the test

The embryo in the seed of the genera of this group consists of two large, in some cases (e.g. *Gossypium*) crumpled and folded, somewhat fleshy cotyledons and a short embryonic axis.

At the start of germination, the primary root pushes through the seed coat and rapidly elongates. In all Cucurbitaceae the primary root soon produces numerous secondary roots, the first ones forming immediately below the hypocotyl[1]. The hypocotyl elongates and the cotyledons come free from the seed coat, expand, turn green and start to photosynthesise. The cotyledons continue to photosynthesise far beyond the seedling stage. The epicotyl does not develop within the prescribed test period and the terminal bud is hardly visible between the cotyledons.

Differentiation of normal and abnormal seedlings

Normal seedlings	
Seedling as a whole	
all essential structures	are **normal** as detailed in the following:
root system	
the primary root	is **intact** *or* shows **acceptable defects** – • discoloured or necrotic spots • healed cracks or splits • superficial cracks or splits[2]
A seedling with a defective primary root is classed as normal, if sufficient normal secondary roots have developed.	
shoot system	
the hypocotyl	is **intact** *or* shows **acceptable defects** – • discoloured or necrotic spots • healed cracks and splits • superficial cracks and splits[2] • loose twists
the terminal bud and surrounding tissues	(usually not visible) are **intact**
the cotyledons	are **intact** *or* show **acceptable defects** – • up to 50 % of tissue not functioning normally • only one intact cotyledon • three cotyledons

1 Please note: The primary root of *Gossypium* grows for some time, without producing secondary roots.
2 Superficial cracks and splits: not affecting the conducting tissues.

Abnormal seedlings	
Seedling as a whole	
the seedling	is **abnormal** if it • is deformed • is fractured • has cotyledons emerging before the primary root • consists of fused twin seedlings • is yellow or white • is spindly • is glassy • is decayed as a result of primary infection
one or more of the essential structures	are **abnormal**, as detailed in the following:
root system	
the primary root	is **defective** and secondary roots are insufficient if it • is stunted or stubby • is retarded • is missing • is broken • is split from the tip • is trapped in the seed coat[3] • is negatively geotropic • is constricted • is spindly • is glassy • is decayed as a result of primary infection
A seedling with a defective primary root is classed as normal, if sufficient normal secondary roots have developed.	
shoot system	
the hypocotyl	is **defective** if it • is short and/or thick • is deeply cracked or broken[4] • is split right through • is missing • is bent over or forming a loop • is tightly twisted or forming a spiral • is constricted • is spindly or glassy • is decayed as a result of primary infection
the terminal bud or surrounding tissues	(usually not visible) are **defective**
the cotyledons	are **defective** if they • are **defective to such an extent, that less than 50 %** of the original tissue (or estimated tissue) is functioning normally • are swollen, curled or deformed • are broken or otherwise damaged • are separate or missing • are discoloured or necrotic • are glassy • are decayed as a result of primary infection
Damage or decay of the cotyledons at the point of attachment to the seedling axis or near the terminal bud render a seedling abnormal, irrespective of the 50 % rule.	

Supplementary remarks

None

3 A seedling with its primary root trapped in the seed coat is considered normal, if by the end of the test the root tip has found its way out of the seed coat.

4 Deeply cracked: affecting the conducting tissue.

Normal **Abnormal** **Abnormal**

Figure 16.1 *Cucumis sativus* (abnormal type 11/04): the primary root is missing and the secondary roots are not sufficient to compensate so seedlings are abnormal.

Figure 16.2 *Cucumis sativus*: the primary root is missing but there is sufficient secondary root growth to compensate so seedlings are normal.

Normal **Abnormal** **Abnormal**

Figure 16.3 *Gossypium* spp. (abnormal types 31/03 and 31/05): more than 50 % of the cotyledon tissue is broken or otherwise damaged (seedling in the middle), and discoloured or necrotic (seedling on the right).

Normal **Abnormal** **Abnormal** **Abnormal**

Figure 16.4 *Gossypium* spp. (abnormal type 11/12): the root is decayed as a result of primary infection.

Section 17: Seedling Type E – Seedling Group A-2-1-4-3

A-2-1-4-3

Dicotyledons
with epigeal germination
with tuberous hypocotyl

There are several seminal roots instead of a primary root

Representative genus: *Cyclamen*

The seedling part that grows towards the light is the hypocotyl with two cotyledons attached that turn green.

The shoot system consists of a swollen, tuberous hypocotyl and a single cotyledon, which is borne on a petiole. The terminal bud lies at the base of the petiole.

The root system consists of several seminal roots, developing more or less simultaneously at the distal end of the hypocotyl.

Development of the seedling during the test

The seed of *Cyclamen* contains a small, straight embryo embedded in endosperm.

At the start of germination the seed coat is pierced by a small, pink vaulted structure. The lower part of the hypocotyl then pushes through this structure and the pink, nearly transparent hypocotyl swells and forms a tuber.

At the base of the hypocotyl several fine seminal roots develop, one of which may be slightly larger than the others. When the hypocotyl has completely emerged from the seed coat, it is followed by the stout petiole of the single cotyledon (normally there is no second cotyledon) and finally by the dark green, heart-shaped blade of the cotyledon. This, however, seldom occurs during the prescribed test period.

The terminal bud is visible on top of the tuberous hypocotyl at the base of the cotyledon, but it takes some time before the first foliage leaf starts to develop.

Differentiation of normal and abnormal seedlings

Normal seedlings	
Seedling as a whole	
all essential structures	are **normal** as detailed in the following:
root system	
at least two seminal roots	are **intact** *or* show **acceptable defects** – • discoloured or necrotic spots
shoot system	
the hypocotyl	is **intact and forms a tuber** *or* shows **acceptable defects** – • discoloured or necrotic spots • healed cracks or splits • superficial cracks or splits[1]
The presence of a tuber is essential.	
the terminal bud and the surrounding tissues	are **intact**
the cotyledon petiole	is **intact** *or* shows **acceptable defects** – • discoloured or necrotic spots • healed cracks or splits • superficial cracks or splits[1] • two petioles (cotyledons)

1 Superficial cracks or splits: not affecting the conducting tissues.

Abnormal seedlings	
Seedling as a whole	
the seedling	is **abnormal** if it • is deformed • is fractured • consists of fused twin seedlings • is yellow or white • is spindly • is glassy • is decayed as a result of primary infection
one or more of the essential structures	are **abnormal**, as detailed in the following:
root system	
the seminal roots	are **defective** if they • are stunted or stubby • are retarded • are missing • show negative geotropism • are spindly • are glassy • are decayed as a result of primary infection
A seedling with fewer than two intact seminal roots is considered abnormal.	
shoot system	
the hypocotyl	is **defective** if it • does not form a tuber • is deeply cracked or split • is constricted • is spindly • is glassy • is decayed as a result of primary infection
the cotyledon petiole	is **defective** if it • is broken • is split right through • is constricted • is necrotic • is decayed as a result of primary infection
Attention must be paid to signs of decay at the point where the cotyledon petiole leaves the seed coat.	
the terminal bud	is **defective** if it • is deformed • is missing

Supplementary remarks

None

Figure 17.1 *Cyclamen persicum*: normal seedling development.

Normal **Abnormal** **Abnormal**

Figure 17.2 *Cyclamen persicum* (abnormal type 12/01): there is only one or no seminal root.

Normal **Abnormal** **Abnormal**

Figure 17.3 *Cyclamen persicum* (abnormal type 21/02): the hypocotyl has not formed a tuber.

Section 18: Seedling Type F – Seedling Group A-2-1-2-2

A-2-1-2-2

Dicotyledons
with epigeal germination
with epicotyl elongation

The primary root may be replaced by secondary roots

Representative genus: *Phaseolus* (except *P. coccineus*)

Additional illustrated genus: *Arachis*

The seedling part that grows towards the light and turns green is the hypocotyl with two cotyledons and the epicotyl with the primary leaves.

The shoot system consists of the elongated hypocotyl, two cotyledons and a more or less elongated epicotyl with two primary leaves developing around the terminal bud.

The root system consists of the primary root, usually with root hairs, and secondary roots, which are taken into account in seedling evaluation if the primary root is defective.

Development of the seedling during the test

The embryo in the seed of the genera of this group consists of two large, fleshy cotyledons in which the seedling's food reserves are stored. The cotyledons are attached to an embryonic axis with the radicle and the plumule. The radicle is curved in some genera (e.g. in *Phaseolus*), or short and straight (e.g. in *Arachis*). The plumule consists of two differentiated primary leaves, which are folded between the cotyledons.

At the start of germination, the primary root breaks through the seed coat, rapidly elongates and soon produces numerous secondary roots. The hypocotyl elongates and the cotyledons are freed from the seed coat. The cotyledons of many of the genera of this group expand, become green and photosynthesise (e.g. *Glycine*), but those of others (e.g. *Phaseolus*), hardly expand and soon shrivel and abscise. By the end of the prescribed test period there may be some elongation of the epicotyl and expansion of the primary leaves.

Differentiation of normal and abnormal seedlings

Normal seedlings	
Seedling as a whole	
all essential structures	are **normal** as detailed in the following:
root system	
the primary root	is **intact** *or* shows **acceptable defects** – • discoloured or necrotic spots • healed cracks or splits • superficial cracks or splits[1]
A seedling with a defective primary root is classed as normal, if sufficient normal secondary roots have developed.	
shoot system	
the hypocotyl and the epicotyl	are **intact** *or* show **acceptable defects** – • discoloured or necrotic spots • healed cracks and splits • superficial cracks and splits[1] • loose twists
the terminal bud	is **intact**
the cotyledons	are **intact** *or* show **acceptable defects** – • up to 50 % of tissue not functioning normally • only one (intact) cotyledon • three cotyledons

1 Superficial cracks or splits: not affecting the conducting tissues.

Normal seedlings	
shoot system	
the primary leaves	are **intact** *or* show **acceptable defects** – • up to 50 % of tissue not functioning normally • only one (intact) primary leaf • three primary leaves • normal shape but retarded growth

Abnormal seedlings	
Seedling as a whole	
the seedling	is **abnormal** if it • is deformed • is fractured • has cotyledons emerging before the primary root • consists of fused twin seedlings • is yellow or white • is spindly or glassy • is decayed as a result of primary infection
one or more of the essential structures	are **abnormal**, as detailed in the following:
root system	
the primary root	is **defective** if it • is stunted or stubby • is retarded • is missing • is broken • is split from the tip • is trapped in the seed coat[2] • shows negative geotropism • is constricted • is spindly or glassy • is decayed as a result of primary infection
A seedling with a defective primary root is classed as normal, if sufficient normal secondary roots have developed.	
shoot system	
the hypocotyl and/or the epicotyl	are **defective** if they • are short and/or thick • are deeply cracked[3] • are split right through • are missing • are bent over or forming a loop • are forming a spiral • are tightly twisted • are constricted • are spindly or glassy • are decayed as a result of primary infection
If there is no damage causing the loop on the hypocotyl (on the upper or lower part), then the seedling is considered as normal.	
the terminal bud	is **defective** or **missing**
the cotyledons	are **defective** if they • are **defective to such an extent, that less than 50 %** of the original tissue (or estimated tissue) is functioning normally • are deformed • are broken or otherwise damaged • are separate or missing • are discoloured or necrotic • are glassy • are decayed as a result of primary infection

2 A seedling with its primary root trapped in the seed coat is considered normal, if by the end of the test the root tip has found its way out of the seed coat.
3 Deeply cracked: affecting the conducting tissue.

Abnormal seedlings	
shoot system	
Damage or decay of the cotyledons at the point of attachment to the seedling axis or near the terminal bud render a seedling abnormal – irrespective of the 50 % rule.	
the primary leaves	are **defective** if they • are **defective to such an extent, that less than 50 %** of the original leaf area (or estimated area) is functioning normally • are curled or otherwise deformed • are damaged • are separate or missing • are discoloured • are necrotic • are of normal shape, but less than ¼ of normal size • are decayed as a result of primary infection
Irrespective of the presence of auxiliary buds arising from the axils of the cotyledons or the primary leaves, the seedling is classed as abnormal if the main shoot fails to develop normally.	

Supplementary remarks

***Phaseolus* spp.**

Table 5A of the ISTA Rules permits 'between paper' (BP) and 'in sand' (S) as substrates for germinating *Phaseolus* seed. Depending on the method chosen seedlings may look very different, even when temperature and duration of the test are the same. In order to obtain uniform results, appropriate interpretation of the ISTA Rules is required.

1. Germination in sand

Phaseolus seedlings grown for nine days in sand and given light will have developed the following essential structures:

- a large root system comprising a long primary root and numerous secondary roots;
- an elongated, straight hypocotyl;
- an elongated, straight epicotyl;
- two expanded and enlarged primary leaves; and
- two cotyledons.

The cotyledons of *Phaseolus* and *Vigna* do not increase their size after germination and show little photosynthetic activity. The nutrients stored in them are soon consumed by the growing seedling, and their function expires. By the end of the test period the cotyledons may have shrivelled or even have dropped from the plant. Therefore they need not be taken into account in seedling evaluation. On the other hand, the cotyledons of *Glycine* and *Lupinus* considerably increase in size during and after germination. They remain green and continue photosynthesising far beyond the seedling stage. Consequently, they must be evaluated according to the 50 % rule.

For all genera, the primary leaves expand and take over photosynthesis. This renders them decisive, essential structures for the seedling and they must be evaluated. Primary leaves are considered normal, as long as they show their normal shape and are not smaller than one quarter of the average leaf size of a normal seedling in the same test[4]. However, if the primary leaves are damaged or deformed, their leaf area must comply with the 50 % rule.

2. Germination in rolled towels

Phaseolus seedlings grown for nine days in rolled towels will have developed the following essential structures:

- a primary root, usually with a large number of young secondary roots;
- two cotyledons, swollen, fleshy and usually light yellow or green;
- a moderately elongated hypocotyl; usually bent due to the natural seedling rotation (or sometimes due to restrictive conditions within the rolled towel);
- a slightly elongated epicotyl, just visible between the cotyledons; and
- two small, folded, yellow or green primary leaves, enclosed between the cotyledons or just protruding somewhat.

At this seedling stage, the cotyledons are essential and must comply with the 50 % rule. The soundness of the secondary leaves must be checked by opening the cotyledons at evaluation.

4 Very small primary leaves are often the consequence of damaged, necrotic or decayed cotyledons.

Primary leaves evaluation: 25 % rule
When the primary leaves are properly formed, the seedling is considered as abnormal if the primary leaves are less than a quarter of normal size.

Normal **Abnormal** **Abnormal** **Abnormal**

Figure 18.1 *Phaseolus vulgaris* (abnormal type 33/06): measurement areas on primary leaves for evaluation of the 25 % rule.

Training on the 25 % rule
For training, the following quick calculation can help the analyst to gain experience on the 25 % rule.

First step: measure the Length (L) and the Width (W) of a normal standard leaf from a seedling of the same test.

Second step: measure the length (l) and the width (w) of one of the leaves of the doubtful seedlings.

Third step: if $l < L/2$ and if $w < W/2$, then the seedling is abnormal.

In addition, to check the 25 % ratio, calculate:

$(l \times w) / (L \times W) \times 100$

If <25 % seedling is abnormal, if >25 % the seedling is normal.

Routine analysis
When there is a doubt whether the leaves are normal or abnormal, place the small leaves on a normal standard leaf and then evaluate if they are more or less than 25 %.

Evaluation of the loop on the hypocotyl
If there is no damage causing the loop on the hypocotyl (on the upper or lower part), then the seedling has to be considered as normal.

To be sure, the seedlings may be left in light for one more day. The loops usually disappear.

Arachis hypogaea

Unlike *Phaseolus*, *Arachis* has a comparatively short and rather stout hypocotyl. The cotyledons remain attached to the seedling for a long time and, as essential structures, they have to be evaluated according to the 50 % rule. During the test period a short epicotyl and two pinnate primary leaves develop between the cotyledons. Beside the main shoot, two auxiliary shoots normally develop. The main shoot, however, is essential and if it is missing or defective, the seedling must be considered abnormal.

***Lupinus* spp.**

Ornamental and flower species of *Lupinus* (*Lupinus polyphyllus*, *Lupinus angustifolius*, *Lupinus nanus*, *Lupinus hartwegii* as examples) do not form a significant amount of secondary roots in TP, BP or S during the germination period, compared to *Lupinus albus* (Figures 18.19 and 18.20). However, some secondary roots do form when the primary root is damaged and the seedling develops normally.

Glycine max

Evaluation of the root system
A seedling with a defective primary root is classed as normal, if sufficient normal secondary roots have developed.

For *Glycine max*, when the primary root is defective (see Figure 18.22):

a. At least three secondary roots which do not show any defect listed for primary root should be present.
b. These secondary roots should be equal to or greater than half of the length of the hypocotyl.
c. In seedlings with under developed hypocotyls, the hypocotyl and each secondary root should have at least an equal length as the major axis of the cotyledon.

Normal Abnormal Abnormal

Figure 18.2 *Phaseolus vulgaris* (abnormal type 00/06): the seedling is yellow or white (sand or compost test).

Normal Abnormals

Figure 18.3 *Phaseolus vulgaris* (abnormal type 21/05): the hypocotyl is missing (sand or compost test).

Normal Abnormal Abnormal Abnormal

Figure 18.4 *Phaseolus vulgaris* (abnormal type 21/04): the hypocotyl is split right through (sand or compost test).

Normal Abnormal Abnormal

Figure 18.5 *Phaseolus vulgaris* (abnormal type 22/03): the terminal bud is missing (sand or compost test).

Normal Abnormal Abnormal

Figure 18.6 *Phaseolus vulgaris* (abnormal type 33/06): the primary leaves are of normal shape but are less than one quarter normal size so seedlings are abnormal (sand or compost test).

Normal Abnormal Abnormal

Figure 18.7 *Phaseolus vulgaris* (abnormal type 33/02): the primary leaves are damaged; less than 50 % of their area is functioning so seedlings are abnormal (sand or compost test).

Normal Abnormal Abnormal

Figure 18.8 *Phaseolus vulgaris* (abnormal type 22/02): the terminal bud is damaged (paper towel test).

Normal Abnormal Abnormal

Figure 18.9 *Phaseolus vulgaris* (abnormal type 31/03): the cotyledons are deeply cracked or broken (paper towel test).

Figure 18.10 *Phaseolus vulgaris* (abnormal type 11/03): the primary root is retarded (sand test).

Figure 18.11 *Phaseolus vulgaris* (abnormal type 31/02): the cotyledons are deformed (sand test).

Figure 18.12 *Phaseolus vulgaris* (abnormal type 22/01): the terminal bud is deformed (sand test).

Figure 18.13 *Phaseolus vulgaris* (abnormal type 22/03): the terminal bud is missing (sand test).

Figure 18.14 *Phaseolus vulgaris* (abnormal type 21/03): the epicotyl is deeply cracked or broken (sand test).

Figure 18.15 *Phaseolus vulgaris* (abnormal type 21/06): the hypocotyl is bent over or forming a loop (sand test). The seedling at the top is normal (loop without damage) and the seedling at the bottom is abnormal (loop with damage).

Normal **Abnormal**

Figure 18.16 *Arachis hypogaea* (abnormal type 11/04): the primary root is missing and there are insufficient secondary roots.

Normal **Abnormal** **Abnormal**

Figure 18.17 *Arachis hypogaea* (abnormal type 21/06): the hypocotyl is forming a loop.

Normal **Abnormal** **Abnormal**

Figure 18.18 *Arachis hypogaea* (abnormal type 22/02): the terminal bud is damaged.

Figure 18.19 Development of a seedling of *Lupinus polyphyllus* (substrate: paper).

Lupinus polyphyllus ***Lupinus nanus***

Lupinus albus ***Lupinus hartwegii*** ***Lupinus* hybrids**

Figure 18.20 Development of a seedling of *Lupinus albus* and of several flower species of the same genus (substrate: sand). In the flower species, no secondary roots are produced (or only a few and only very late); in *Lupinus albus*, secondary roots are normally produced during the test period.

Section 18: Type F – Group A-2-1-2-2 (Phaseolus)

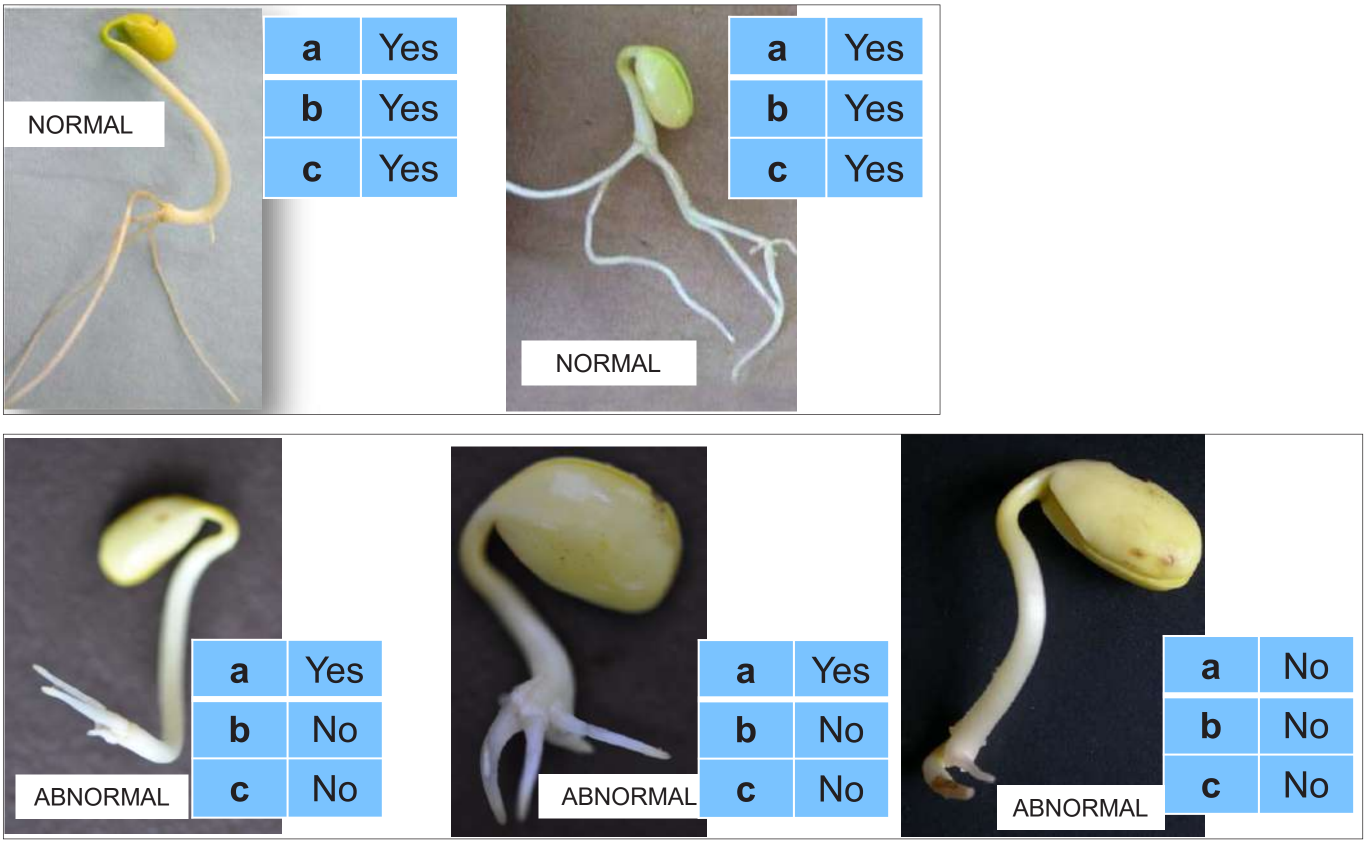


Figure 18.21 *Glycine max*: seedling growth evaluation (where, a: At least three secondary roots which do not show any defect listed for primary root should be present; b: These secondary roots should be equal to or greater than half of the length of the hypocotyl; c: In seedlings with under developed hypocotyls, the hypocotyl and each secondary root should have at least an equal length as the major axis of the cotyledon).

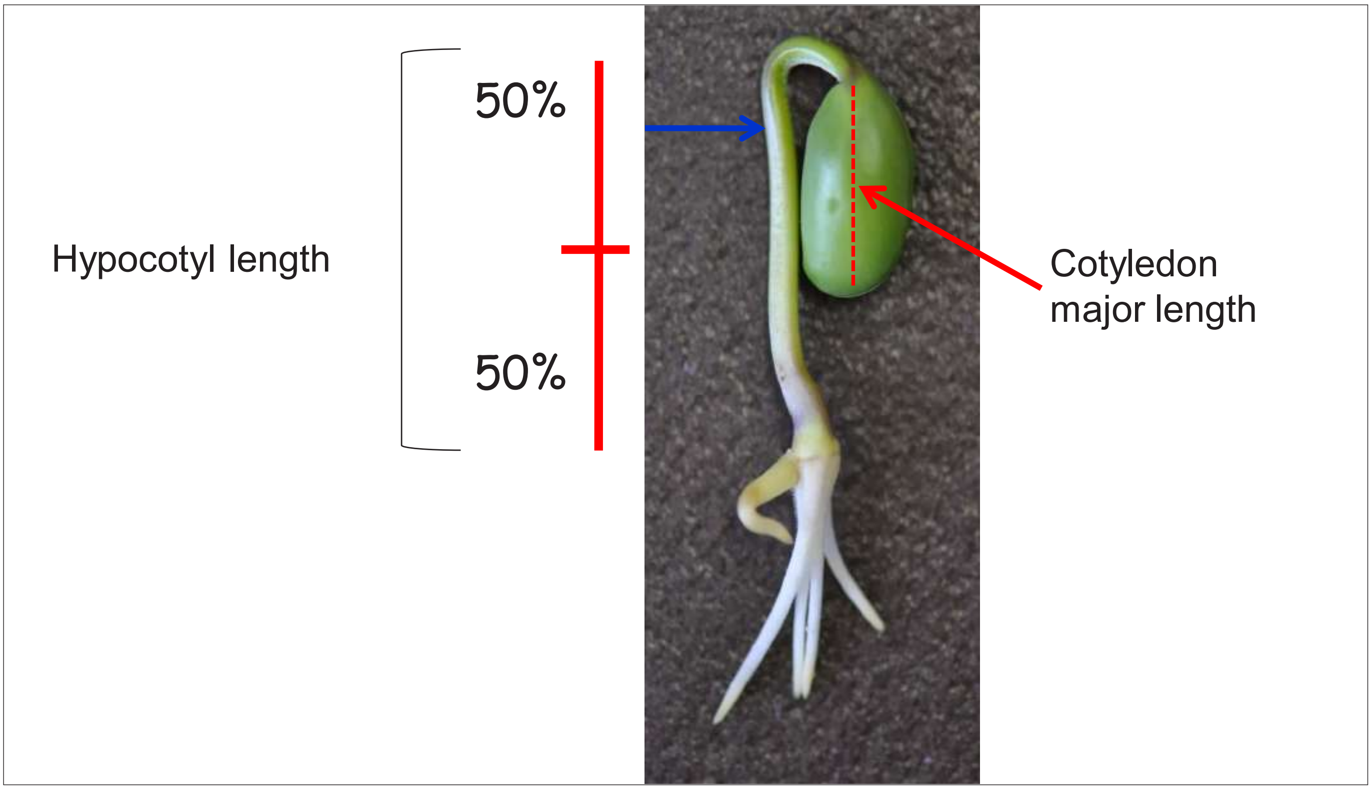


Figure 18.22 *Glycine max*: cotyledon and hypocotyl length proportion.

Section 18: Type F – Group A-2-1-2-2 (Phaseolus)

Section 19:
Seedling Type G – Seedling Group A-2-2-2-2

A-2-2-2-2

Dicotyledons
with hypogeal germination
with epicotyl elongation

The primary root may be replaced by secondary roots

Representative genus: *Pisum*

Additional illustrated genus: *Vicia*

The seedling part that grows towards the light and turns green is the epicotyl with the primary leaves.

The shoot system consists of the elongated epicotyl and the terminal bud with developing primary leaves. The cotyledons usually remain within the seed coat and the hypocotyl is hardly discernible.

The root system consists of a primary root, usually with root hairs, and secondary roots, which are taken into account if the primary root is defective.

Development of the seedling during the test

The embryos of the seed of the genera in this group, have two large, fleshy cotyledons containing food reserves. The cotyledons are attached to a short embryonic axis with the radicle and the plumule.

At the start of germination, the primary root pushes through the seed coat and rapidly elongates; secondary roots soon develop. The hypocotyl is hardly discernible, but the epicotyl elongates considerably. The epicotyl usually carries some scale leaves below the primary foliage leaves and the terminal bud. The buds in the axils of the cotyledons are usually dormant, except if the terminal bud is damaged.

Differentiation of normal and abnormal seedlings

Normal seedlings	
Seedling as a whole	
all essential structures	are **normal** as detailed in the following:
root system	
the primary root	is **intact** *or* shows **acceptable defects** – • discoloured or necrotic spots • healed cracks or splits • superficial cracks or splits[1]
A seedling with a defective primary root is classed as normal, if sufficient secondary roots have developed.	
shoot system	
the cotyledons	are **intact** *or* show **acceptable defects** – • at least 50 % of tissue functioning normally • only one intact cotyledon • three cotyledons
the epicotyl	is **intact** *or* shows **acceptable defects** – • discoloured or necrotic spots • healed cracks or splits • superficial cracks or splits[1] • loose twists
the terminal bud	is **intact**
the primary leaves (as far as they have developed)	are **intact** *or* show at least 50 % of tissue functioning normally

1 Superficial cracks or splits: not affecting the conducting tissues.

Abnormal seedlings	
Seedling as a whole	
the seedling	is **abnormal** if it • is deformed • is fractured • consists of fused twin seedlings • is yellow or white • is spindly or glassy • is decayed as a result of primary infection
one or more of the essential structures	are **abnormal**, as detailed in the following:
root system	
the primary root	is **defective** if it • is stunted or stubby • is retarded • is missing • is broken • is split from the tip • is trapped in the seed coat[2] • shows negative geotropism • is constricted • is spindly or glassy • is decayed as a result of primary infection
A seedling with a defective primary root is classed as normal, if sufficient secondary roots have developed.	
shoot system	
the cotyledons	are **defective** if they • are **defective to such an extent, that less than 50 %** of the original tissue (or estimated tissue) is functioning normally • are deformed • are broken or otherwise damaged (e.g. by insects) • are discoloured or necrotic • are decayed as a result of primary infection
Attention must be paid to signs of decay at the point of attachment of the cotyledons to the seedling axis. Such decay renders the seedling abnormal.	
the epicotyl	is **defective** if it • is too short and/or thick • is deeply cracked or broken[3] • is split right through • is missing • is bent over or forming a loop • is forming a spiral • is tightly twisted • is constricted • is spindly or glassy • is decayed as a result of primary infection
the terminal bud	is **defective** or **missing**
the primary leaves (as far as they have developed)	are **defective** if they • are **defective to such an extent, that less than 50 %** of the original leaf area (or estimated area) is functioning normally • are deformed • are damaged • are separate or missing • are discoloured • are necrotic • are decayed as a result of primary infection
The seedling must be classed as abnormal, if the main shoot or the terminal bud are defective, even if auxiliary shoots may have developed.	

2 A seedling with its primary root trapped in the seed coat is considered normal, if by the end of the test the root tip has found its way out of the seed coat.
3 Deeply cracked: affecting the conducting tissue.

Supplementary remarks

None

Normal Abnormal Abnormal

Figure 19.1 *Pisum sativum* (abnormal type 00/06): the seedling is yellow or white.

Normal Abnormal Abnormal

Figure 19.2 *Pisum sativum* (abnormal type 22/01): the terminal bud is deformed.

Normal Abnormal Abnormal

Figure 19.3 *Pisum sativum* (abnormal type 22/03): the terminal bud is missing; even though auxiliary shoots are present, the seedlings are abnormal.

Normal Abnormals

Figure 19.4 *Pisum sativum* (abnormal type 31/05): more than 50 % of cotyledon tissue is necrotic.

Figure 19.5 *Pisum sativum* (abnormal type 21/03): the epicotyl is deeply cracked or broken.

Figure 19.6 *Pisum sativum* (abnormal type 11/06): the primary root is split from the tip.

Figure 19.7 *Pisum sativum* (abnormal type 11/02): the primary root is thickened and stubby. Generally this type of abnormal seedling is a result of phytotoxic response to seed treatment; the type of abnormal is 'phytotoxic seedling' (00/10).

Figure 19.8 *Pisum sativum* (abnormal type 11/03): the primary root is retarded.

Section 19: Type G – Group A-2-2-2-2 (Pisum)

Normal **Abnormal**

Figure 19.9 *Pisum sativum* (abnormal type 11/08): the primary root shows negative geotropism.

Normal **Abnormal**

Figure 19.10 *Pisum sativum* (abnormal type 21/01): the epicotyl is too short and/or thick. Generally this type of abnormal seedling is a result of phytotoxic response to seed treatment. This type of abnormal is 'phytotoxic' (00/10).

Figure 19.11 *Pisum sativum* (abnormal type 21/06): the epicotyl is forming a loop.

Figure 19.12 *Pisum sativum* (abnormal type 22/01): the terminal bud is deformed.

Figure 19.13 *Pisum sativum* (abnormal type 00/02): the seedling is fractured.

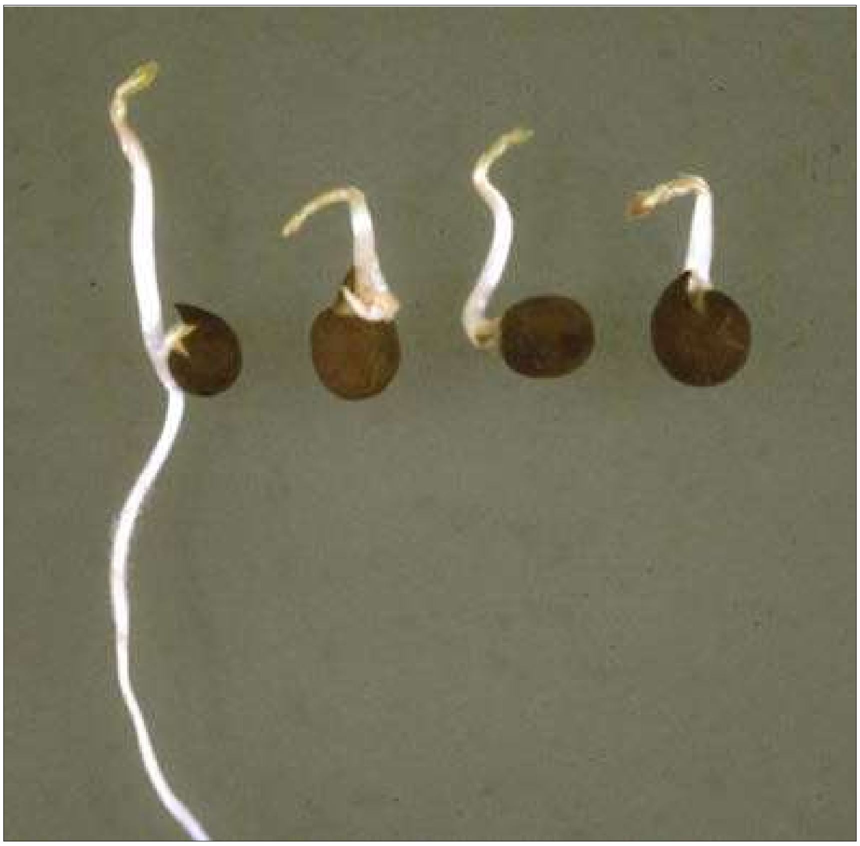
Figure 19.14 *Pisum sativum* (abnormal type 00/01): the seedling is deformed.

Normal Normal Normal Abnormal

Figure 19.15 *Vicia sativa* (abnormal type 11/04): the primary root is missing. The central two seedlings have sufficient secondary roots and are normal but the seedling on the right does not and is abnormal.

Normal Abnormal Abnormal Abnormal

Figure 19.16 *Vicia sativa* (abnormal type 22/02): the terminal bud is damaged.



Normal Abnormal Abnormal Abnormal

Figure 19.17 *Vicia sativa* (abnormal type 11/04): the primary root is missing.

Seedling evaluation according to groups

Category B: Trees and shrubs

This section deals with the evaluation of seedlings of the individual groups germinated on artificial substrates.

In order to show the group-specific properties and to facilitate a correct seedling evaluation, each group is dealt with under the following aspects:

- The group number is briefly explained;
- The specific morphology of the seedling type is described;
- A genus, which may serve as a representative of the group, is named;
- The development of the seedling during the prescribed test period is described;
- A survey of normal and abnormal seedlings typical of the respective group is given;
- Where necessary, supplementary remarks are added; and
- Illustrations of normal and abnormal seedlings of the representative genus and – where available – of additional genera belonging to the group conclude the group description.
- Figures: in general, the seedling on the left is a normal seedling that was obtained in the same test as the abnormal seedlings to the right of it. Normal or abnormal is indicated under each seedling.

At the end of the Handbook (Appendix 2) there is an index of seedling groups for the all genera covered by the ISTA Rules. The group number given to each indicates the criteria by which seed of that genus must be evaluated by in a germination test. An index is also given of different types of seedling abnormalities.

Section 20: Seedling Type E – Seedling Group B-2-1-1-1

B-2-1-1-1

Dicotyledons with epigeal germination without epicotyl elongation

The primary root is essential

Representative genus: *Robinia*

Additional illustrated genera: *Fagus*, *Ulmus*

The seedling part that grows towards the light is the hypocotyl with two cotyledons attached that turn green.

The shoot system consists of an elongated hypocotyl and two cotyledons with the terminal bud lying between them. There is usually no epicotyl elongation within the test period; epicotyl and terminal bud are usually not discernible.

The root system consists of a primary root, usually with root hairs, which is essential. Secondary roots may occasionally develop during the test period, but they are not taken into account in seedling evaluation.

Development of the seedling during the test

The seeds of the genera in this group include embryos of various sizes and shapes, which may be embedded in endosperm or contain the food reserves in their cotyledons.

At the start of germination the primary root pushes through the seed coat and rapidly elongates. The hypocotyl elongates and grows towards the cotyledons, which expand, become green and start photosynthesis. The epicotyl and the terminal bud remain between the cotyledons and do not usually develop during the test period.

Most of the species of this group do not normally develop secondary roots within the test period and certain genera of this group may occasionally show slight epicotyl elongation.

Differentiation of normal and abnormal seedlings

Normal seedlings	
Seedling as a whole	
all essential structures	are **normal** as detailed in the following:
root system	
the primary root	is **intact** *or* shows **acceptable defects** – • discoloured or necrotic spots • healed cracks or splits • superficial cracks or splits[1]
shoot system	
the hypocotyl	is **intact** *or* shows **acceptable defects** – • discoloured or necrotic spots • loose twists
the terminal bud and surrounding tissues	are **intact** (but not usually visible)
the cotyledons	are **intact** *or* show **acceptable defects** – • up to 50 % of tissue not functioning normally • only one (intact) cotyledon • three cotyledons

1 Superficial cracks or splits: not affecting the conducting tissues.

Abnormal seedlings	
Seedling as a whole	
the seedling	is **abnormal** if it • is deformed • is fractured • has cotyledons emerging before the primary root • consists of fused twin seedlings • is yellow or white • is spindly • is glassy • is decayed as a result of primary infection
one or more of the essential structures	are **abnormal**, as detailed in the following:
root system	
the primary root	is **defective** if it • is stunted or stubby • is retarded • is missing • is broken • is split from the tip • is trapped in the seed coat[2] • with negative geotropism • is constricted • is spindly or glassy • is decayed as a result of primary infection
A seedling is classed as abnormal if the primary root is defective, even if secondary roots may have developed.	
shoot system	
the hypocotyl	is **defective** if it • is short and/or thick • is deeply cracked or broken[3] • is split right through • is missing • is bent over or forming a loop • is forming a spiral • is tightly twisted • is constricted • is spindly or glassy • is decayed as a result of primary infection
the terminal bud and the surrounding tissues	(are usually not visible)
the cotyledons	are **defective** if they • are **defective to such an extent, that less than 50 %** of the original tissue (or estimated tissue) is functioning normally • are swollen or curled • are deformed • are broken or otherwise damaged • are separate or missing • are discoloured or necrotic • are glassy • are decayed as a result of primary infection
Damage or decay of the cotyledons at the point of attachment to the seedling axis or near the terminal bud render a seedling abnormal, irrespective of the 50 % rule.	

Supplementary remarks

Seeds of tree and shrub species with epigeal germination often take a long time to germinate, and the seedlings develop very slowly. In order to reduce the duration of the germination test such seedlings may be evaluated at an earlier stage of development than is usually required for seedlings with epigeal germination.

Thus, seedlings may be evaluated as normal, when the sum of primary root and hypocotyl exceeds four times the length of the seed – provided the essential structures that have developed are normal.

2 A seedling with its primary root trapped in the seed coat is considered normal, if by the end of the test the root tip has found its way out of the seed coat.
3 Deeply cracked: affecting the conducting tissue.

Normal **Abnormal** **Abnormal** **Abnormal**

Figure 20.1 *Robinia pseudoacacia* (abnormal type 00/01): the seedlings are deformed, the primary root is missing and the hypocotyl is broken; (abnormal type 00/03) cotyledons are released from the seed coat before the primary root.

Normal **Abnormal** **Abnormal**

Figure 20.2 *Robinia pseudoacacia* (abnormal type 31/04): the cotyledons are separate or missing.

Normal **Abnormal**

Figure 20.3 *Robinia pseudoacacia* (abnormal type 22/04): the terminal bud and surrounding tissues are necrotic.

Normal Abnormal

Figure 20.4 *Robinia pseudoacacia* (abnormal type 31/05): the cotyledons are discoloured or necrotic.

Normal Abnormal Abnormal Abnormal

Figure 20.5 *Robinia pseudoacacia* (abnormal type 11/06): the primary root is split; (11/07) the primary root is trapped in the seed coat; (11/03) the primary root is retarded.

Normal Abnormal

Figure 20.6 *Robinia pseudoacacia* (abnormal type 00/09): the seedling is decayed as a result of primary infection.

Normal **Abnormal** **Abnormal**

Figure 20.7 *Fagus sylvatica* (abnormal type 11/02): the primary root is stubby.

Normal **Abnormal**

Figure 20.8 *Fagus sylvatica* (abnormal type 11/04): the primary root is missing; even though secondary roots are present, the seedling is abnormal.

Normal **Abnormal** **Abnormal**

Figure 20.9 *Fagus sylvatica* (abnormal type 31/05): the cotyledons are discoloured or necrotic.

Figure 20.10 *Fagus sylvatica* (abnormal type 00/01): the seedlings are deformed. The primary root is missing and the hypocotyl is short and thick.

Normal **Abnormal** **Abnormal**

Figure 20.11 *Ulmus americana* (abnormal type 00/09): the seedlings are decayed as a result of primary infection.

Normal **Abnormal** **Abnormal**

Figure 20.12 *Ulmus americana* (abnormal type 11/03): the primary root is retarded.

Section 21: Seedling Type G – Seedling Group B-2-2-2-2

B-2-2-2-2

Dicotyledons
with hypogeal germination
with epicotyl elongation

The primary root may be replaced by secondary roots

Representative genus: *Quercus*

The seedling part that grows towards the light and turns green is the epicotyl with the primary leaves.

The shoot system consists of an elongated epicotyl and the terminal bud with developing primary leaves. The cotyledons usually remain within the seed coat; the hypocotyl is hardly discernible.

The root system consists of a primary root, usually with root hairs, and secondary roots, which are taken into account in seedling evaluation if the primary root is defective.

Development of the seedling during the test

The embryos of seeds in this group have two large, fleshy cotyledons containing the food reserves. The cotyledons are attached to a short embryonic axis with the radicle and plumule. At the start of germination the primary root pushes through the seed coat and elongates; secondary roots soon develop. The hypocotyl is hardly discernible but the epicotyl elongates considerably, though it may remain underdeveloped due to epicotyl dormancy. The epicotyl usually carries some scale leaves below the primary foliage leaves and the terminal bud.

Differentiation of normal and abnormal seedlings

Normal seedlings	
Seedling as a whole	
all essential structures	are **normal** as detailed in the following:
root system	
the primary root	is **intact** *or* shows **acceptable defects** – • discoloured or necrotic spots • healed cracks or splits • superficial cracks or splits[1]
A seedling with a defective primary root is classed as normal, if sufficient secondary roots have developed.	
shoot system	
the cotyledons	are **intact** *or* show **acceptable defects** – • up to 50 % of tissue not functioning normally • only one intact cotyledon • three cotyledons
the epicotyl	is **intact** *or* shows **acceptable defects** – • discoloured or necrotic spots • healed cracks or splits • superficial cracks or splits[1] • loose twists
If the epicotyl is retarded or missing due to epicotyl dormancy, the seedling is considered normal, if it is otherwise normal.	
the terminal bud	is **intact**
the primary leaves (as far as they have developed)	are **intact** *or* show at least 50 % of tissue functioning normally

1 Superficial cracks or splits: not affecting the conducting tissues.

Abnormal seedlings	
Seedling as a whole	
the seedling	is **abnormal** if it • is deformed • is fractured • consists of fused twin seedlings • is yellow or white • is spindly • is glassy • is decayed as a result of primary infection
one or more of the essential structures	are **abnormal**, as detailed in the following:
root system	
the primary root	is **defective** if it • is stunted or stubby • is retarded • is missing • is broken • is split from the tip • is trapped in the seed coat[2] • shows negative geotropism • is spindly or glassy • is decayed as a result of primary infection
A seedling with a defective primary root is considered normal, if sufficient secondary roots have developed.	
shoot system	
the cotyledons	are **defective** if they • are **defective to such an extent, that less than 50 %** of the original leaf area (or estimated area) is functioning normally • are deformed • are broken or otherwise damaged (e.g. by insects) • are discoloured or necrotic • are decayed as a result of primary infection
Damage or decay of the cotyledons at the point of attachment to the seedling axis or near the terminal bud render a seedling abnormal, irrespective of the 50 % rule.	
the epicotyl	is **defective** if it • is deeply cracked or broken[3] • is split right through • is bent over or forming a loop • is forming a spiral • is tightly twisted • is constricted • is spindly or glassy • is decayed as a result of primary infection
If the epicotyl is retarded or missing due to epicotyl dormancy, the seedling is considered normal.	
the terminal bud	is **defective** or **missing**
the primary leaves (as far as they have developed)	are **defective** if they • are **defective to such an extent, that less than 50 %** of the original leaf area (or estimated area) is functioning normally • are deformed • are damaged • are necrotic • are decayed as a result of primary infection
The seedling must be classed as abnormal if the main shoot is defective, even if auxiliary shoots may have developed.	

Supplementary remarks

None

2 A seedling with its primary root trapped in the seed coat is considered normal, if by the end of the test the root tip has found its way out of the seed coat.
3 Deeply cracked: affecting the conducting tissue.

Normal

Figure 21.1 *Quercus petraea* (normal seedling): the primary root is missing but there are sufficient secondary roots.

Normal **Abnormal**

Figure 21.2 *Quercus petraea* (abnormal type 33/07): the primary leaves are decayed as a result of primary infection.

Abnormal

Figure 21.3 *Quercus petraea* (abnormal type 00/01): the seedling is deformed. The terminal bud and the epicotyl are missing.

Normal **Abnormal**

Figure 21.4 *Quercus petraea* (abnormal type 00/06): the seedling is yellow or white.

Normal **Abnormal**

Figure 21.5 *Quercus petraea* (abnormal type 22/02): the terminal bud is damaged and even though one axiliary shoot is present, the seedling is abnormal.

Normal **Abnormal**

Figure 21.6 *Quercus petraea* (abnormal type 33/07): the primary leaves are decayed and even though two axiliary shoots are present, the seedling is abnormal.

Section 22: Seedling Type H – Seedling Group B-3-1-1-1

B-3-1-1-1

Conifers with epigeal germination without epicotyl elongation

The primary root is essential

Representative genus: *Pinus*

Additional illustrated genus: *Abies*

The seedling part that grows towards the light and turns green is the hypocotyl with the cotyledons.

The shoot system consists of an elongating hypocotyl and a specific number of long, narrow cotyledons, depending on the genus being tested. The epicotyl does not elongate, and the terminal bud in the centre of the whorl of cotyledons usually does not show growth during the test period.

The root system consists of a primary root, normally without discernible root hairs, which must be normal. Any secondary roots, which might develop during the test period, are not taken into account in seedling evaluation.

Development of the seedling during the test

The seeds of the genera in this group vary in size and shape, but they all possess a straight embryo that is embedded in gametophyte tissue, the nutrient storing tissue of the conifers. At the start of germination the primary root emerges from the seed and elongates. Usually no secondary roots are formed during the prescribed test period. The hypocotyl elongates and grows towards the cotyledons with the seed coat. The cotyledons remain with their tips embedded in the seed coat to absorb nutrients from the gametophyte tissue until it is exhausted. Then the seed coat is shed and the shoot apex becomes visible in the centre of the expanding whorl of long, narrow cotyledons, but it usually does not show any growth during the test period.

Most of the species of this group do not normally develop secondary roots within the test period and certain genera of this group may occasionally show slight epicotyl elongation.

Differentiation of normal and abnormal seedlings

Normal seedlings	
Seedling as a whole	
all essential structures	are **normal** as detailed in the following:
root system	
the primary root	is **intact** *or* shows **acceptable defects** – • discoloured or necrotic spots • superficial cracks or splits[1]
shoot system	
the hypocotyl	is **intact** *or* shows **acceptable defects** – • discoloured or necrotic spots • healed cracks or splits • superficial cracks or splits[1] • loose twists
the terminal bud and surrounding tissues	are **intact**
the cotyledons	are **intact** *or* show at least 50 % of tissue functioning normally

1 Superficial cracks or splits: not affecting the conducting tissues.

Abnormal seedlings	
Seedling as a whole	
the seedling	is **abnormal** if it • is deformed • is fractured • has cotyledons emerging before the primary root • consists of fused twin seedlings • bears a persisting 'endosperm-collar' • is yellow or white • is spindly • is glassy • is decayed as a result of primary infection
one or more of the essential structures	are **abnormal**, as detailed in the following:
root system	
the primary root	is **defective** if it • is stunted or stubby • is retarded • is missing • is broken • is split from the tip • is trapped in the seed coat[2] • shows negative geotropism • is constricted • is spindly or glassy • is decayed as a result of primary infection
A seedling is classed as abnormal if the primary root is defective, even if secondary roots may have developed.	
shoot system	
the hypocotyl	is **defective** if it • is short and/or thick • is deeply cracked or broken[3] • is split right through • is missing • is bent over or forming a loop • is forming a spiral • is tightly twisted • is constricted • is spindly or glassy • is decayed as a result of primary infection
the terminal bud	is **defective** or **missing**
the cotyledons	are **defective** if they • are **defective to such an extent, that less than 50 %** of the original leaf area (or estimated area) is functioning normally • are deformed • are broken or otherwise damaged • are separate or missing • are discoloured • are necrotic • are glassy • are decayed as a result of primary infection
Attention must be paid to signs of decay at the point where the cotyledons leave the seed coat.	

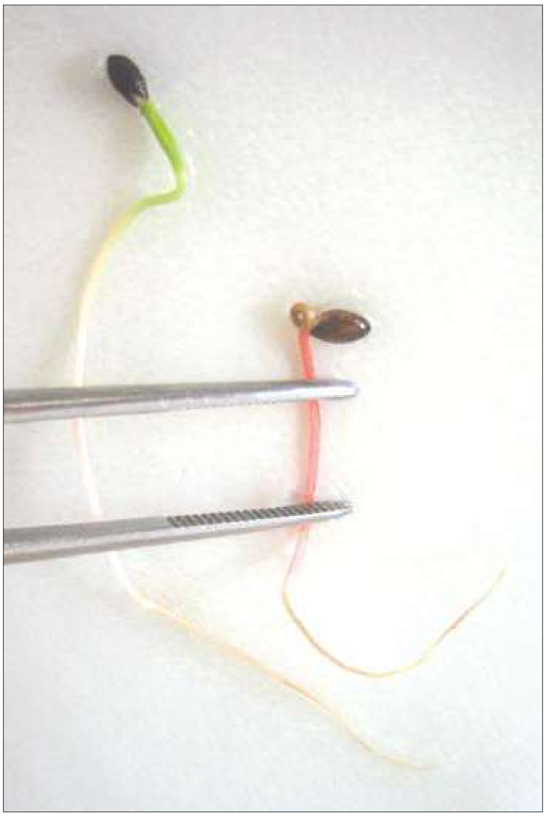
Supplementary remarks

None

2 A seedling with its primary root trapped in the seed coat is considered normal, if by the end of the test the root tip has found its way out of the seed coat.
3 Deeply cracked: affecting the conducting tissue.

Normal **Abnormal**

Figure 22.1 *Pinus* sp. (abnormal type 11/05): the primary root is broken or otherwise damaged.



Normal **Abnormal**

Figure 22.2 *Pinus* sp. (abnormal type 21/06): the hypocotyl is forming a loop.

Normal **Abnormal**

Figure 22.3 *Pinus* sp. (abnormal type 11/02): the primary root is stubby.

Normal **Abnormal**

Figure 22.4 *Pinus* sp. (abnormal type 00/08): the seedling is glassy. The primary root is stunted (11/01).

Normal Abnormal Abnormal

Figure 22.5 *Pinus sylvestris* (abnormal type 11/03): the primary root is retarded.

Normal Abnormal Abnormal

Figure 22.6 *Pinus sylvestris* (abnormal type 11/07): the primary root is trapped in the seed coat.

Normal Abnormal

Figure 22.7 *Pinus pinaster* (abnormal type 00/06): the seedling is yellow or white.

Normal Abnormal

Figure 22.8 *Pinus maritima* (abnormal type 00/09): the seedling is decayed as a result of primary infection.

Normal **Abnormal** **Abnormal** **Abnormal**

Figure 22.9 *Pinus pinaster* (abnormal type 11/02): the primary root is stubby. The primary root is retarded (11/03).

Normal **Abnormal**

Figure 22.10 *Pinus pinea* (abnormal type 00/03): the cotyledons are released from the seed coat before the primary root.

Figure 22.11 *Abies lasiocarpa*: normal seedling development.

Normal **Abnormal**

Figure 22.12 *Abies nordmanniana* (abnormal type 00/06): the seedling is yellow or white.

Normal **Abnormal**

Figure 22.13 *Abies nordmanniana* (abnormal type 00/03): the cotyledons are released from the seed coat before the primary root.

Normal **Abnormal** **Abnormal**

Figure 22.14 *Abies nordmanniana* (abnormal type 00/09): the seedlings are decayed as a result of primary infection.

Normal **Abnormal** **Abnormal**

Figure 22.15 *Abies nordmanniana* (abnormal type 11/12): the primary root is decayed as a result of primary infection.

Appendix 1: Glossary

50 % rule The 50 % rule states that normal seedlings may have deformed or damaged cotyledons or leaves providing that more than half the total cotyledon or leaf area can function normally. Damage or decay of the cotyledon or leaves at the point of attachment to the seedling axis or adjacent to the shoot apex render a seedling abnormal, irrespective of the 50 % rule.

Abnormal seedling A seedling that is damaged, deformed or decayed to such an extent, that normal development cannot be expected. See also ‘normal seedling’.

Achene Term applied to simple, dry, one-seeded, indehiscent fruit, with seed attached to ovary wall at one point only

Adventitious root Secondary root not arising from another root but from another seedling part (e.g. from the hypocotyl). See also ‘secondary root’.

Aerobic respiration The release of energy from food using oxygen. Involves a series of enzyme mediated reactions where carbohydrates are oxidised releasing energy: the by-products are carbon dioxide and water.

CARBOHYDRATES + OXYGEN
= ENERGY + CARBON DIOXIDE + WATER

Agriculture Production of plants and animals useful to human beings.

Aleurone A type of cell with high protein content which occurs in a layer surrounding the storage cells of the endosperm of grasses (Poaceae). During germination, it secretes hydrolytic enzymes used in the breakdown of the food reserves in the endosperm.

Alternating temperatures A diurnal regime of fluctuating temperatures used as a dormancy-breaking treatment for many species, e.g. 16 hours at 20 °C and 8 hours at 30 °C is the temperature regime used to germinate most *Brassica* spp. in the seed laboratory.

Androecium The collective name given to the male (♂) parts of the flower, consisting of the stamens. See also ‘gynoecium’.

Angiosperms The collective name for species of plants which bear seed within an ovary. See also ‘gymnosperms’.

Annual A type of plant that normally starts from a seed, produces its flowers, fruits and seeds and then dies within one growing season.

Anther The sac-like structure of the male part of a flower in which pollen is formed. Anthers normally have two lobes that dehisce and allow the pollen to disperse.

Apical meristem Growing point (zone of cell division) at the tip of the root and stem in vascular plants.

Apparent abnormality Seedling abnormality due to inappropriate germination conditions in the laboratory.

Artificial propagation Vegetative propagation of plants by man. Cuttings, grafting and tissue culture are examples of the different techniques employed.

Asexual reproduction The production of a new individual by a single organism without the production of gametes.

Assimilation Absorption and building up of simple foodstuffs, or products of digestion of foodstuffs, into complex constituents of the organism.

Autotroph Organism which is independent of outside sources of organic substances for provision of its own organic constituents, which it can manufacture from inorganic material, e.g. green plants synthesise organic materials using radiant energy and the process of photosynthesis. See also ‘heterotrophic’.

Auxiliary bud A bud in the axil of a leaf.

Auxiliary shoot A shoot developing from an auxiliary bud

Awn A slender, straight or bent bristle-like appendage often associated with seeds. In grasses it is usually a continuation of the mid-nerve of the lemmas or glumes.

Biennial A type of plant that produces only vegetative growth during its first growing season. After a period of storage or over-wintering out-of-doors, flowers, fruits and seed are produced during the second growing season and the plant then dies, i.e. species of plant that require two years to complete their life-cycles.

Binomial system of nomenclature The scientific method of naming species. The name is in Latin and is in two parts: one part, the generic name, designating the genus to which it belongs; the other, the specific name, particular to the species within the genus.

Blade The expanded part of a leaf.

Branched Refers to the morphology of plants. Whereas animals are of a definite shape, plants are branched and their shape depends largely on environmental conditions.

Calyx A collective term for all the sepals surrounding a flower and forms part of the covering of some seeds.

Carbohydrates Any of a large group of organic compounds, including sugars and starch, that contain, carbon, hydrogen and oxygen with the general formula $C_m(H_2O)_n$. They are produced in plants during photosynthesis and are a source of food and energy for plants and animals.

Carpel Female reproductive organ of flowering plants consisting of stigma, style and ovary. One, several or many carpels may occur in a flower and they may be separate or fused. Collectively the carpels are called the gynoecium.

Caryopsis The fruit (grain) of the grass family (Poaceae); dry one-seeded indehiscent fruit with the fruit wall (pericarp) fused with the very thin seed coat (testa).

Catalyst A substance whose presence increases the rate of a chemical reaction without itself undergoing any permanent change.

Cell A unit mass of living matter whether independent, as in the simplest plants or animals, or associated with other cells to form a more complex organism. Plant cells are surrounded by a cell wall.

Cell division Division of the cell into two. Cell division occurs by means of the process of mitosis or meiosis.

Cell wall Limiting layer of a plant cell usually formed of cellulose. The cell wall encloses the protoplasm and gives mechanical support to plant tissue.

Chlorophyll A pigment found in chloroplasts that gives plants their green colour. It is vital in the process by which radiant energy is transformed into chemical energy during photosynthesis.

Chloroplast In higher plants chlorophyll is located in small rounded lens-shaped structures called chloroplasts. Chloroplasts are the site of photosynthesis in higher plants.

Chromosome Thin rod or thread-like structures inside the nucleus of all cells. They contain the hereditary material in the form of a series of individual genes or 'coded' DNA.

Class A term used in the classification of organisms. Consists of a number of similar orders; similar classes are grouped into a phylum.

Classification Means by which organisms are classified scientifically in a hierarchical series of groups. The basic group used is the species. Species are grouped in a genus; similarly, genera are grouped into families, families into orders, orders into classes, classes into phyla and phylum into kingdoms, the highest taxonomic rank.

Cluster A densely crowded inflorescence or in *Beta* spp: part of an inflorescence. It is a seed-like structure containing more than one seed.

Coleoptile The sheath surrounding and protecting the shoot initial (terminal bud) of the embryo and young seedling of certain monocotyledons (e.g. Poaceae). It is the basal part of the cotyledon and protects the plumule as it emerges through the soil. The coleoptile is photosensitive and stops growth when exposed to light, allowing the plumule to break through and continue growth.

Coleorhiza The sheath surrounding the root tip(s) in the first seedling stage of the grass family (Poaceae). It is present in the embryo and protects the emerging root(s) from damage.

Compensation point The light intensity at which, for a given plant, the rate of photosynthesis is equal to the rate of respiration, assuming all other factors are constant.

Competition The simultaneous demand for the same material or resources by two or more organisms of the same or different species. The struggle between organisms for the necessities of life.

Conducting tissue The tissues transporting substances necessary for the maintenance and growth of a plant. A term used for both xylem and phloem.

Continuous internal changes A characteristic of living matter revealed externally by the gaseous exchanges associated with respiration.

Corolla The inner floral envelope composed of the petals.

Cotyledon(s) The first (non-foliage) leaf or leaves of an embryo and seedling. See also 'primary leaves'.

Crop Selected plants grown on cultivated land to produce food, food-stuffs or raw materials; also crop is the term used for produce from a given area of cultivated land, i.e. a field of barley yields a crop of barley.

Cultivar A plant type within a cultivated species that can be distinguished by one or more characters.

Decay, decayed Breakdown of organic tissue usually associated with the presence and activity of fungi or bacteria.

Defective seedling A seedling that is damaged, deformed or decayed to such an extent that normal development is prevented. See also 'abnormal seedling'.

Dehiscent The splitting open spontaneously at maturity by pods or capsules along definite lines or sutures. See also 'indehiscent'.

Denatured Permanent structural changes produced in proteins by heat or various chemicals. These structural changes render them less soluble than in their original condition.

Dicotyledoneae Larger of the two classes into which flowering plants (Angiospermae) are divided; distinguished from the smaller class, Monocotyledoneae, by the presence of two seed leaves (cotyledons) in the embryo and by other structural features, e.g. net-veined leaves.

Dicotyledons A group of plants so classified because the embryo normally has two cotyledons. See also 'monocotyledons'.

Differentiation Process of change in cell, tissue or organ during development resulting in the appearance of the variety of structure and function found in the adult.

Discoloration Alteration or loss of colour in a seedling structure.

Diseased Seedling (or seedling part) showing signs of infection with fungi or bacteria.

DNA Short for Deoxyribonucleic Acid, the carrier of genetic information in cells. It consists of two chains of phosphate, sugar molecules (deoxyribose) and organic bases (purines and pyrimidines) arranged in a double helix or spiral. The DNA molecule is capable of self-replication.

Dormancy/Dormant A 'resting' phase where growth and metabolism appear to be suspended. A physical or physiological condition of viable seed that prevents germination even in the presence of otherwise favourable germination conditions. See also 'quiescence'.

Dry fruit Ripened ovary of the flower, enclosing seed(s). In its mature state it is desiccated having a dry outer covering protecting the seed, e.g. the fruits of the Poaceae and the Fabaceae.

Embryo Young, still undeveloped plant contained in a seed, normally consisting of a more or less differentiated axis and attached cotyledon(s). The generative part of a seed that develops from the union of pollen cell with ovum and during germination becomes the growing plant.

Embryo sac The female gametophyte; an 8-nucleate structure within the nucellus of the ovule, ready for fertilization.

Endosperm Nutritive tissue derived from the double fertilisation of the angiosperms. It provides for the nutrition of the embryo and is retained in certain seeds until maturity as a storage tissue for food reserves.

Endospermic Mature seed with food reserves stored in endosperm.

Enforced Term used to describe a type of dormancy where viable seeds do not germinate because of a limitation in the external environment. Once the limitation is removed, the seeds germinate.

Enzyme A biological catalyst produced in living matter. Enzymes are specialised proteins capable of promoting, regulating and controlling metabolic processes without being permanently changed or destroyed.

Epicotyl The part of the seedling axis immediately above the cotyledons and below the primary leaf or pair of leaves.

Epigeal The type of germination in which the cotyledons are raised above soil level by elongation of the hypocotyl. See also 'hypogeal germination'.

Etiolation Phenomenon exhibited by green plants when grown in darkness. Such plants are pale yellow, their stems are exceptionally long and their leaves reduced in size.

Evolution Cumulative change in characteristics of populations or organisms, occurring in the course of successive generations related by descent.

False fruit A false fruit is formed from other parts of the plants as well as the ovary e.g. both apple and strawberry are false fruits being formed from the receptacle as well as the ovary.

Family One of the kinds of groups used in classifying organisms. Consists of a number of similar genera.

Fertilisation The union and fusion of male and female reproductive cells (gametes) to form a zygote.

Field capacity The maximum quantity of water that is retained by the soil matrix after free drainage for 24 hours.

Filament The stalk of an anther. Together filament and anther are called a stamen in a flower.

Flaw Collective term for 'slight defects and small deficiencies'.

Fleshy fruit Ripened ovary of a flower, enclosing seed(s). In its mature state it has a fleshy outer covering protecting the seed(s), e.g. berries (tomato and orange) and drupes (plum and cherry).

Floret A small individual flower in a dense inflorescence. In grasses (Poaceae) it consists of the lemma, palea, stamens and pistil.

Fruit Mature ovary (often with additional floral organs involved), which contains the seeds, protects them and may contribute to their dispersal.

Fungi Simple organisms lacking chlorophyll. Made up of tubular filaments called hyphae, and reproducing by forming spores. They are heterotrophs obtaining food by saprophytic or parasitic means.

Gamete Reproductive cell which can fuse with another gamete (constituting fertilisation), the resulting cell (zygote) developing into a new individual.

Gametes Male and female reproductive cells.

Gametophyte tissue The nutritive tissue occurring within conifer seeds; it exists before fertilisation occurs, therefore it is sometimes called primary endosperm. See also 'endosperm' and 'perisperm'.

Gene Unit of the material of inheritance. An area on a chromosome which determines the presence or absence of certain characteristics in an organism.

Genetic variability More or less pronounced diversity of features among the individuals of a species.

Genus [pl. Genera] One of the kinds of groups used in classifying organisms. Consists of a number of similar species. Similar genera are grouped in a family.

Geotropism Plant growth response to gravity.
- Positive geotropism: growth according to gravity (e.g. a normal primary root).
- Negative geotropism: growth against gravity (e.g. a normal shoot).

Germination Germination is the active growth of the embryo that results in the rupture of the seed coat. In this Handbook seed germination also includes the emergence of the seedling, which develops into the young plant.

Gibberellins Class of promotive plant hormones, involved in numerous plant activities, e.g. germination and cell elongation.

Glume One of the usually two sterile bracts which occur at the base of a grass spikelet, often completely enclosing it.

Growth Increase in size. Can be due to cell expansion and elongation or be due to mitotic division of cells.

Gymnosperms Sub-division of the seed plants, comprising those plants that are characterised by the ovules lying open on carpels. See also 'angiosperms'.

Gynoecium Collective name for the carpels of a flower: the female (♀) parts of a flower. See also 'androecium'.

Heat damage or Drying damage Damage caused to the germinability of seed by drying at too high a temperature or due to the heating of a moist grain as a result of the activities of microflora and microfauna.

Heterotroph An organism requiring a supply of organic material (food) from its environment, e.g. all animals and fungi.

Heterotrophic Organisms (animals, fungi) dependent on outside sources of organic substances (food) needed for life See also 'autotrophic'.

Hilum The scar remaining on the seed at the place of its detachment from the seed stalk.

Hook Compaction of stem apex of some seeds during germination. The hook pushes the plumule above the soil where the apex straightens and leaf enlargement begins.

Hormone A chemical substance that is produced in one part of an organism and used in minute quantities to induce a growth response in another part.

Hydration To combine with water.

Hydrophilic Readily absorbing and retaining moisture and often showing a change of form as a result.

Hyphae Fine tubular threads which form fungus mycelium. The vegetative part of a fungus.

Hypocotyl The part of the seedling axis immediately above the primary root and below the cotyledons

Hypogeal germination A type of germination in which the cotyledon(s) or comparable structure (e.g. scutellum) remain in the soil and within the seed; the shoot is carried above soil level by the elongating epicotyl (dicotyledons) or mesocotyl (some monocotyledons). See also 'epigeal germination'.

Imbibition The initial step in seed germination involving the uptake of moisture by absorption from the germination media and hydration of seed tissue.

Indehiscent Fruits that do not split open naturally at maturity to release seed. See also 'dehiscent'.

Induced Term used to describe a type of dormancy where viable seed do not germinate because of restriction in the germination environment. Once the restriction is removed, the seeds will not germinate until they have undergone dormancy-breaking treatments.

Infection Entrance and spread of disease organisms in living material (e.g. seedling structures), not necessarily, but often causing disease symptoms and decay.

- Primary infection: disease organisms present and active in or on the seed itself.
- Secondary infection: disease organisms spreading from other seeds or seedlings.

Inflorescence A flowering shoot bearing more than one flower: the arrangement of flowers on such as a shoot.

Inhibitors Chemical substances which retard or prevent a growth process such as germination.

Innate Term used to describe the most common type of seed dormancy. Innate dormancy is a genetic characteristic and there are three types: (a) immaturity of the embryo, (b) an after-ripening requirement, and (c) a requirement for a specific environmental stimulus.

Insect pollination Process by which pollen is transferred from the anther, where it is produced, to the stigma of a flower by an insect. Plants relying on insect pollination usually have bright coloured flowers and nectaries to attract insects. The pollen is usually sticky.

Intact seedling A seedling in which all the essential structures are complete, well balanced and healthy. See also 'defective seedling'.

Integuments Covers (in angiosperms usually two) around the nucellus, serve to protect the ovule and eventually form the seed coat (testa).

Irritability The unique susceptibility to stimuli possessed by living tissues.

ISTA-plants All plants covered by the tables of the ISTA Rules.

Lateral root A root arising from the primary root or from another root. See also 'secondary root'.

Leaf One of the lateral organs developed from the stem or axis of the plant below its growing point. Major site of photosynthesis and water loss in most plants.

Lemma The lower (outer) of two bracts usually enclosing the fruit in Poaceae. It is located on the side nearest the embryo and opposite the rachilla. See also 'palea'.

Lesion A term used to describe a wound or damaged tissue.

Life-cycle Progressive series of changes undergone by an organism from fertilisation to death.

Light Form of electro-magnetic radiation. Usually used to describe radiation in the visible spectrum: the agent by which objects are rendered visible.

Linnaeus An eighteenth-century botanist who developed the binomial system of nomenclature and was first to adopt the species as the basic unit of classification.

Longevity Length of life or viability of organisms.

Looped structure Seedling structure (e.g. hypocotyl, coleoptile) forming a permanent curve crossing itself.

Marsh spot A disorder of pea (*Pisum sativum*) seed caused by a deficiency of available manganese in the soil in which the parent plant is grown. There is usually a discoloured area at the centre of the cotyledons and during germination the plumular bud may not develop.

Mechanical damage Damaged caused to the germinability of a seed by a mechanical process.

Meiosis The form of cell division that takes place when cells divide to produce gametes.

Meristem Localised region of plant cells capable of cell division – in contrast to permanent tissues with special functions, the cells of which have lost their ability to divide; in the seedling it is found mainly in the root tips and in the terminal bud.

Mesocotyl In some highly specialised monocotyledons (e.g. certain Poaceae) the part of the seedling axis between the point of attachment of the scutellum and the shot apex; it is recognised to be a compound structure, formed by addition of part of the cotyledon to the hypocotyls.

Metabolism, Metabolic Physiological–biochemical processes occurring in living organisms.

Microfauna Collective name for athropods (mites and insects).

Microflora Collective name for fungi, bacteria and unicellular plants.

Micro-organism Microscopically small organism: unicellular plant, animal, fungus or bacterium.

Micropyle The integumentary opening of the ovule through which the pollen tube enters prior to fertilisation. Recognisable in mature seed as a pore in the seed coat through which water enters when a seed begins to germinate.

Monocotyledoneae Smaller of the two classes into which flowering plants (Angiospermae) are divided; distinguished from the larger class (Dicotyledoneae) by the presence of a single leaf (cotyledon) in the embryo and by other structural features, e.g. parallel veined leaves.

Monocotyledons Class of seed plants comprising those plants that are characterised by an embryo with one cotyledon. See also 'dicotyledons'.

Mycelium Collective term for mass of hyphae that constitutes the vegetative part of a fungus.

Necrosis Death of tissue marked by areas of discoloration and dead tissue.

Nectary Gland secreting sugary fluid (nectar) which is attractive to insects. Nectaries are found in many insect-pollinated flowers.

Nematode Round-, thread- and eel-worms. Phylum of animals not closely related to any other. Many are parasitic to plants and animals.

Nomenclature The accurate naming of species.

Non-endospermic Refers to seeds, which at maturity do not have their food reserves in the form of endosperm tissue. See also 'endospermic'.

Normal seedling A seedling with all essential structures present and capable of developing into a plant in favourable conditions in the field. Certain defects may be present provided they are judged not to be severe enough to impede continued development. See also 'abnormal seedling'.

Nucellus Main cell mass enclosed by (usually two) integuments and filling up the ovule prior to fertilisation. See also 'perisperm'.

Order A term used in classifying organisms. Consists of a number of similar families. Similar orders are grouped into a class.

Ovary Floral organ developing into the fruit and containing the ovule(s).

Ovule The structure within the ovary of the flower which becomes the seed following fertilisation and development.

Ovum An unfertilised egg cell.

Palea The upper (inner) of two bracts usually enclosing the caryopsis in the Poaceae. It is located on the side opposite the embryo. See also 'lemma'.

Parasite Organism living in or on another organism (its host) from which it obtains food.

Pathogen Parasite which causes disease.

Pedicel The small stalk of each single flower in an inflorescence.

Perennial Plant that continues its growth from year to year. In some perennials aerial parts may die away in autumn and new shoots replace these in the following year from underground structures, e.g. *Elytrigia* and rhubarb. In woody perennials permanent woody stems above the ground form the starting point for each year's new growth, e.g. shrubs and trees.

Perianth A collective term for petals and sepals taken together.

Pericarp The fruit wall which encloses the seeds and is derived from the ovary wall. It may be: dry, membranous, or hard, e.g. achene and nut; or fleshy, e.g. berry.

Perisperm A type of nutritive storage tissue in mature seed which develops from the nucellus of the parent plant. *Beta* is an example of a species with nutritive tissue in the form of well-developed perisperm tissue. See also 'endosperm' and 'gametophyte tissue'.

Petal Usually brightly coloured and conspicuous parts of the flower collectively known as the corolla.

Petiole The stalk of a leaf.

Phenotype The sum of the characteristics manifested by an organism. These characteristics are due to an interaction between the genetic make-up of the organism and the environment.

Phloem The type of conducting tissue that transports synthesised food through a plant.

Photoblastic Refers to light requirement for germination. The germination of positively photoblastic seed is enhanced by light; in negatively photoblastic seeds, germination is inhibited by light.

Photosynthesis In plants, the synthesis of organic compounds from water and carbon dioxide using energy absorbed by chlorophyll from sunlight. One important by-product of this process is oxygen.

Phylum A term used in the classification of organisms. Consists of a number of similar classes, similar phyla are grouped into kingdoms.

Physiological germination When the radicle or the plumule has ruptured the seed coat, physiological germination has occurred.

Phytochrome Photo-reversible pigment which is responsible for the photoperiodic control of flowering and seed germination. It exists in two forms in plants, the biologically active PF–R and the biologically inactive PR.

Phytotoxic A compound that is poisonous to plants.

Pigment Colouring naturally occurring in plant cells (e.g. chlorophyll).

Pistil Female reproductive organ in the flower, consisting of ovary, style and stigma.

Plant hormones Regulator substances naturally occurring in plants, that control physiological processes in plants.

Plumule The terminal bud of the embryo. The major leaf bud of the seed or seedling. The part of the embryonic plant axis above the point of attachment of the cotyledons. See also 'terminal bud'.

Pollen Material which is produced and borne within the stamens of flowers and contain the male gametes.

Pollen grain An individual unit of pollen.

Pollen tube A microscopic tube that grows down the stigma from the pollen grain to the embryo sac.

Pollination The process by which pollen is transferred from the anther, where it is produced, to the stigma of a flower.

Potassium nitrate A 0.2–0.3 % solution of potassium nitrate is often employed in the germination medium of grasses (Poaceae) as a dormancy-breaking agent.

Pre-chilling The practice of exposing imbibed seed to a temperature of between 5–10 °C for a few days prior to germination in order to break dormancy.

Pre-drying The practice of exposing dry seed to a temperature of 35 °C for a few days prior to germination in order to break dormancy.

Pre-washing A technique employed to remove inhibitors from the seed coats of some species of seed, e.g. in *Beta* removal of these inhibitors breaks dormancy.

Primary infection Disease organisms present and active in or on the seed itself.

Primary leaf The first foliage leaf (or pair of leaves) of a seedling. Occurring above the cotyledon(s).

Primary root The main root of a seedling, developing from the radicle of the embryo.

Promoter Term given to chemical compounds that stimulate dormant seed into growth. Gibberellins and ethylene are examples of promoters.

Protein An essential constituent of all living cells. Proteins occur naturally and are complex combinations of amino acids linked by peptide bonds.

Quiescence The absence of growth, usually inferring the absence of environmental conditions favouring growth. Quiescence is distinguished from dormancy, which implies the inability to germinate even in environmental conditions favouring growth. See also 'dormancy'.

Rachilla The central axis of a grass floret: the axis that bears the floret. Where grass seeds are retained within the lemma, the upper end of the rachilla may be present as a structure on the ventral side of the seed.

Radicle The (main) root initial of the embryo, developing into the primary root after emergence through the seed coat during germination.

Receptacle The apex of the flower stalk to which the flower parts are attached (perianth, stamens, and carpels). Sometimes forms part of the mature fruits as in apple and strawberry.

Reproduction Ability to produce new individuals of the same species.
- Sexual reproduction: involves the fusion of male and female gametes.
- Vegetative reproduction: production of genetically identical individuals by means of vegetative propagules (bulbils, tubers, runners, etc.).

Respiration The metabolic process by which organisms obtain energy from their food.

Retarded growth Of a seedling structure (e.g. the primary root): too short and weak to be in balance with the other structures of the seedling.

Retarded root A root usually with an intact tip, but much too short and weak to be in balance with the other structures of the seedling.

Root cap Cap of loosely arranged cells covering the apex of the growing point of a root and protecting it as it is forced through soil.

Root hair Fine tubular growth of the outermost root cells, absorbing water and mineral salts from the soil and growing medium; produced in large numbers behind region of active cell division at the root tip.

Saprophyte In seed testing: non-pathogenic fungi.

Saprophytes Organisms which obtain organic matter from dead and decaying tissues of plants or animals.

Scale leaf A small, reduced leaf, usually attached to the epicotyl of some hypogeal seedlings (e.g. in *Asparagus* and *Pisum*).

Scarification Process of mechanically or chemically abrasing a seed coat to make it more permeable to air and water.

Scutellum Highly specialised part of the cotyledon of some monocotyledons, mainly of the Poaceae, transformed into a shield-shaped structure that absorbs nutrients for the embryo from the endosperm. See also 'coleoptile'.

Secondary infection Disease organisms originating and spreading from other seeds or seedlings.

Secondary root Term used in seed testing to denote any root type other than the primary root. See also 'adventitious root' and 'lateral root'.

Seed
- In the botanical sense: a mature ovule consisting of an embryonic plant and food reserves with a protective coat;
- In the popular sense and in seed testing: a seed (or seed unit) may also include the fruit coat and remains of floral organs.

Seed-borne Of fungi or diseases: carried on or within the seed.

Seed quality Combination of various characteristics of seed, analysed in Seed Testing Laboratories.

Seed-borne disease A disease in a seedling or a crop which can result from a pathogen which is carried in or on seed.

Seedling A young plant developing from the embryo in a seed.

Seminal roots Seedling root system including the primary root and a number of adventitious roots, all appearing more or less simultaneously in germination. Occurs in a range of different species such as *Triticum* and *Cyclamen*.

Sepal Small, often leaf-like, structure forming the outer whorl of flowers and known collectively as the calyx. Usually green in colour.

Sexual reproduction Refers to reproduction involving the fusion of male and female gametes giving rise to a zygote.

Sheath Lower, often tubular part of the leaf or leaf-like structures (e.g. coleoptile) in grasses (Poaceae).

Shoot Aerial part of germinating seedling. Aerial growth of a plant.

Shoot apex The main growing point of the seedling axis.

Species A term used in classification of organisms. A group of organisms possessing many features in common and capable of breeding with each other. Similar species form a genus.

Spikelets The unit of a grass inflorescence comprising one or more florets subtended by one or two glumes.

Spiralled structure Seedling structure (e.g. hypocotyl, coleoptile) that has turned with one end fixed around an external axis. See also 'twisted structure'.

Stamen The flower's male reproductive organ. Each stamen consists of a filament and an anther.

Stem The whole of the axis above the primary leaves of a young plant.

Stigma [pl. Stigmata] Top part of the carpel, which can receive the pollen grains in pollination.

Stratification The practice of exposing imbibed seed to cool temperature conditions prior to germination in order to break dormancy. During this cool period it is thought that there are changes in the balance of endogenous promoters and inhibitors.

Stubby root The kind of root characteristic for seedlings showing phytotoxic symptoms: the roots are short and club-shaped, though often showing an intact tip. See also 'stunted root'.

Stunted root Root with a defective or missing tip, irrespective of the length of the root. See also 'stubby root'.

Style Connection between ovary and stigma in a pistil.

Symptom An indication of the existence of a causal agent.

Synergistic Combined activity of agencies, e.g. hormones, which separately influence a certain process in the same direction, such that an effect is produced greater than the sum of the effects of each agency acting alone.

Systematics Department of biology describing and differentiating the diversity of plants and arranging the groups in a hierarchic system.

Taxonomy Study of the classification of organisms according to their resemblances and differences.

Terminal bud The shoot apex enveloped by several more or less differentiated leaves.

Testa The outer covering of seeds; the usually hard and dry seed coat formed from integument(s). See also 'pericarp'.

Tetrazolium test A biochemical test that may be used to make a rapid assessment of seed viability.

True fruit Ripened ovary of a flower containing seeds. Contains no other floral or plant part apart from the ovary, e.g. a pod of peas (*Pisum sativum*).

Turgid State of cell in which the cell wall is rigid; it has stretched due to an increase in volume of the vacuole and protoplasm during absorption of water.

Twisted structure Seedling structure (e.g. hypocotyl, coleoptile) that has turned – with one end fixed – around its inherent axis.

- Loosely twisted: with few (1–2) turns involving the whole of the structure.
- Tightly twisted: with several turns involving a short section of the structure.

Vacuole Fluid-filled space within the cytoplasm of a cell. The vacuole of plant cells takes up most of the volume of the cell.

Vascular bundle Longitudinal strand of conducting tissue consisting mainly of xylem and phloem.

Vascular system Plant tissue consisting mainly of xylem and phloem which forms a continuous system throughout all parts of higher plants. It conducts water, mineral salts and synthesised food materials.

Vegetative propagation Asexual reproduction of a plant. Can be either natural or artificial (induced by man). Examples of natural vegetative propagation are bulbs, rhizomes and runners, whereas cuttings and grafts are examples of artificial vegetative propagation. With vegetative propagation the resultant plants are genetically identical.

Viable Alive. Seed viability indicates that a seed contains structures and substances, including enzyme systems, which give it the capacity to germinate under favourable conditions in the absence of dormancy.

Whorl A number of leaves arranged in a circle around an axis (e.g. seedling axis, floral axis).

Wind pollination Process by which pollen is blown, or drifts in the wind from the anther where it is produced to the stigma of a flower. Plants relying on wind pollination usually have dense inflorescences with small inconspicuous flowers. The pollen is aerodynamic.

Xylem The type of conducting tissue that transports water and mineral salts through a plant.

Zygote The cell produced when male and female gametes fuse: a fertilised egg that can grow into a new individual.

Appendix 2: Index of seedling groups

Genus	Family	Seedling Type	Seedling Group
Abelmoschus	Malvaceae	E	A-2-1-1-2
Abies	Pinaceae	H	B-3-1-1-1
Abutilon	Malvaceae	E	A-2-1-1-2
Acacia	Fabaceae	E	B-2-1-1-1
Acer (without *A. saccharum*)	Aceraceae	E	B-2-1-1-1
Acer saccharum	Aceraceae	G	B-2-2-2-2
Achillea	Asteraceae	E	A-2-1-1-1
Adonis	Ranunculaceae	E	A-2-1-1-1
Aesculus	Hippocastanaceae	G	B-2-2-2-2
Ageratum	Asteraceae	E	A-2-1-1-1
Agrimonia	Rosaceae	E	A-2-1-1-1
Agropyron	Poaceae	D	A-1-2-3-1
Agrostemma	Caryophyllaceae	E	A-2-1-1-1
Agrostis	Poaceae	D	A-1-2-3-1
Ailanthus	Simaroubaceae	E	B-2-1-1-1
Alcea	Malvaceae	E	A-2-1-1-2
Allium	Liliaceae	A	A-1-1-1-1
Alnus	Betulaceae	E	B-2-1-1-1
Alopecurus	Poaceae	D	A-1-2-3-1
Althaea	Malvaceae	E	A-2-1-1-2
Alysicarpus	Fabaceae	E	A-2-1-1-1
Alyssum	Brassicaceae	E	A-2-1-1-1
Amaranthus	Amaranthaceae	E	A-2-1-1-1
Amberboa	Asteraceae	E	A-2-1-1-1
Ammobium	Asteraceae	E	A-2-1-1-1
Amorpha	Fabaceae	E	B-2-1-1-1
Anagallis	Primulaceae	E	A-2-1-1-1
Anchusa	Boraginaceae	E	A-2-1-1-1
Andropogon	Poaceae	D	A-1-2-3-1
Anemone	Ranunculaceae	E	A-2-1-1-1
Anethum	Apiaceae	E	A-2-1-1-1
Angelica	Apiaceae	E	A-2-1-1-1
Anthoxanthum	Poaceae	D	A-1-2-3-1
Anthriscus	Apiaceae	E	A-2-1-1-1
Anthyllis	Fabaceae	E	A-2-1-1-1
Antirrhinum	Scrophulariaceae	E	A-2-1-1-1
Apium	Apiaceae	E	A-2-1-1-1
Aquilegia	Ranunculaceae	E	A-2-1-1-1
Arabis	Brassicaceae	E	A-2-1-1-1
Arachis	Fabaceae	F	A-2-1-2-2
Arctium	Asteraceae	E	A-2-1-1-1
Arctotis	Asteraceae	E	A-2-1-1-1
Armeria	Plumbaginaceae	E	A-2-1-1-1
Arnica	Asteraceae	E	A-2-1-1-1
Arrhenatherum	Poaceae	D	A-1-2-3-1
Artemisia	Asteraceae	E	A-2-1-1-1
Asclepias	Asclepiadaceae	E	A-2-1-1-1
Asparagus	Liliaceae	C	A-1-2-2-1
Aster	Asteraceae	E	A-2-1-1-1
Astragalus	Fabaceae	E	A-2-1-1-1
Astrebla	Poaceae	D	A-1-2-3-1
Atriplex	Chenopodiaceae	E	A-2-1-1-1
Atropa	Solanaceae	E	A-2-1-1-1
Aubrieta	Brassicaceae	E	A-2-1-1-1

Genus	Family	Seedling Type	Seedling Group
Avena	Poaceae	D	A-1-2-3-3
Axonopus	Poaceae	D	A-1-2-3-1
Beckmannia	Poaceae	D	A-1-2-3-1
Begonia	Begoniaceae	E	A-2-1-1-1
Bellis	Asteraceae	E	A-2-1-1-1
Beta	Chenopodiaceae	E	A-2-1-1-1
Betula	Betulaceae	E	B-2-1-1-1
Borago	Boraginaceae	E	A-2-1-1-1
Bothriochloa	Poaceae	D	A-1-2-3-1
Bouteloua	Poaceae	D	A-1-2-3-1
Brachiaria	Poaceae	D	A-1-2-3-1
Brachyscome	Asteraceae	E	A-2-1-1-1
Brassica	Brassicaceae	E	A-2-1-1-1
Briza	Poaceae	D	A-1-2-3-1
Bromus	Poaceae	D	A-1-2-3-1
Browallia	Solanaceae	E	A-2-1-1-1
Brunnera	Boraginaceae	E	A-2-1-1-1
Cajanus	Fabaceae	G	A-2-2-2-2
Calceolaria	Scrophulariaceae	E	A-2-1-1-1
Calendula	Asteraceae	E	A-2-1-1-1
Callistephus	Asteraceae	E	A-2-1-1-1
Calocedrus	Cupressaceae	H	B-3-1-1-1
Calopogonium	Fabaceae	E	A-2-1-1-1
Camelina	Brassicaceae	E	A-2-1-1-1
Campanula	Campanulaceae	E	A-2-1-1-1
Cannabis	Cannabaceae	E	A-2-1-1-1
Capsicum	Solanaceae	E	A-2-1-1-1
Caragana	Fabaceae	E	A-2-1-1-1
Carica	Caricaceae	E	A-2-1-1-2
Carpinus	Betulaceae	E	B-2-1-1-1
Carthamus	Asteraceae	E	A-2-1-1-1
Carum	Apiaceae	E	A-2-1-1-1
Cassia	Fabaceae	E	A-2-1-1-1
Castalis	Asteraceae	E	A-2-1-1-1
Castanea	Fagaceae	G	B-2-2-2-2
Catalpa	Bignoniaceae	E	B-2-1-1-1
Cedrela	Meliaceae	E	B-2-1-1-1
Cedrus	Pinaceae	H	B-3-1-1-1
Celosia	Amaranthaceae	E	A-2-1-1-1
Cenchrus	Poaceae	D	A-1-2-3-1
Centaurea	Asteraceae	E	A-2-1-1-1
Centrosema	Fabaceae	E	A-2-1-1-1
Cerastium	Caryophyllaceae	E	A-2-1-1-1
Chamaecyparis	Cupressaceae	H	B-3-1-1-1
Cheiranthus	Brassicaceae	E	A-2-1-1-1
Chelidonium	Papaveraceae	E	A-2-1-1-1
Chloris	Poaceae	D	A-1-2-3-1
Chrysanthemum	Asteraceae	E	A-2-1-1-1
Cicer	Fabaceae	G	A-2-2-2-2
Cichorium	Asteraceae	E	A-2-1-1-1
Citrullus	Cucurbitaceae	E	A-2-1-1-2
Clarkia	Onagraceae	E	A-2-1-1-1
Claytonia	Portulacaceae	E	A-2-1-1-1
Cleome	Capparaceae	E	A-2-1-1-1
Cnicus	Asteraceae	E	A-2-1-1-1
Cobaea	Polemoniaceae	E	A-2-1-1-1
Coïx	Poaceae	D	A-1-2-3-1
Coleus	Lamiaceae	E	A-2-1-1-1

Genus	Family	Seedling Type	Seedling Group
Consolida	Ranunculaceae	E	A-2-1-1-1
Convolvulus	Convolvulaceae	E	A-2-1-1-1
Corchorus	Tiliaceae	E	A-2-1-1-1
Coreopsis	Asteraceae	E	A-2-1-1-1
Coriandrum	Apiaceae	E	A-2-1-1-1
Cornus	Cornaceae	E	B-2-1-1-1
Coronilla	Fabaceae	E	A-2-1-1-1
Corylus	Betulaceae	G	B-2-2-2-2
Cosmos	Asteraceae	E	A-2-1-1-1
Cotoneaster	Rosaceae	E	B-2-1-1-1
Crataegus	Rosaceae	E	B-2-1-1-1
Crotalaria	Fabaceae	E	A-2-1-1-1
Cryptomeria	Taxodiaceae	H	B-3-1-1-1
Cucumis	Cucurbitaceae	E	A-2-1-1-2
Cucurbita	Cucurbitaceae	E	A-2-1-1-2
Cuminum	Apiaceae	E	A-2-1-1-1
Cupressus	Cupressaceae	H	B-3-1-1-1
Cyamopsis	Fabaceae	F	A-2-1-2-2
Cyclamen	Primulaceae	E	A-2-1-4-3
Cydonia	Rosaceae	E	B-2-1-1-1
Cymbalaria	Scrophulariaceae	E	A-2-1-1-1
Cynara	Asteraceae	E	A-2-1-1-1
Cynodon	Poaceae	D	A-1-2-3-1
Cynoglossum	Boraginaceae	E	A-2-1-1-1
Cynosurus	Poaceae	D	A-1-2-3-1
Cytisus	Fabaceae	E	B-2-1-1-1
Dactylis	Poaceae	D	A-1-2-3-1
Dahlia	Asteraceae	E	A-2-1-1-1
Datura	Solanaceae	E	A-2-1-1-1
Daucus	Apiaceae	E	A-2-1-1-1
Delphinium	Ranunculaceae	E	A-2-1-1-1
Dendranthema	Asteraceae	E	A-2-1-1-1
Deschampsia	Poaceae	D	A-1-2-3-1
Desmodium	Fabaceae	E	A-2-1-1-1
Dianthus	Caryophyllaceae	E	A-2-1-1-1
Dichanthium	Poaceae	D	A-1-2-3-1
Dichondra	Convolvulaceae	E	A-2-1-1-1
Digitalis	Scrophulariaceae	E	A-2-1-1-1
Digitaria	Poaceae	D	A-1-2-3-1
Dimorphotheca	Asteraceae	E	A-2-1-1-1
Dizygotheca	Araliaceae	E	A-2-1-1-1
Doronicum	Asteraceae	E	A-2-1-1-1
Dorotheanthus	Aizoaceae	E	A-2-1-1-1
Echinacea	Asteraceae	E	A-2-1-1-1
Echinochloa	Poaceae	D	A-1-2-3-1
Echinops	Asteraceae	E	A-2-1-1-1
Echium	Boraginaceae	E	A-2-1-1-1
Ehrharta	Poaceae	D	A-1-2-3-1
Elaeagnus	Elaeagnaceae	E	B-2-1-1-1
Eleusine	Poaceae	D	A-1-2-3-1
Elymus	Poaceae	D	A-1-2-3-1
Elytrigia	Poaceae	D	A-1-2-3-1
Eragrostis	Poaceae	D	A-1-2-3-1
Erigeron	Asteraceae	E	A-2-1-1-1
Eruca	Brassicaceae	E	A-2-1-1-1
Erysimum	Brassicaceae	E	A-2-1-1-1
Eschscholzia	Papaveraceae	E	A-2-1-1-1
Eucalyptus	Myrtaceae	E	B-2-1-1-1

Genus	Family	Seedling Type	Seedling Group
Euonymus	Celastraceae	E	B-2-1-1-1
Eustoma	Gentianaceae	E	A-2-1-1-1
Fagopyrum	Polygonaceae	E	A-2-1-1-1
Fagus	Fagaceae	E	B-2-1-1-1
Fatsia	Araliaceae	E	A-2-1-1-1
Felicia	Asteraceae	E	A-2-1-1-1
Festuca	Poaceae	D	A-1-2-3-1
Festulolium	Poaceae	D	A-1-2-3-1
Foeniculum	Apiaceae	E	A-2-1-1-1
Fragaria	Rosaceae	E	A-2-1-1-1
Fraxinus	Oleaceae	E	B-2-1-1-1
Freesia	Iridaceae	B	A-1-2-1-1
Gaillardia	Asteraceae	E	A-2-1-1-1
Galega	Fabaceae	E	A-2-1-1-1
Galeopsis	Lamiaceae	E	A-2-1-1-1
Gazania	Asteraceae	E	A-2-1-1-1
Gentiana	Gentianaceae	E	A-2-1-1-1
Geranium	Geraniaceae	E	A-2-1-1-1
Gerbera	Asteraceae	E	A-2-1-1-1
Geum	Rosaceae	E	A-2-1-1-1
Gilia	Polemoniaceae	E	A-2-1-1-1
Ginkgo	Ginkgoaceae	H	B-3-1-1-1
Gleditsia	Fabaceae	E	A-2-1-1-1
Glycine	Fabaceae	F	A-2-1-2-2
Godetia	Onagraceae	E	A-2-1-1-1
Gomphrena	Amaranthaceae	E	A-2-1-1-1
Goniolimon	Plumbaginaceae	E	A-2-1-1-1
Gossypium	Malvaceae	E	A-2-1-1-2
Grevillea	Proteaceae	E	A-2-1-1-1
Gypsophila	Caryophyllaceae	E	A-2-1-1-1
Hedysarum	Fabaceae	E	A-2-1-1-1
Helenium	Asteraceae	E	A-2-1-1-1
Helianthemum	Cistaceae	E	A-2-1-1-1
Helianthus	Asteraceae	E	A-2-1-1-1
Helichrysum	Asteraceae	E	A-2-1-1-1
Heliopsis	Asteraceae	E	A-2-1-1-1
Heliotropium	Boraginaceae	E	A-2-1-1-1
Helipterum	Asteraceae	E	A-2-1-1-1
Hesperis	Brassicaceae	E	A-2-1-1-1
Heuchera	Saxifragaceae	E	A-2-1-1-1
Hibiscus	Malvaceae	E	A-2-1-1-2
Hippeastrum	Amaryllidaceae	A	A-1-1-1-1
Holcus	Poaceae	D	A-1-2-3-1
Hordeum	Poaceae	D	A-1-2-3-3
Hypericum	Clusiaceae	E	A-2-1-1-1
Hyssopus	Lamiaceae	E	A-2-1-1-1
Iberis	Brassicaceae	E	A-2-1-1-1
Ilex	Aquifoliaceae	E	B-2-1-1-1
Impatiens	Balsaminaceae	E	A-2-1-1-2
Inula	Asteraceae	E	A-2-1-1-1
Ipomoea	Convolvulaceae	E	A-2-1-1-1
Juniperus	Cupressaceae	H	B-3-1-1-1
Kalanchoë	Crassulaceae	E	A-2-1-1-1
Kniphofia	Liliaceae	B	A-1-2-1-1
Kochia	Chenopodiaceae	E	A-2-1-1-1
Koeleria	Poaceae	D	A-1-2-3-1
Koelreuteria	Sapindaceae	E	B-2-1-1-1
Lablab	Fabaceae	F	A-2-1-2-2

Genus	Family	Seedling Type	Seedling Group
Laburnum	Fabaceae	E	B-2-1-1-1
Lactuca	Asteraceae	E	A-2-1-1-1
Lagenaria	Cucurbitaceae	E	A-2-1-1-2
Larix	Pinaceae	H	B-3-1-1-1
Lathyrus	Fabaceae	G	A-2-2-2-2
Lavandula	Lamiaceae	E	A-2-1-1-1
Lavatera	Malvaceae	E	A-2-1-1-2
Legousia	Campanulaceae	E	A-2-1-1-1
Lens	Fabaceae	G	A-2-2-2-2
Leontopodium	Asteraceae	E	A-2-1-1-1
Leonurus	Lamiaceae	E	A-2-1-1-1
Lepidium	Brassicaceae	E	A-2-1-1-1
Lespedeza	Fabaceae	E	A-2-1-1-1
Leucaena	Fabaceae	E	A-2-1-1-1
Leucanthemum	Asteraceae	E	A-2-1-1-1
Levisticum	Apiaceae	E	A-2-1-1-1
Liatris	Asteraceae	E	A-2-1-1-1
Ligustrum	Oleaceae	E	B-2-1-1-1
Lilium	Liliaceae	A	A-1-1-1-1
Limonium	Plumbaginaceae	E	A-2-1-1-1
Linaria	Scrophulariaceae	E	A-2-1-1-1
Linum	Linaceae	E	A-2-1-1-1
Liquidambar	Hamamelidaceae	E	B-2-1-1-1
Liriodendron	Magnoliaceae	E	B-2-1-1-1
Lobelia	Lobeliaceae	E	A-2-1-1-1
Lobularia	Brassicaceae	E	A-2-1-1-1
Lolium	Poaceae	D	A-1-2-3-1
Lonas	Asteraceae	E	A-2-1-1-1
Lotononis	Fabaceae	E	A-2-1-1-1
Lotus	Fabaceae	E	A-2-1-1-1
Luffa	Cucurbitaceae	E	A-2-1-1-2
Lunaria	Brassicaceae	E	A-2-1-1-1
Lupinus	Fabaceae	F	A-2-1-2-2
Lychnis	Caryophyllaceae	E	A-2-1-1-1
Lycopersicon	Solanaceae	E	A-2-1-1-1
Macroptilium	Fabaceae	F	A-2-1-2-2
Macrotyloma	Fabaceae	F	A-2-1-2-2
Mahonia	Berberidaceae	E	B-2-1-1-1
Malcolmia	Brassicaceae	E	A-2-1-1-1
Malope	Malvaceae	E	A-2-1-1-2
Malus	Rosaceae	E	B-2-1-1-1
Malva	Malvaceae	E	A-2-1-1-2
Marrubium	Lamiaceae	E	A-2-1-1-1
Matricaria	Asteraceae	E	A-2-1-1-1
Matthiola	Brassicaceae	E	A-2-1-1-1
Medicago	Fabaceae	E	A-2-1-1-1
Melilotus	Fabaceae	E	A-2-1-1-1
Melinis	Poaceae	D	A-1-2-3-1
Melissa	Lamiaceae	E	A-2-1-1-1
Mentha	Lamiaceae	E	A-2-1-1-1
Mimosa	Fabaceae	E	A-2-1-1-1
Mimulus	Scrophulariaceae	E	A-2-1-1-1
Mirabilis	Nyctaginaceae	E	A-2-1-1-1
Molucella	Lamiaceae	E	A-2-1-1-1
Momordica	Cucurbitaceae	E	A-2-1-1-2
Morus	Moraceae	E	B-2-1-1-1
Mucuna	Fabaceae	G	A-2-2-2-2
Myosotis	Boraginaceae	E	A-2-1-1-1

Genus	Family	Seedling Type	Seedling Group
Nasturtium	Brassicaceae	E	A-2-1-1-1
Nemesia	Scrophulariaceae	E	A-2-1-1-1
Nemophila	Hydrophyllaceae	E	A-2-1-1-1
Nepeta	Lamiaceae	E	A-2-1-1-1
Nicandra	Solanaceae	E	A-2-1-1-1
Nicotiana	Solanaceae	E	A-2-1-1-1
Nierembergia	Solanaceae	E	A-2-1-1-1
Nigella	Ranunculaceae	E	A-2-1-1-1
Nothofagus	Fagaceae	E	B-2-1-1-1
Ocimum	Lamiaceae	E	A-2-1-1-1
Oenothera	Onagraceae	E	A-2-1-1-1
Onobrychis	Fabaceae	E	A-2-1-1-1
Origanum	Lamiaceae	E	A-2-1-1-1
Ornithopus	Fabaceae	E	A-2-1-1-1
Oryza	Poaceae	D	A-1-2-3-2
Oryzopsis	Poaceae	D	A-1-2-3-1
Osteospermum	Asteraceae	E	A-2-1-1-1
Panicum	Poaceae	D	A-1-2-3-1
Papaver	Papaveraceae	E	A-2-1-1-1
Pascopyrum	Poaceae	D	A-1-2-3-1
Paspalum	Poaceae	D	A-1-2-3-1
Pastinaca	Apiaceae	E	A-2-1-1-1
Pelargonium	Geraniaceae	E	A-2-1-1-1
Pennisetum	Poaceae	D	A-1-2-3-1
Penstemon	Scrophulariaceae	E	A-2-1-1-1
Perilla	Lamiaceae	E	A-2-1-1-1
Petroselinum	Apiaceae	E	A-2-1-1-1
Petunia	Solanaceae	E	A-2-1-1-1
Phacelia	Hydrophyllaceae	E	A-2-1-1-1
Phalaris	Poaceae	D	A-1-2-3-1
Pharbitis	Convolvulaceae	E	A-2-1-1-1
Phaseolus (without *P. coccineus*)	Fabaceae	F	A-2-1-2-2
Phaseolus coccineus	Fabaceae	G	A-2-2-2-2
Phleum	Poaceae	D	A-1-2-3-1
Phlox	Polemoniaceae	E	A-2-1-1-1
Physalis	Solanaceae	E	A-2-1-1-1
Picea	Pinaceae	H	B-3-1-1-1
Pimpinella	Apiaceae	E	A-2-1-1-1
Pinus	Pinaceae	H	B-3-1-1-1
Piptantherum	Poaceae	D	A-1-2-3-1
Pisum	Fabaceae	G	A-2-2-2-2
Plantago	Plantaginaceae	E	A-2-1-1-1
Platanus	Platanaceae	E	A-2-1-1-1
Poa	Poaceae	D	A-1-2-3-1
Populus	Salicaceae	E	B-2-1-1-1
Portulaca	Portulacaceae	E	A-2-1-1-1
Primula	Primulaceae	E	A-2-1-1-1
Prunus (without *P. serotina*)	Rosaceae	E	B-2-1-1-1
Prunus serotina	Rosaceae	G	B-2-2-2-2
Pseudorogneria	Poaceae	D	A-1-2-3-1
Pseudotsuga	Pinaceae	H	B-3-1-1-1
Psophocarpus	Fabaceae	G	A-2-2-2-2
Psyllostachys	Plumbaginaceae	E	A-2-1-1-1
Pueraria	Fabaceae	E	A-2-1-1-1
Pulsatilla	Ranunculaceae	E	A-2-1-1-1
Pyrus	Rosaceae	E	B-2-1-1-1
Quamoclit	Convolvulaceae	E	A-2-1-1-1
Quercus	Fagaceae	G	B-2-2-2-2

Genus	Family	Seedling Type	Seedling Group
Ranunculus	Ranunculaceae	E	A-2-1-1-1
Raphanus	Brassicaceae	E	A-2-1-1-1
Rapistrum	Brassicaceae	E	A-2-1-1-1
Reseda	Resedaceae	E	A-2-1-1-1
Rheum	Polygonaceae	E	A-2-1-1-1
Ricinus	Euphorbiaceae	E	A-2-1-1-1
Robinia	Fabaceae	E	B-2-1-1-1
Rosa	Rosaceae	E	B-2-1-1-1
Rosmarinus	Lamiaceae	E	A-2-1-1-1
Rudbeckia	Asteraceae	E	A-2-1-1-1
Rumex	Polygonaceae	E	A-2-1-1-1
Ruta	Rutaceae	E	A-2-1-1-1
Saintpaulia	Gesneriaceae	E	A-2-1-1-1
Salix	Salicaceae	E	B-2-1-1-1
Salpiglossis	Solanaceae	E	A-2-1-1-1
Salvia	Lamiaceae	E	A-2-1-1-2
Sanguisorba	Rosaceae	E	A-2-1-1-1
Sanvitalia	Asteraceae	E	A-2-1-1-1
Saponaria	Caryophyllaceae	E	A-2-1-1-1
Satureja	Lamiaceae	E	A-2-1-1-1
Scabiosa	Dipsacaceae	E	A-2-1-1-1
Schizanthus	Solanaceae	E	A-2-1-1-1
Scorzonera	Asteraceae	E	A-2-1-1-1
Secale	Poaceae	D	A-1-2-3-3
Senecio	Asteraceae	E	A-2-1-1-1
Senna	Fabaceae	E	A-2-1-1-1
Sequoia	Taxodiaceae	H	B-3-1-1-1
Sequoiadendron	Taxodiaceae	H	B-3-1-1-1
Sesamum	Pedaliaceae	E	A-2-1-1-1
Sesbania	Fabaceae	E	A-2-1-1-1
Setaria	Poaceae	D	A-1-2-3-1
Silene	Caryophyllaceae	E	A-2-1-1-1
Silybum	Asteraceae	E	A-2-1-1-1
Sinapis	Brassicaceae	E	A-2-1-1-1
Sinningia	Gesneriaceae	E	A-2-1-1-1
Solanum	Solanaceae	E	A-2-1-1-1
Sophora	Fabaceae	E	B-2-1-1-1
Sorbus	Rosaceae	E	B-2-1-1-1
Sorghastrum	Poaceae	D	A-1-2-3-1
Sorghum	Poaceae	D	A-1-2-3-2
Spartium	Fabaceae	E	B-2-1-1-1
Spergula	Caryophyllaceae	E	A-2-1-1-1
Spinacia	Chenopodiaceae	E	A-2-1-1-1
Stachys	Lamiaceae	E	A-2-1-1-1
Stipa	Poaceae	D	A-1-2-3-1
Stylosanthes	Fabaceae	E	A-2-1-1-1
Syringa	Oleaceae	E	B-2-1-1-1
Taeniatherum	Poaceae	D	A-1-2-3-1
Tagetes	Asteraceae	E	A-2-1-1-1
Tanacetum	Asteraceae	E	A-2-1-1-1
Taraxacum	Asteraceae	E	A-2-1-1-1
Taxodium	Taxodiaceae	H	B-3-1-1-1
Taxus	Taxaceae	H	B-3-1-1-1
Tectona	Verbenaceae	E	B-2-1-1-1
Tetragonia	Aizoaceae	E	A-2-1-1-1
Thuja	Cupressaceae	H	B-3-1-1-1
Thunbergia	Acanthaceae	E	A-2-1-1-1
Thymus	Lamiaceae	E	A-2-1-1-1

Genus	Family	Seedling Type	Seedling Group
Tilia	Tiliaceae	E	B-2-1-1-1
Torenia	Scrophulariaceae	E	A-2-1-1-1
Tragopogon	Asteraceae	E	A-2-1-1-1
Trifolium	Fabaceae	E	A-2-1-1-1
Trigonella	Fabaceae	E	A-2-1-1-1
Tripleurospermum	Asteraceae	E	A-2-1-1-1
Trisetum	Poaceae	D	A-1-2-3-1
Triticosecale	Poaceae	D	A-1-2-3-3
Triticum	Poaceae	D	A-1-2-3-3
Tropaeolum	Tropaeolaceae	G	A-2-2-2-2
Tsuga	Pinaceae	H	B-3-1-1-1
Ulmus	Ulmaceae	E	B-2-1-1-1
Urochloa	Poaceae	D	A-1-2-3-1
Vaccaria	Caryophyllaceae	E	A-2-1-1-1
Valeriana	Valerianaceae	E	A-2-1-1-1
Valerianella	Valerianaceae	E	A-2-1-1-1
Verbascum	Scrophulariaceae	E	A-2-1-1-1
Verbena	Verbenaceae	E	A-2-1-1-1
Viburnum	Adoxaceae	E	B-2-1-1-1
Vicia	Fabaceae	G	A-2-2-2-2
Vigna (without *V. subterraneae* and *V. angularis*)	Fabaceae	F	A-2-1-2-2
Vigna subterraneae and *V. angularis*	Fabaceae	G	A-2-2-2-2
Vinca	Apocynaceae	E	A-2-1-1-1
Viola	Violaceae	E	A-2-1-1-1
Xeranthemum	Asteraceae	E	A-2-1-1-1
Zea	Poaceae	D	A-1-2-3-2
Zelkova	Ulmaceae	E	B-2-1-1-1
Zinnia	Asteraceae	E	A-2-1-1-1
Zoysia	Poaceae	D	A-1-2-3-1

Appendix 3: Index of seedling abnormalities

One or a combination of the following defects render a seedling abnormal:

0 Overall abnormalities		
00 Seedling		
Abnormal type	**00/01**	is deformed
Abnormal type	**00/02**	is fractured
Abnormal type	**00/03**	releases the cotyledons before the primary root from the seed coat
Abnormal type	**00/04**	consists of fused twin seedlings
Abnormal type	**00/05**	bears an ‘endosperm collar’
Abnormal type	**00/06**	is yellow or white
Abnormal type	**00/07**	is spindly
Abnormal type	**00/08**	is glassy
Abnormal type	**00/09**	is decayed as a result of primary infection
Abnormal type	**00/10**	shows phytotoxic symptoms
Abnormal type	**00/11**	is unbalanced
Abnormal type	**00/12**	in Poaceae, detached endosperm

1 Abnormalities of the root system		
11 Primary root		
Abnormal type	**11/01**	is stunted
Abnormal type	**11/02**	is stubby
Abnormal type	**11/03**	is retarded
Abnormal type	**11/04**	is missing
Abnormal type	**11/05**	is deeply cracked or broken
Abnormal type	**11/06**	is split from the tip or split right through
Abnormal type	**11/07**	is trapped in the seed coat
Abnormal type	**11/08**	shows negative geotropism
Abnormal type	**11/09**	is constricted
Abnormal type	**11/10**	is spindly
Abnormal type	**11/11**	is glassy
Abnormal type	**11/12**	is decayed as a result of primary infection
Abnormal type	**11/13**	is split right through
Secondary roots showing one or more of the above defects are considered abnormal, and cannot replace an abnormal primary root in cases where the presence of several normal secondary roots (e.g. in *Cucumis*) determines the value of a seedling.		

12 Seminal roots (only in groups A-1-2-3-3 and A-2-1-4-3)		
Abnormal type	**12/01**	are stubby, weak or missing
At least one strong seminal root (e.g. *Triticum*), or two strong seminal roots (i.e. *Cyclamen*) are required for a normal seedling.		

2 Abnormalities of the shoot system		
21 Hypocotyl, epicotyl or mesocotyl		
Abnormal type	**21/01**	is too short and/or thick (except *Cyclamen*)
Abnormal type	**21/02**	does not form a tuber (only in *Cyclamen*)
Abnormal type	**21/03**	is deeply cracked or broken
Abnormal type	**21/04**	is split right through
Abnormal type	**21/05**	is missing
Abnormal type	**21/06**	is bent over or forms a loop
Abnormal type	**21/07**	forms a spiral
Abnormal type	**21/08**	is tightly twisted
Abnormal type	**21/09**	is constricted
Abnormal type	**21/10**	is spindly
Abnormal type	**21/11**	is glassy
Abnormal type	**21/12**	is decayed as a result of primary infection
Abnormal type	**21/13**	shows negative phototropism

22 Terminal bud and surrounding tissues		
Abnormal type	**22/01**	are deformed
Abnormal type	**22/02**	are damaged
Abnormal type	**22/03**	are missing
Abnormal type	**22/04**	are necrotic
Abnormal type	**22/05**	are decayed as a result of primary infection
Irrespective of the presence of auxiliary buds (e.g. *Phaseolus*) or auxiliary shoots (e.g. *Pisum*) arising from the axils of the cotyledons or of the primary leaves, the seedling is considered abnormal if the main shoot fails to develop normally.		

3 Abnormalities of the cotyledons and primary leaves		
31 Cotyledons (apply the 50 % rule; see Appendix 6)		
Abnormal type	**31/01**	are swollen or curled
Abnormal type	**31/02**	are deformed
Abnormal type	**31/03**	are broken or otherwise damaged
Abnormal type	**31/04**	are separate or missing
Abnormal type	**31/05**	are discoloured or necrotic
Abnormal type	**31/06**	are glassy
Abnormal type	**31/07**	are decayed as a result of primary infection
Abnormal type	**31/08**	are fused on both sides
Damage or decay of the cotyledons at the point of attachment to the seedling axis or near the terminal bud render a seedling abnormal, irrespective of the 50 % rule.		

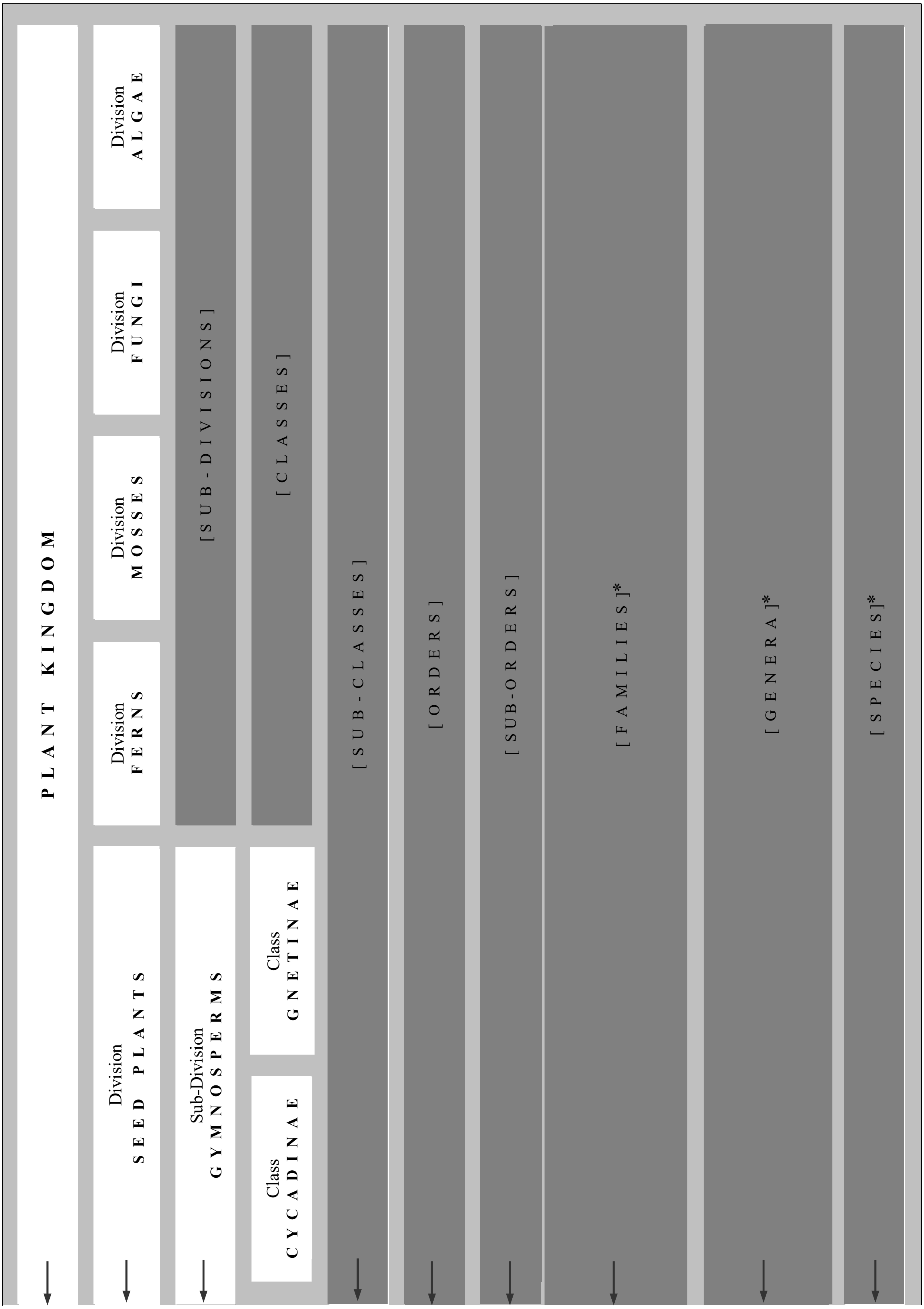
32 Cotyledon of group A-1-1-1-1		
Abnormal type	**32/01**	is short and thick
Abnormal type	**32/02**	is bent over or forms a loop
Abnormal type	**32/03**	forms a spiral
Abnormal type	**32/04**	does not show a definite ‘knee’
Abnormal type	**32/05**	is constricted
Abnormal type	**32/06**	is spindly

33 Primary leaves (apply the 50 % rule; see Appendix 6)		
Abnormal type	**33/01**	are deformed
Abnormal type	**33/02**	are damaged
Abnormal type	**33/03**	are missing
Abnormal type	**33/04**	are discoloured
Abnormal type	**33/05**	are necrotic
Abnormal type	**33/06**	are of normal shape, but less than one-quarter normal size (only in *Phaseolus*)
Abnormal type	**33/07**	are decayed as a result of primary infection

4 Abnormalities of the coleoptile and primary leaf		
41 Coleoptile		
Abnormal type	**41/01**	is stubby or otherwise deformed
Abnormal type	**41/02**	is broken
Abnormal type	**41/03**	is missing
Abnormal type	**41/04**	is defective or has no tip
Abnormal type	**41/05**	is strongly bent over or forms a loop
Abnormal type	**41/06**	forms a spiral
Abnormal type	**41/07**	is tightly twisted
Abnormal type	**41/08**	is split for more than one-third of the length from the tip
Abnormal type	**41/09**	is spindly
Abnormal type	**41/10**	is decayed as a result of primary infection
Abnormal type	**41/11**	is split other than from the tip
Abnormal type	**41/12**	is trapped under the lemma or the seed coat

42 Primary leaf		
Abnormal type	**42/01**	extends less than halfway up the coleoptile
Abnormal type	**42/02**	is missing
Abnormal type	**42/03**	is shredded or otherwise deformed
Abnormal type	**42/04**	protrudes from the lower part of the coleoptile
Abnormal type	**42/05**	is yellow or white (no chlorophyll)
Abnormal type	**42/06**	is decayed as a result of primary infection

Appendix 4: Systematics of the ISTA Families (IF)



* ISTA refers to Families, Genera and Species.

PLANT KINGDOM → Division SEED PLANTS → [SUB-CLASSES] → [ORDERS] → [SPECIES]

Sub-Division	Class	Code	Family	Genera
GYMNOSPERMS	GINKGOINAE	**IF01**	*Ginkgoaceae*	*Ginkgo*
GYMNOSPERMS	CONIFERS	**IF02**	*Pinaceae*	*Abies*, *Calocedrus*, *Cedrus*, *Larix*, *Picea*, *Pinus*, *Pseudotsuga*, *Tsuga*
GYMNOSPERMS	CONIFERS	**IF03**	*Taxodiaceae*	*Cryptomeria*, *Sequoia*, *Sequoiadendron*, *Taxodium*
GYMNOSPERMS	CONIFERS	**IF04**	*Cupressaceae*	*Chamaecyparis*, *Cupressus*, *Juniperus*, *Thuja*
GYMNOSPERMS	CONIFERS	**IF05**	*Taxaceae*	*Taxus*
ANGIOSPERMS	MONOCOTYLEDONS	**IF06**	*Liliaceae*	*Allium*, *Asparagus*, *Kniphofia*, *Lilium*
ANGIOSPERMS	MONOCOTYLEDONS	**IF07**	*Amaryllidaceae*	*Hippeastrum*
ANGIOSPERMS	MONOCOTYLEDONS	**IF08**	*Iridaceae*	*Freesia*
ANGIOSPERMS	MONOCOTYLEDONS	**IF09**	*Poaceae*	*Agropyron*, *Agrostis*, *Alopecurus*, *Andropogon*, *Anthoxanthum*, *Arrhenatherum*, *Astrebla*, *Avena*, *Axonopus*, *Beckmannia*, *Bothriochloa*, *Bouteloua*, *Brachiaria*, *Briza*, *Bromus*, *Cenchrus*, *Chloris*, *Coïx*, *Cynodon*, *Cynosurus*, *Dactylis*, *Deschampsia*, *Dichantium*

Appendix 4: Systematics of ISTA Families

PLANT KINGDOM

Division SEED PLANTS

Sub-Division ANGIOSPERMS

[SUB-CLASSES] [ORDERS] [SPECIES]

Class MONOCOTYLEDONS

IF09	*Poaceae*	*Digitaria*, *Echinochloa*, *Ehrharta*, *Eleusine*, *Elymus*, *Elytrigia*, *Eragrostis*, *Festuca*, × *Festulolium*, *Holcus*, *Hordeum*, *Koeleria*, *Lolium*, *Melinis*, *Oryza*, *Oryzopsis*, *Panicum*, *Pascopyrum*, *Paspalum*, *Pennisetum*, *Phalaris*, *Phleum*, *Piptantherum*, *Poa*, *Pseudorogneria*, *Secale*, *Setaria*, *Sorghastrum*, *Sorghum*, *Stipa*, *Taeniatherum*, *Trisetum*, *Triticum*, × *Triticosecale*, *Urochloa*, *Zea*, *Zoysia*

Class DICOTYLEDONS

IF10	*Magnoliaceae*	*Liriodendron*
IF11	*Ranunculaceae*	*Adonis*, *Anemone*, *Aquilegia*, *Consolida*, *Delphinium*, *Nigella*, *Pulsatilla*, *Ranunculus*
IF12	*Berberidaceae*	*Mahonia*
IF13	*Papaveraceae*	*Chelidonium*, *Eschscholzia*, *Papaver*
IF14	*Nyctaginaceae*	*Mirabilis*
IF15	*Aizoaceae*	*Dorotheanthus*, *Tetragonia*
IF16	*Amaranthaceae*	*Amaranthus*, *Celosia*, *Gomphrena*

Division SEED PLANTS → Sub-Division ANGIOSPERMS → Class DICOTYLEDONS → [SUB-CLASSES] → [ORDERS] → Families → [SPECIES]

IF17	*Chenopodiaceae*	*Atriplex*, *Beta*, *Kochia*, *Spinacia*
IF18	*Portulacaceae*	*Claytonia*, *Portulaca*
IF19	*Caryophyllaceae*	*Agrostemma*, *Cerastium*, *Dianthus*, *Gypsophila*, *Lychnis*, *Saponaria*, *Silene*, *Spergula*, *Vaccaria*
IF20	*Polygonaceae*	*Fagopyrum*, *Rheum*, *Rumex*
IF21	*Plumbaginaceae*	*Armeria*, *Goniolimon*, *Limonium*, *Psyllostachys*
IF22	*Hamamelidaceae*	*Liquidambar*
IF23	*Platanaceae*	*Platanus*
IF24	*Betulaceae*	*Alnus*, *Betula*, *Carpinus*, *Corylus*
IF25	*Fagaceae*	*Castanea*, *Fagus*, *Nothofagus*, *Quercus*
IF26	*Ulmaceae*	*Ulmus*, *Zelkova*
IF27	*Moraceae*	*Morus*
IF28	*Cannabaceae*	*Cannabis*
IF29	*Crassulaceae*	*Kalanchoë*
IF30	*Saxifragaceae*	*Heuchera*
IF31	*Rosaceae*	*Agrimonia*, *Cotoneaster*, *Crataegus*, *Cydonia*, *Fragaria*, *Geum*, *Malus*, *Prunus*, *Pyrus*, *Rosa*, *Sanguisorba*, *Sorbus*
IF32	*Fabaceae*	*Acacia*, *Alysicarpus*, *Amorpha*, *Anthyllis*

PLANT KINGDOM → Division SEED PLANTS → Sub-Division ANGIOSPERMS → Class DICOTYLEDONS → [SUB-CLASSES] → [ORDERS] → [SPECIES]

IF32	*Fabaceae*	*Arachis*
		Astragalus
		Cajanus
		Calopogonium
		Caragana
		Cassia
		Centrosema
		Cicer
		Coronilla
		Crotalaria
		Cyamopsis
		Cytisus
		Desmodium
		Galega
		Gleditsia
		Glycine
		Hedysarum
		Lablab
		Laburnum
		Lathyrus
		Lens
		Lespedeza
		Leucaena
		Lotononis
		Lotus
		Lupinus
		Macroptilium
		Macrotyloma
		Medicago
		Melilotus
		Mimosa
		Mucuna
		Onobrychis
		Ornithopus
		Phaseolus
		Pisum
		Psophocarpus
		Pueraria
		Robinia
		Senna
		Sesbania
		Sophora
		Spartium
		Stylosanthes
		Trifolium
		Trigonella
		Vicia
		Vigna
IF33	*Elaeagnaceae*	*Elaeagnus*
IF34	*Proteaceae*	*Grevillea*
IF35	*Myrtaceae*	*Eucalyptus*
IF36	*Onagraceae*	*Clarkia*
		Godetia
		Oenothera
IF37	*Rutaceae*	*Ruta*
IF38	*Meliaceae*	*Cedrela*
IF39	*Simaroubaceae*	*Ailanthus*
IF40	*Sapindaceae*	*Koelreuteria*

Appendix 4: Systematics of ISTA Families

PLANT KINGDOM

Division **SEED PLANTS**

Sub-Division **ANGIOSPERMS**

Class **DICODYLEDONS**

[SUB-CLASSES]

[ORDERS]

		[SPECIES]
IF41	*Hippocastanaceae*	*Aesculus*
IF42	*Aceraceae*	*Acer*
IF43	*Linaceae*	*Linum*
IF44	*Geraniaceae*	*Geranium*
		Pelargonium
IF45	*Tropaeolaceae*	*Tropaeolum*
IF46	*Balsaminaceae*	*Impatiens*
IF47	*Celastraceae*	*Euonymus*
IF48	*Euphorbiacea*	*Ricinus*
IF49	*Araliaceae*	*Dizygotheca*
		Fatsia
IF50	*Apiaceae*	*Anethum*
		Angelica
		Anthriscus
		Apium
		Carum
		Coriandrum
		Cuminum
		Daucus
		Foeniculum
		Levisticum
		Pastinaca
		Petroselinum
		Pimpinella
IF51	*Clusiaceae*	*Hypericum*
IF52	*Cistaceae*	*Helianthemum*
IF53	*Violaceae*	*Viola*
IF54	*Begoniaceae*	*Begonia*
IF55	*Capparaceae*	*Cleome*
IF56	*Brassicaceae*	*Alyssum*
		Arabis
		Aubrieta
		Brassica
		Camelina
		Cheiranthus
		Eruca
		Erysimum
		Hesperis
		Iberis
		Lepidium
		Lobularia
		Lunaria
		Malcolmia
		Matthiola
		Nasturtium
		Raphanus
		Rapistrum
		Sinapis
IF57	*Resedaceae*	*Reseda*
IF58	*Salicaceae*	*Populus*
		Salix

Appendix 4: Systematics of ISTA Families

PLANT KINGDOM

Division
SEED PLANTS

Sub-Division
ANGIOSPERMS

Class
DICOTYLEDONS

[SUB-CLASSES]

[ORDERS]

IF59	*Cucurbitaceae*	*Citrullus*
		Cucumis
		Cucurbita
		Lagenaria
		Luffa
		Momordica
IF60	*Tiliaceae*	*Corchorus*
		Tilia
IF61	*Malvaceae*	*Abelmoschus*
		Abutilon
		Alcea
		Althaea
		Gossypium
		Hibiscus
		Lavatera
		Malope
		Malva
IF62	*Primulaceae*	*Anagallis*
		Cyclamen
		Primula
IF63	*Aquifoliaceae*	*Ilex*
IF64	*Cornaceae*	*Cornus*
IF65	*Adoxaceae*	*Viburnum*
IF66	*Valerianaceae*	*Valeriana*
		Valerianella
IF67	*Dipsacaceae*	*Scabiosa*
IF68	*Oleaceae*	*Fraxinus*
		Ligustrum
		Syringa
IF69	*Gentianaceae*	*Gentiana*
IF70	*Apocynaceae*	*Vinca*
IF71	*Asclpiadaceae*	*Asclepias*
IF72	*Solanaceae*	*Atropa*
		Browallia
		Capsicum
		Datura
		Lycopersicon
		Nicandra
		Nicotiana
		Nierembergia
		Petunia
		Physalis
		Salpiglossis
		Schizanthus
		Solanum
IF73	*Convolvulaceae*	*Convolvulus*
		Dichondra
		Ipomoea
		Pharbitis
		Quamoclit
IF74	*Polemoniaceae*	*Cobaea*
		Gilia
		Phlox

[SPECIES]

Appendix 4: Systematics of ISTA Families

PLANT KINGDOM → Division SEED PLANTS → Class DICOTYLEDONS → Class DICOTYLEDONS → [SUB-CLASSES] → [ORDERS] → [SPECIES]

IF75	*Hydrophyllaceae*	*Nemophila*
		Phacelia
IF76	*Boraginaceae*	*Anchusa*
		Borago
		Brunnera
		Cynoglossum
		Echium
		Heliotropium
		Myosotis
IF77	*Scrophulariaceae*	*Antirrhinum*
		Calceolaria
		Cymbalaria
		Digitalis
		Linaria
		Mimulus
		Nemesia
		Penstemon
		Torenia
		Verbascum
IF78	*Gesneriaceae*	*Saintpaulia*
		Sinningia
IF79	*Acanthaceae*	*Thunbergia*
IF80	*Pedaliaceae*	*Sesamum*
IF81	*Bignoniaceae*	*Catalpa*
IF82	*Plantaginaceae*	*Plantago*
IF83	*Verbenaceae*	*Tectona*
		Verbena
IF84	*Lamiaceae*	*Coleus*
		Galeopsis
		Hyssopus
		Lavandula
		Leonurus
		Marrubium
		Melissa
		Mentha
		Moluccella
		Nepeta
		Ocimum
		Origanum
		Perilla
		Rosmarinus
		Salvia
		Satureja
		Stachys
		Thymus
IF85	*Campanulaceae*	*Campanula*
		Legousia
IF86	*Lobeliaceae*	*Lobelia*
IF87	*Asteraceae*	*Achillea*
		Ageratum
		Amberboa
		Ammobium
		Arctium
		Arctotis
		Arnica
		Artemisia

Appendix 4: Systematics of ISTA Families

PLANT KINGDOM	Division **SEED PLANTS**	Sub-Division **ANGIOSPERMS**	Class **DICOTYLEDONS**	[SUB-CLASSES]	[ORDERS]	**IF87**	*Asteraceae*	*Aster*, *Bellis*, *Brachyscome*, *Calendula*, *Callistephus*, *Carthamus*, *Castalis*, *Centaurea*, *Chrysanthemum*, *Cichorium*, *Cnicus*, *Coroepsis*, *Cosmos*, *Cynara*, *Dahlia*, *Dendranthema*, *Dimorphotheca*, *Doronicum*, *Echinacea*, *Echinops*, *Erigeron*, *Gaillardia*, *Gazania*, *Gerbera*, *Helenium*, *Helianthus*, *Helichrysum*, *Heliopsis*, *Helipterum*, *Inula*, *Lactuca*, *Leontopodium*, *Leucanthemum*, *Liatris*, *Lonas*, *Matricaria*, *Osteospermum*, *Rudbeckia*, *Sanvitalia*, *Scorzonera*, *Senecio*, *Silybum*, *Tagetes*, *Tanacetum*, *Taraxacum*, *Tragopogon*, *Tripleurospermum*, *Xeranthemum*, *Zinnia*	[SPECIES]

Appendix 5: Illustrative standard operating procedures

A5.1 Introduction

In order to promote uniformity of results and reduce variation within and between laboratories Table 5A of the Germination chapter of the ISTA Rules[1] prescribes temperature regimes that must be used when carrying out germination tests on different species when reporting on ISTA Certificates. The chapter also prescribes temperatures to be used, when pre-chilling or pre-heating are used for breaking dormancy and stipulates specifications for various attributes of growing media used in germination tests.

ISTA accredited laboratories are required to have procedures in place for the measurement and monitoring of temperatures. They are also expected to have in place procedures that ensure that media used in germination tests conforms to the specifications prescribed in the ISTA Rules.

This Appendix of the Handbook provides an illustrative Standard Operation Procedure (SOP) that can be used by laboratories when they are developing procedures and arrangements for temperature measurement in their own laboratory. It also contains illustrative SOPs that could be used to measure the attributes of the growing media used in their laboratory. Laboratories are expected to develop their own procedures and the SOPs are included as guidance to laboratories as to what meets the requirements of the ISTA Accreditation Standard[2]. They are simply examples and should not be regarded as a requirement or criterion that will be applied by auditors when assessing the procedures used in individual laboratories.

These illustrative SOPs should be referred to in conjunction with current copies of the ISTA Rules and the ISTA Accreditation Standard.

A5.2 Germination procedures – temperature measurement and control in the germination laboratory

A5.2.1 Introduction

Temperature is one of the most commonly measured physical quantities but its basis is not widely understood. Whereas the units of other quantities, such as mass and time units are based on real physical entities temperature is founded on a theoretical set of conditions. So whilst the perfect kilogram is in Paris and time is based on atomic transitions in a caesium atom, temperature is based on the thermodynamics of perfect systems, such as ideal gases. This results in the thermodynamic temperature scale measured in kelvins (K), which is unattainable. To overcome this we do the next best thing and use imperfect thermodynamic systems to achieve a working temperature scale as near to the theoretical one as we can get.

Temperature is an important factor in a number of tests carried out by ISTA Member Laboratories and this standard operating procedure has been drawn up to assist laboratories and auditors when they are considering the arrangement for temperature measurement.

A5.2.2 Temperature specification

The ISTA Rules state that:

‘Temperatures prescribed in Table 5A are those the seed is exposed to on, or inside the substrate. They should be as uniform as possible throughout the germination apparatus, cabinet or room germinator. It is recommended that for tests, either in darkness or under an artificial source of light or in indirect daylight, variation from the prescribed temperature, due to the apparatus, should not be more than ±2 °C.

‘Where alternating temperatures are indicated, the lower temperature should usually be maintained for 16 hours and the higher temperature for eight hours. A gradual changeover lasting three hours may be satisfactory, but a sharp changeover lasting one hour or less, or transference of the tests to another germinator at a lower temperature, may be necessary for seeds which are likely to be dormant.’

A5.2.3 Temperature measurement

The two most common methods of measuring temperature are using thermometers or thermocouples.

A5.2.3.1 Thermometers

A thermometer (Figure A5.1) is an instrument that measures the temperature of a system in a quantitative way. The most direct ‘regular’ way is a linear one. For example, the element mercury (Figure A5.2) is liquid in the temperature range of –38.9 °C to 356.7 °C. As a liquid, mercury expands as it gets warmer. Its expansion rate is linear and can be accurately calibrated.

1 In the Appendix 5, ‘ISTA Rules’ refers to the International Rules for Seed Testing, Edition 2006.
2 In the Appendix 5, ‘ISTA Accreditation Standard’ refers to the ISTA Seed Testing Laboratory Accreditation Standard, Version 4.0.

Figure A5.1 Range of different types of temperature measuring equipment.

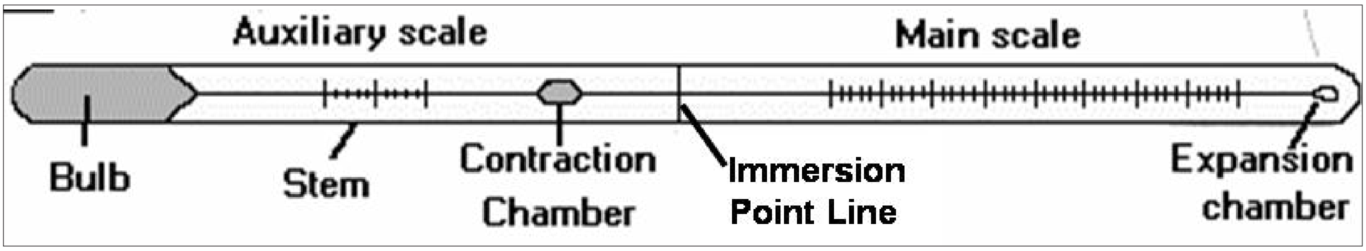


Figure A5.2 The mercury-in-glass thermometer contains a bulb filled with mercury that is allowed to expand into a capillary. Its rate of expansion is calibrated on the glass.

A5.2.3.2 Thermocouples

When wires of different metals are fused at one end and heated, a current flows from one to the other. The electromotive force generated can be quantitatively related to the temperature and hence, the system can be used as a thermometer – known as a thermocouple. The thermocouple is used in many electronic/digital thermometers and many different metals are used, for example platinum and platinum-rhodium or nickel–chromium and nickel–aluminium (Figure A5.3).

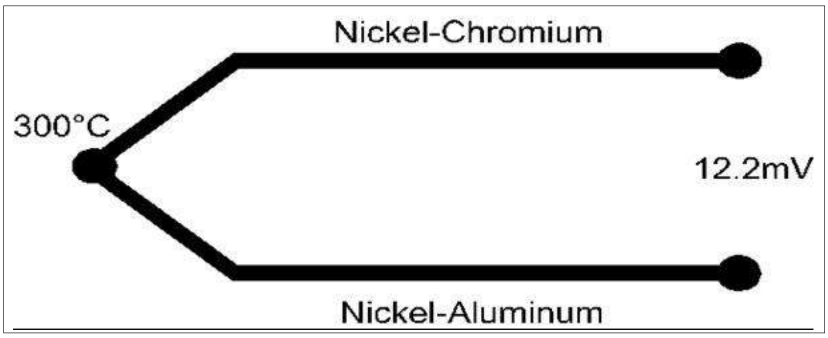


Figure A5.3 A nickel–chromium / nickel–aluminium thermocouple which generates 12.2 mV when heated to 300 °C.

A5.2.3.3 Total immersion and partial immersion thermometers

Total immersion thermometers (Figure A5.4a) are designed to be totally submerged in the media whose temperature is being measured. They are distinguished by the engraved suffix '/ TOTAL' or '/ TOT IMM'.

Partial immersion thermometers (Figure A5.4b) should only be immersed to the indicated depth. They have a suffix that indicates the immersion depth, e.g. '/ 100 MM IMM' should be immersed to the depth of 10 cm. They may also have an engraved ring on the stem indicating the immersion depth.

Figure A5.4 Total (**a**) and partial (**b**) immersion thermometers.

A5.2.3.4 Sensitivity/accuracy of probes

The sensitivity (Figure A5.5) and accuracy of probes (thermometers or thermocouples) varies. Some give readings with accuracy of ±0.1 °C; with others it would be difficult to achieve a temperature reading with an accuracy of ±2 °C.

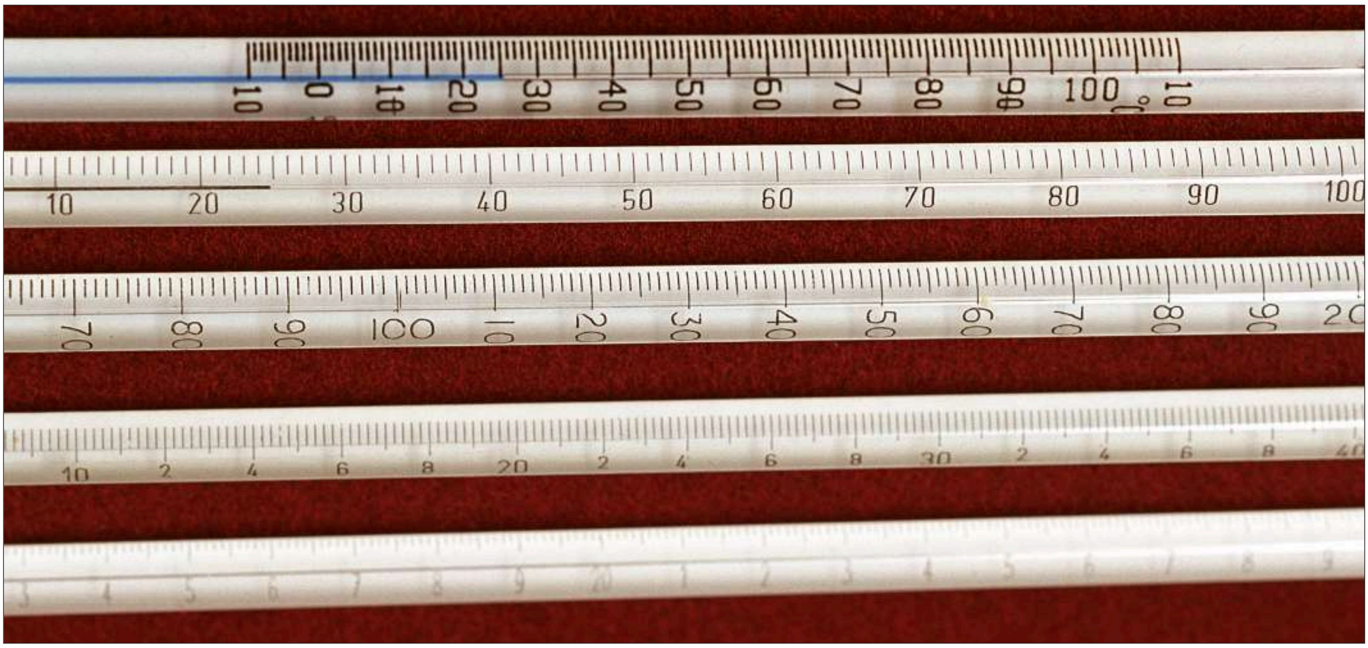


Figure A5.5 Thermometers with different sensitivities.

A5.2.3.5 Sensitivity and accuracy of temperature measurement in ISTA germination laboratories

Laboratories should use temperature measurement instruments that conform to ISO 386 with a scale range divided into units of at least 0.5 °C. The measurement uncertainties of thermometers that meet these criteria are given in Table A5-A. These depend on the range of the thermometer and whether the thermometer is total immersion or partial immersion.

Table A5-A Measurement uncertainties of total immersion and partial immersion thermometers.

Thermometer scale divided into (in degree Celsius)	Uncertainties of thermometers (in degree Celsius)[3]							
	Scale range[4]							
	Total immersion				Partial immersion			
	–25 °C to 100 °C	–10 °C to 50 °C	–10 °C to 100 °C	–10 °C to 200 °C	–25 °C to 100 °C	–10 °C to 50 °C	–10 °C to 100 °C	–10 °C to 200 °C
0.01	–	±0.005	±0.01	–	–	±0.005	±0.01	–
0.02	±0.02	±0.01	±0.02	–	±0.02	±0.01	±0.02	–
0.05	±0.05	±0.02	±0.02	±0.05	±0.05	±0.05	±0.05	±0.05
0.1	±0.1	±0.02	±0.02	±0.05	±0.1	±0.05	±0.05	±0.1
0.2	±0.1	±0.05	±0.05	±0.05	±0.1	±0.05	±0.05	±0.1
0.5	±0.2	±0.05	±0.05	±0.1	±0.5	±0.1	±0.1	±0.1

The sensitivity and uncertainty of thermocouples used in the germination laboratory should be similar to the specifications given above for thermometers.

A5.2.3.6 Total immersion thermometers and thermocouples

Total immersion thermometers and thermocouples can be used to monitor the temperature of incubators, germination cabinets, walk-in germinators, ovens and fridges.

For ease of taking measurements and to reduce dramatic temperature fluctuations that can result from the opening of doors of apparatus, thermometers and probes should be submerged in glycerol or sand (Figure A5.6).

A5.2.3.7 Partial immersion thermometers

Partial immersion thermometers can be used to monitor the temperature of incubators, ovens and fridges through an external aperture on the apparatus, provided that they can be immersed to the required depth within the apparatus (Figure A5.7).

3 Widest limits of measurement uncertainty for compliance with calibration requirements of ISO 386.
4 When the scale range of a thermometer falls within more than one of the ranges given in the table the range with the highest uncertainty is held to apply.

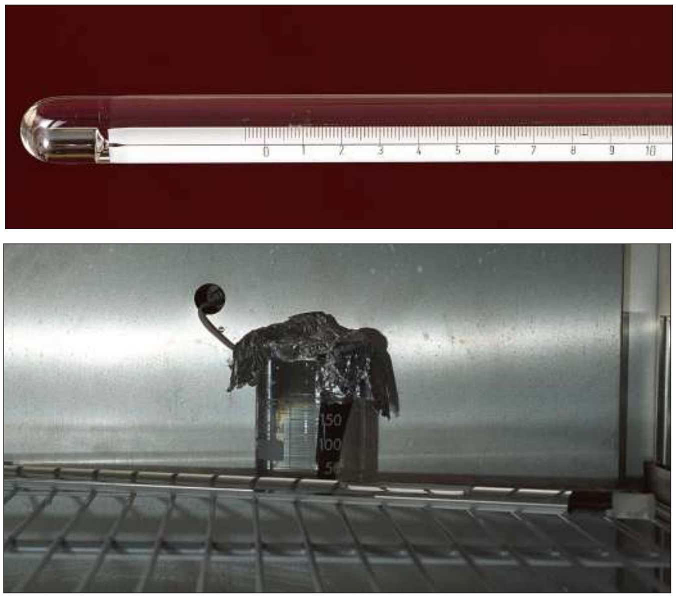


Figure A5.6 Thermometers/probes should be immersed in glycerol or sand to reduce dramatic temperature fluctuations.

Figure A5.7 Monitoring oven temperature using partial immersion thermometers.

A5.2.3.8 Measurement of temperature – Copenhagen tanks

Total and partial immersion thermometers can NOT be used to monitor the temperature of Copenhagen Tanks or germinators since it is not possible to meet immersion requirements. Thermocouples with contact probes must be used (Figure A5.8).

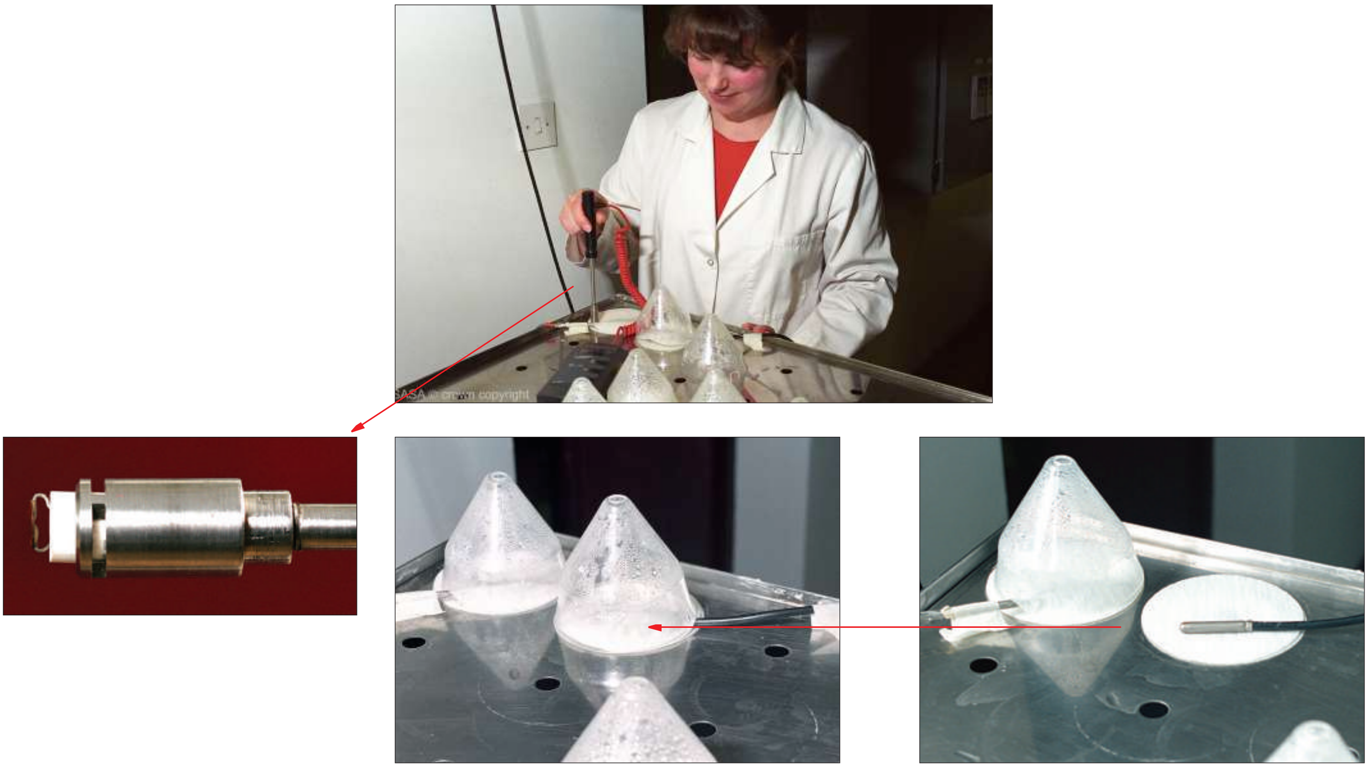


Figure A5.8 Measurement of the temperature of a Copenhagen tank using a contact or surface probe.

Appendix 5: Illustrative SOPs

A5.2.4 Frequency

Many laboratories now use data-loggers, or other temperature monitoring equipment, that constantly monitors temperature and records of readings are available on an hourly basis or less. For manual recording the following are the minimum requirements:

- For constant temperature equipment, at least three readings should be recorded per day at regular pre-set times. If records show that the equipment is stable in terms of temperature, with variations of less than 1.0 °C between readings, the recording frequency can be reduced to once per day. However, if there is any indication of a change in performance, recording frequency should be increased.
- For alternating temperature equipment, at least three readings should be recorded per day at pre-set times. The timing of these readings must be such that both temperature phases are monitored.
- Laboratories should plot readings from individual pieces of apparatus against time as this is the easiest way to notice any changes in the performance.
- For alternating temperature equipment, quarterly checks are required on the change over time between high and low phases. This should be no more than 3 hours.
- Where records show that a piece of apparatus is stable, with day to day variations of less than 1.0 °C, measures to take readings at weekends and public holidays are not required. Where apparatus is unstable, with day to day variations greater than 1.0 °C, measures to take readings at weekends and public holidays are required. This might involve the use of maximum/minimum thermometer or a small individual logger.

A5.2.5 Temperature profiles

Before accepting a piece of temperature controlled equipment (ovens, incubators, fridges, Copenhagen tanks, germinators, propagators, germination rooms, etc.) into service a laboratory must ascertain the temperature profile of the equipment to ensure that it is fit for purpose. There should be a minimum of three check points covering different combinations of height, depth and breadth, as appropriate. For example, for incubators, fridges and ovens temperature should be recorded on top, middle and bottom shelves and on each shelf the temperature should be measured at three points: front (near the door), middle and back. The temperatures of all points measured must be within ±2.0 °C before a piece of equipment is accepted into service.

Once in service profiles are only measured again if a piece of equipment undergoes repair or major service.

A5.2.6 Number of measuring points

At least one temperature measuring point must be used to monitor temperature controlled equipment.

Where there is more than one measuring point the difference in temperature between measuring points should be no more than ±2.0 °C.

If there is more than one measuring point, the mean of the temperatures recorded at the measuring points is used to check whether a specification is met.

A5.2.7 Calibration

The ISTA Accreditation Standard states that:

‘All sampling, measuring and seed testing equipment, for which this is possible, must be adequately calibrated before being placed into service and regularly afterwards, and a log book kept in which is recorded the results of each calibration, service and repairs. Calibration and servicing of equipment must be performed according to an established programme.

‘The overall programme of calibration of equipment must be designed and operated so as to ensure that, wherever applicable, measurements made in the seed testing laboratory are traceable to national and international standards of measurement.

‘Appropriate calibration samples, reference materials and reference standards of measurement must be held by the laboratory, and be used for calibration and reference purpose only. They should, where possible, be traceable to SI units of measurement, or to certified reference materials. Examples include calibration samples for seed blowers, standard buffer solutions for pH meters, calibration weights for balances, and reference collections of seed.’

A5.2.7.1 Calibration of temperature probes in the seed testing laboratory

Calibration can be achieved in a number of ways:

- probes can be calibrated externally;
- externally calibrated reference probes can be used to calibrate working probes;
- probes can be calibrated using ice.

Steam/boiling water must NOT be used to calibrate probes.

A5.2.7.1.1 External calibration of probes

The current working temperature scale is the International Temperature Scale of 1990 (ITS-90) and is measured in degrees Celsius (°C). When a thermometer is calibrated externally it should be to ITS-90 or to an agreed previous temperature scale such as IPTS-68 or even IPTS-48. External calibration should be repeated every five years and this should be supplemented by annual in-house ice point calibration. Any individual probe whose temperature reading differs from the standard by more than ±0.5 °C should be removed from service.

A5.2.7.1.2 Externally calibrated reference probes can be used to calibrate working probes

When calibrating a batch of thermometers against an externally calibrated probe, the calibration should be carried out at temperatures within the normal working range of the thermometers and in conditions where fluctuation in temperature between readings is limited (Figure A5.9). Such calibration should be carried out at least once a year and any probe whose temperature reading differs from the externally calibrated reference probe by more than ±0.5 °C should be removed from service.

A5.2.7.1.3 Calibration of thermometers – international ice point method

When calibrating probes using the ice point, international methodology should be followed. The ice point may be realised in an insulated flask or vessel containing an ice-water melting mixture (Figure A5.10). The ice particles should be no more than a few millimetres in diameter and the water and ice should be pure or prepared from de-ionised water, which is air saturated. For high precision the thermometer should be maintained in the mixture for 10 minutes prior to reading. In theory, accuracies of ±0.001 °C may be achieved but in practice ±0.005 °C is more likely.

Calibration of probes using ice point methodology should take place at least once a year and any probe whose temperature reading differs from the ice point by more than ±0.5 °C should be removed from service.

Only probes that can read 0 °C can be calibrated using ice point methodology (Figure A5.11).

Figure A5.9 Using an externally calibrated reference thermometer to calibrate working thermometers.

Figure A5.10 (far left) Reading an ice point.

Figure A5.11 Probes must be able to read 0 °C for ice point calibrations.

Appendix 5: Illustrative SOPs

A5.2.7.1.4 Calibration of thermometers – steam/boiling water method

Calibration of temperature probes using steam/boiling water **should not be used** by ISTA Member Laboratories. The boiling point of water varies with atmospheric pressure and this is dependent on both weather conditions and altitude (Figure A5.12). At sea level with an atmospheric pressure of 760 mm Hg the boiling point may be 100 °C but at an altitude of 1310 m and atmospheric pressure of 650 mm Hg the boiling point is 95.5 °C.

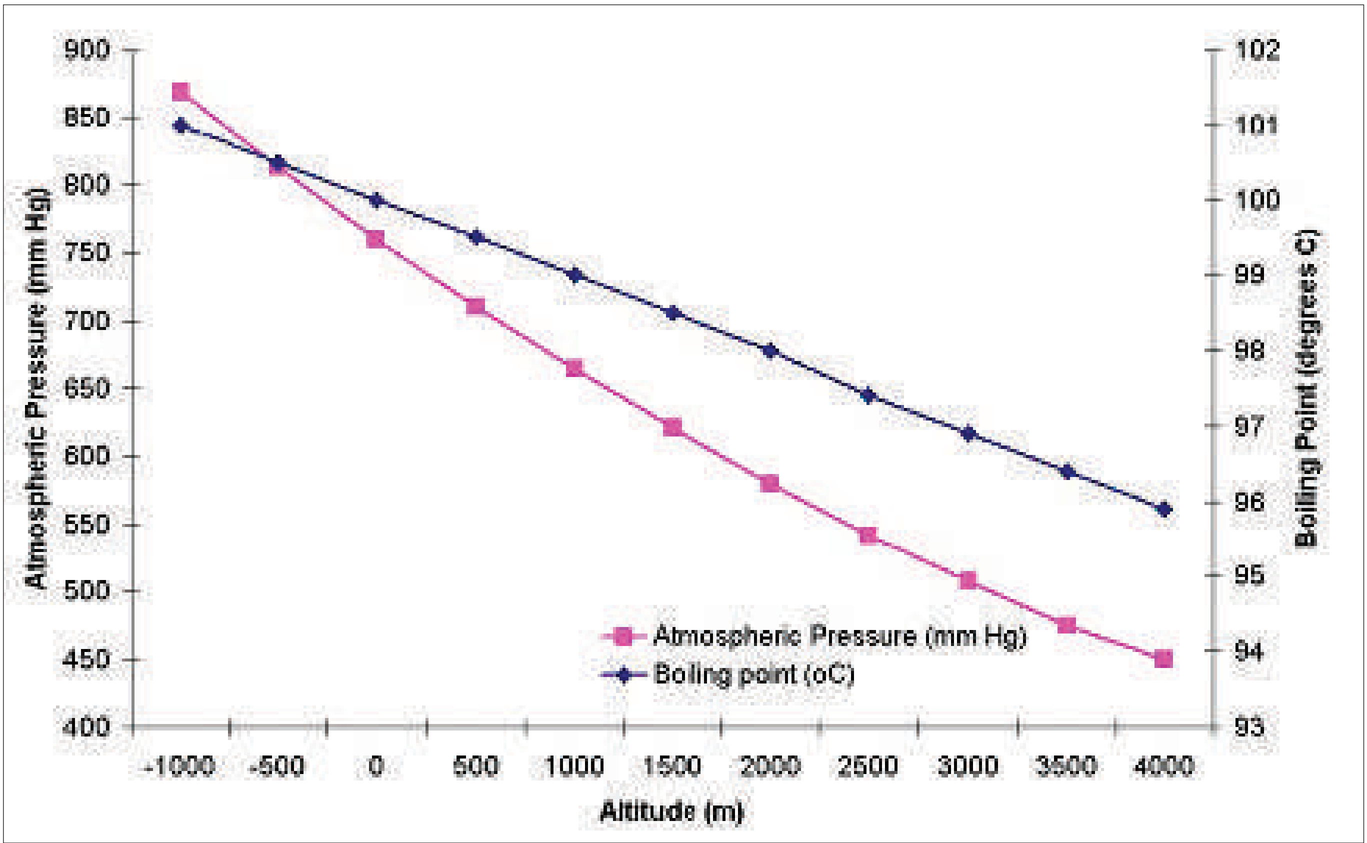


Figure A5.12 Relationship between altitude, atmospheric pressure and the boiling point of water.

A5.2.7.2 Calibration of thermocouples, electronic meters and temperature data loggers

The procedures for the calibration of thermocouples and temperature probes of electronic meters and data loggers (Figure A5.13) should be the same as those adopted for thermometers. In other words they can be calibrated externally, or in-house:

- against a externally calibrated probe or thermometer; or
- using the ice point method.

As with thermometers, any probe that differs by more than ±0.5 °C from the standard should be removed from service unless the probe can be readjusted to give a reading within the ±0.5 °C limit.

A5.2.7.3 Using calibration data to adjust temperature readings and limits

Adjustments should be made for differences between probe temperature and that of the calibrated standard. For example if a probe reads 19.6 °C when the calibrated thermometer/probe reads 20 °C, and the probe is to be used to monitor a germination room which has a performance requirement of 20 ±2 °C, the limits using the probe will be 17.6 to 21.6 °C.

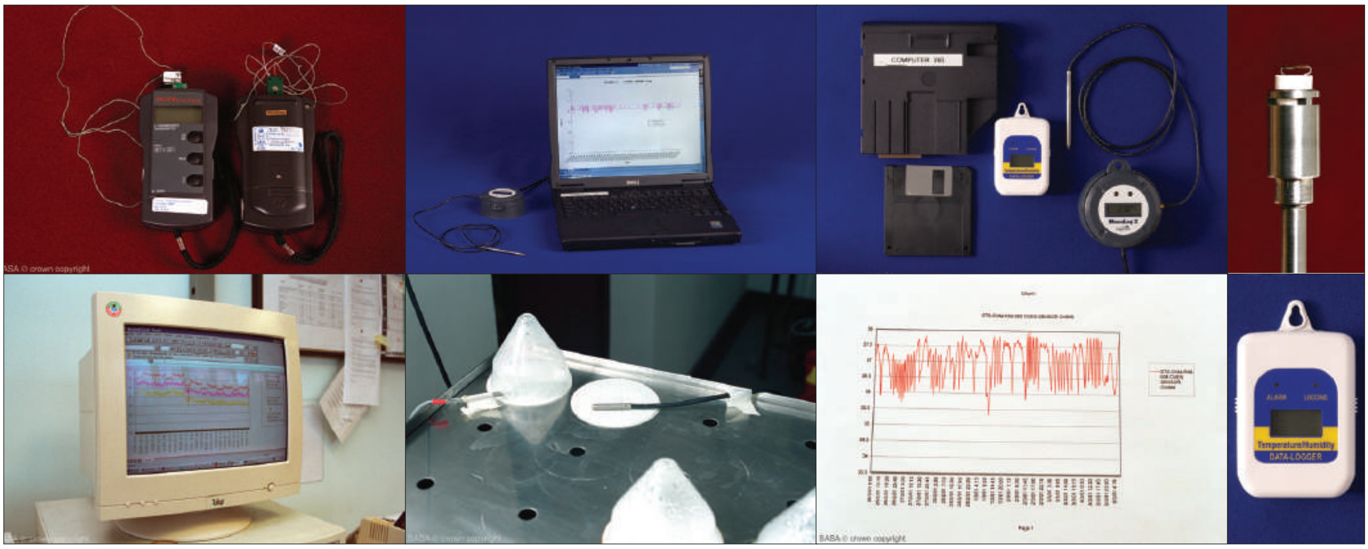


Figure A5.13 Range of different thermocouples, temperature probes, electronic meters and data loggers.

A5.2.7.4 Records of calibration checks

The ISTA Accreditation Standard states that:

'All records of results, calibration, maintenance and repair of equipment and reference materials must be kept for a minimum of six years.'

Calibration checks should be recorded and be available for inspection by auditors (Figure A5.14). The easiest way to do this is in a thermometer/probe register. The temperature records of individual pieces of apparatus must also be recorded and available for inspection by auditors who may want to ascertain the temperature of a particular piece of apparatus on a specific date. Temperature records and calibration checks must be archived for a period of at least six years.

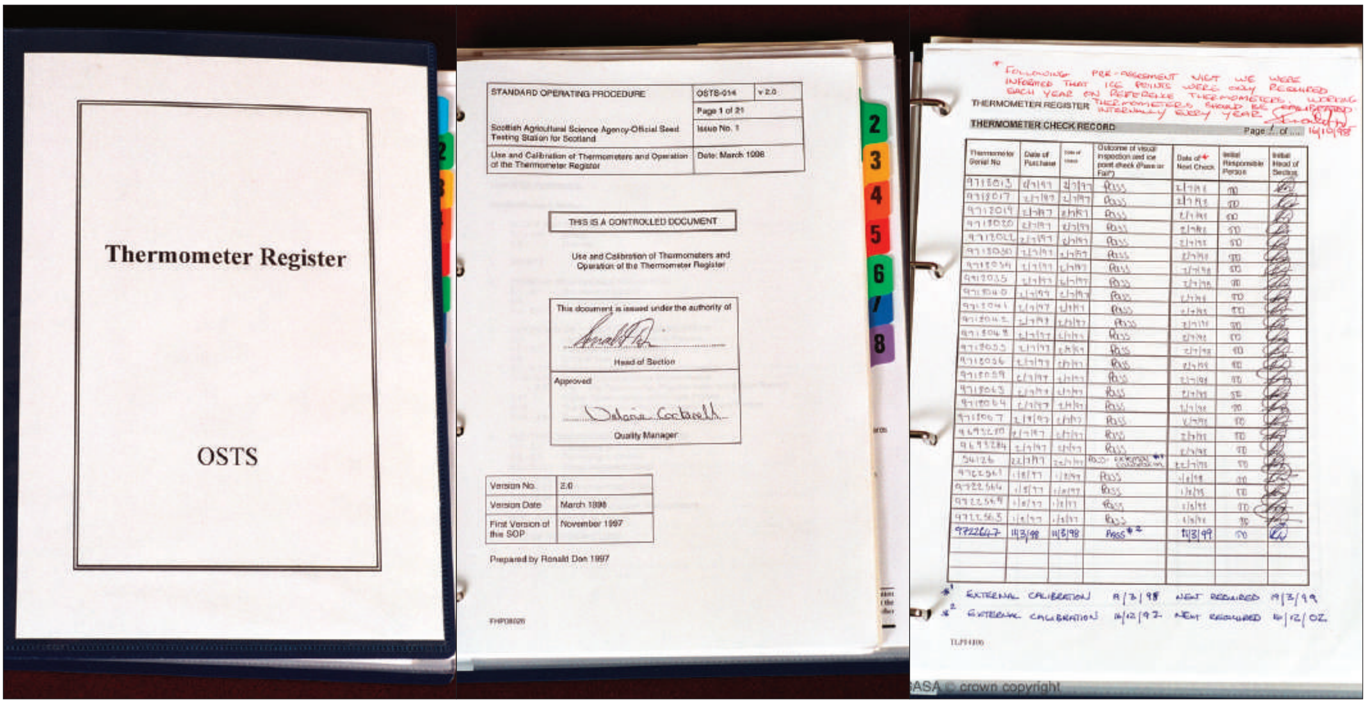


Figure A5.14 Thermometer register containing details of calibration checks.

A5.3 Germination procedures – growing media specification checks: water retention

A5.3.1 Specification

The ISTA Rules give the specification for water retention characteristics of growing media:

'When the appropriate amount of water is added, the particles of the growing media should have the capacity to hold sufficient water to provide continuous movement of water to the seeds and seedlings. It should also provide sufficient pore space for aeration required for optimal germination and root growth. *A priori*, the water content of the growing media shall be adjusted to the maximum water holding capacity. When necessary, for certain species, it can be adjusted to correspond to the needs of a particular species. The water retention shall then be expressed as a percentage of the maximum retention.'

A5.3.2 Measurement principle

The benchmark for the measurement of the water retention characteristics is the ISO method 11274 (1998) (as updated): 'Soil quality – Laboratory methods' for the determination of water retention characteristic.

The general principle is to measure the maximum amount of water held by the substrate and express this as a percentage of the dry weight of substrate.

A5.3.3 Procedure

The moisture content (MC) of the substrate is measured. Moisture content is measured according to the ISTA Rules Chapter 9. The high constant temperature method is used (Figure A5.15).

The water present in the substrate is equal to MC × fresh weight (FW). Weights are measured to one decimal place.

A defined weight of substrate (W_s) is placed in a waterproof container with drainage holes that are covered by a filter, which allows excess water to drain without loss of substrate (Figure A5.16).

Water is added until the substrate becomes saturated and excess water is allowed to filter from the container over a minimum period of 16 hours to a maximum of 24 hours, during which measures are taken to prevent evaporation. At this level of moisture the substrate is said to be at 'field capacity' and its weight is equal to W_{FC} (Figure A5.17).

The amount of water present in the substrate before it is saturated = $(H_2O)_S$

$$\mathbf{(H_2O)_S = W_s \times (MC/100)}$$

The dry weight of the substrate used = $(DW)_S$

$$\mathbf{(DW)_S = W_s - (H_2O)_s}$$

The amount of water present when the substrate is at field capacity = $(H_2O)_{FC}$

$$\mathbf{(H_2O)_{FC} = W_{FC} - W_s + (H_2O)_s}$$

The maximum amount of water held in growing media as percentage of its dry weight = $(H_2O)_{MAX}$

$$\mathbf{(H_2O)_{MAX} = [(H_2O)_{FC} / DW_S] \times 100}$$

For each batch of media at least three measurements should be made on random samples of the batch. The average of the measurements is used to check compliance with the specification for the media.

A5.3.4 Importance of checking water retention capacity of growing media

The water retention capacity of a growing media depends of course on the type of the substrate (paper, sand, organic growing media), but it depends also within the same kind of substrate, when batches are produced.

As the production of batches of media could modify the water retention capacity, it could thus influence the amount of water available for the seeds during germination. It is necessary to check the water retention capacity of each new batch of growing media and to adapt the amount of water to be added for germination.

As an example, if the paper media indicated in Table A5-B is used for germination test and that due to experimental work in the laboratory, it has been demonstrated that the optimal amount of water for germination of *Brassica* species is 20 ml for 10 g of paper (dry weight (DW) basis). Considering that this paper holds a maximum of (467.8/134.2) × 100 = 348.6 g of water for 100 g of paper (DW basis), it means that for this test condition the amount of water added to the paper corresponds to (200/348.6) × 100 = 57 % of the maximum water retention capacity of the media.

If a new batch of the same paper media has a maximum retention capacity of 370 % instead of 348.6 %, then 100 g of dry paper media holds a maximum 370 g of water.

In order to have the same amount of available water for seed germination, you need to adjust the amount of water to 57 % of the maximum water retention capacity. This means that you need to add a different amount of water calculated as the following: 370 × 57/100 = 210.9 g of water for 100 g of dry weight paper (or 21.1 g of water for 10 g).

Table A5-B Example calculations for paper, organic growing media and sand.

	Paper media	Organic growing media	Sand
Moisture content determined using high constant temperature oven method (130 °C for 1 hour) **(MC)**	**7.1 %**	**32.0 %**	**15.5 %**
Weight of substrate used to determine water retention **(W_s)**	**144.5 g**	**257.2 g**	**620 g**
Weight of saturated substrate **(W_{FC})**	**602.0 g**	**508.4 g**	**740.5 g**
$(H_2O)_S$ = W_s × (MC/100)	= 144.5 × 0.071 = **10.3 g**	= 257.2 × 0.32 = **82.3 g**	= 620.0 × 0.155 = **96.1 g**
$(DW)_S$ = W_s − $(H_2O)_s$	= 144.5 − 10.3 = **134.2 g**	= 257.2 − 82.3 = **174.9 g**	= 620 − 96.1 = **523.9 g**
$(H_2O)_{FC}$ = W_{FC} − W_s + $(H_2O)_s$	= 602.0 − 144.5 + 10.3 = **467.8 g**	= 508.4 − 257.2 + 82.3 = **333.5 g**	= 740.5 − 620 + 96.1 = **216.6 g**
$(H_2O)_{MAX}$ = [$(H_2O)_{FC}$ / DW_S] × 100	= (467.8/134.2) × 100 = **348.6 %**	= (333.5/174.9) × 100 = **190.7 %**	= (216.6/523.9) × 100 = **41.3 %**

A5.3.5 Illustrated procedure

Figure A5.15 The moisture content of the germination media is measured using the ISTA constant temperature oven method at 130 °C.

Appendix 5: Illustrative SOPs

Figure A5.16 The germination media is weighed. For sand and organic growing media, a waterproof container with drainage is required. The drainage holes are covered using a material, such as filter paper, that allows water drainage but prevents the loss of material.

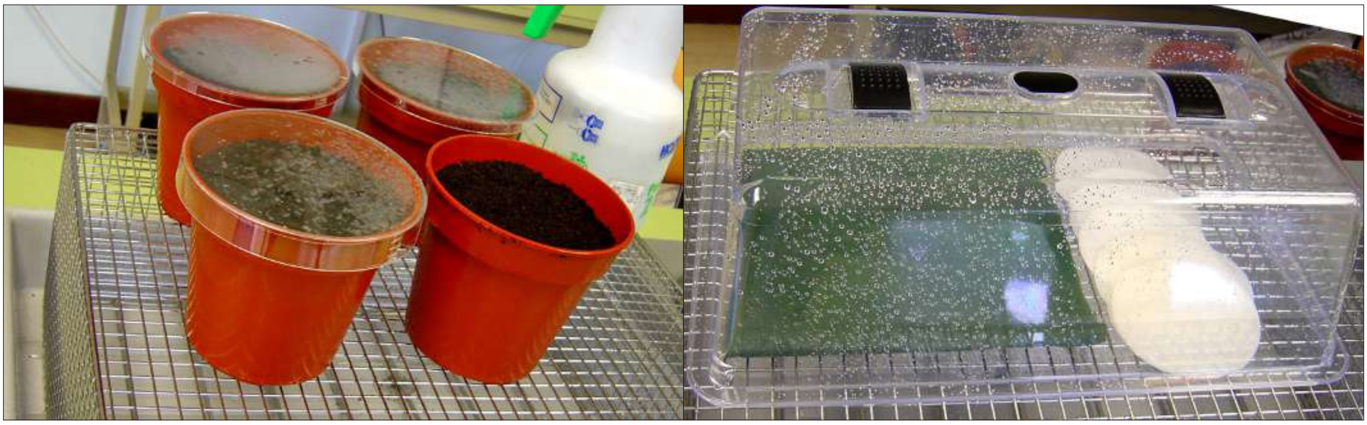


Figure A5.17 The germination media is saturated with water and allowed to freely drain for 12 hours with measures being taken to prevent evaporation. The saturated media is then weighed and the maximum amount of water held in the growing media as percentage of its dry weight is calculated.

A5.3.6 Calculation flow chart

Measure the moisture content of the growing medium using the ISTA high constant oven method (130°C for 1 hour).

The moisture content is the weight of the water in the growing medium divided by the total weight of the growing medium, expressed as a percentage.

↓

Moisture content =
A

A defined weight of growing medium is saturated with water, and excess water is allowed to freely drain over a 12-hour period, during which measures are taken to prevent evaporation

↓

Weight of growing medium =
B

↓

Weight of growing medium after saturation =
C

Calculations

1. Multiply the weight of growing medium by the moisture content:

A x B = **D**

This is the amount of water in the growing media before wetting.

2. Subtract the amount of water in the growing medium before wetting from the weight of the growing medium:

B – D = **E**

This is the dry weight of the medium.

3. Subtract the dry weight of the growing medium from its weight after saturation:

C – E = **F**

This is the maximum amount of water held in the growing medium.

4. Express the maximum amount of water held in the growing medium as a percentage of the dry weight:

(F/E)100

A5.4 Germination procedures – growing media specification checks: pH

A5.4.1 Specification

The ISTA Rules give the specification for the pH of growing media:

'The pH value should be within the range 6.0–7.5 when checked in the substrate.'

A5.4.2 Measurement principle

The benchmark for the measurement of the pH is the ISO method 10390 (1994) (as updated): 'Soil quality – Determination of pH'.

The general principle is to measure the pH of the water available for germination when checked within the substrate.

A5.4.3 Procedure

The preparation for measurement varies according to the germination media.

Organic growing media and sand

Samples of 5 ml or more of the organic growing media or sand are mixed with five volumes of water that is to be used for germination tests[5]. The mixture is stirred for 5 min and then allowed to stand for a minimum of 2 hours and a maximum of 24 hours. After standing the mixture is stirred and the stabilised pH value of the suspension solution measured (Figure A5.18).

Paper Media

Samples of germination paper are moistened with water that is to be used for germination tests[5] and the pH is measured on the surface of the paper.

The pH can be measured using pH paper with an appropriate range (Figures A5.19 and A5.20) or using a calibrated pH meter (Figure A5.21). For paper media when using a pH meter a specific probe manufactured for measuring the pH on the surface of paper must be used (Figures A5.22 and A5.23).

For each batch of media at least three measurements should be made on random samples of the batch.

Should the three measurements differ by more than 0.5 the batch is considered heterogeneous and should be rejected.

The average of the measurements is used to check compliance with the specification.

A5.4.4 Illustrated procedure

Figure 5.18 For sand and organic growing media, one volume of media is mixed with five volumes of water that is to be used for germination tests[5]. The mixture is stirred for 5 min and then allowed to stand for a minimum of 2 hours and a maximum of 24 hours. After standing, the mixture is stirred and the stabilised pH value of the suspension solution measured.

5 It is recommended that the conductivity of the water should be < 0.2 millisiemens/m and its pH should be > 5.6 at 25 °C.

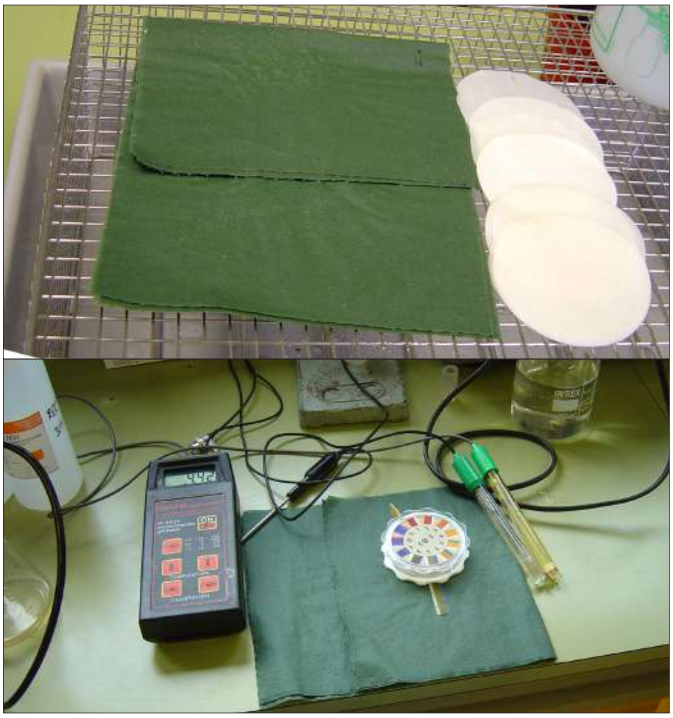


Figure A5.19 Paper media samples are moistened with water that is to be used for germination tests[6] and the pH is measured on the surface of the paper. The pH is measured using a calibrated pH meter or pH paper.

Figure A5.20 Using pH paper to measure the pH of paper germination media.

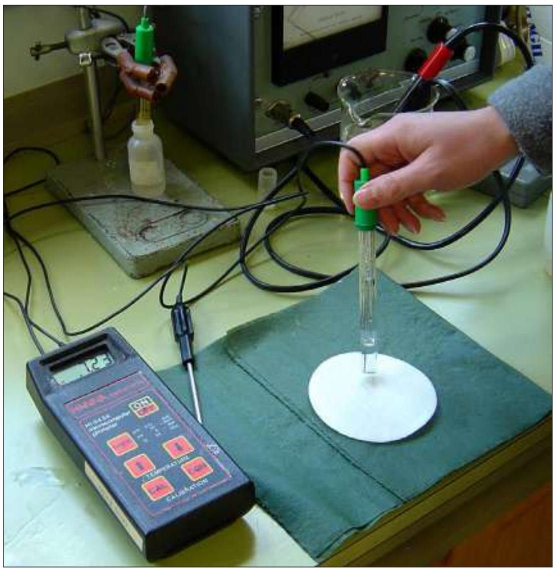


Figure A5.21 A pH meter with a specific probe manufactured for measuring the pH on the surface of paper must be used for paper media.

Figure A5.22 Surface (left) and dip (right) probes for pH meter.

Figure A5.23 Surface probe for measuring the pH of paper.

6 It is recommended that the conductivity of the water should be < 0.2 millisiemens/m and its pH should be > 5.6 at 25 °C.

A5.5 Germination procedures – growing media specification checks: conductivity

A5.5.1 Specifiation

The ISTA Rules give the specification for the conductivity of the growing media:

‘Conductivity: the salinity must be as low as possible and no more than 40 millisiemens per metre.’

A5.5.2 Measurement principle

The benchmark for the measurement of conductivity is the ISO method 11265 (1994) (as updated): ‘Soil quality – Determination of specific electrical conductivity’.

The general principle is to measure the conductivity of solutes in the media.

A5.5.3 Procedure

For paper, sand and organic growing media, 20 g are mixed with 100 ml of water, which is used for germination tests[7], at 20 °C ±1 °C. This is stirred for 30 min before obtaining the solute by passing the mixture through a filter paper (Figure A5.24).

The solute conductivity is measured using a calibrated conductivity meter employing a dip cell (Figure A5.25).

For each batch of media at least three measurements should be made on random samples of the batch.

If the difference between replicates is greater than 5 millisiemens per metre, the batch of media should be rejected.

The average of the measurements is used to check compliance with the specification.

A5.5.4 Illustrated procedure

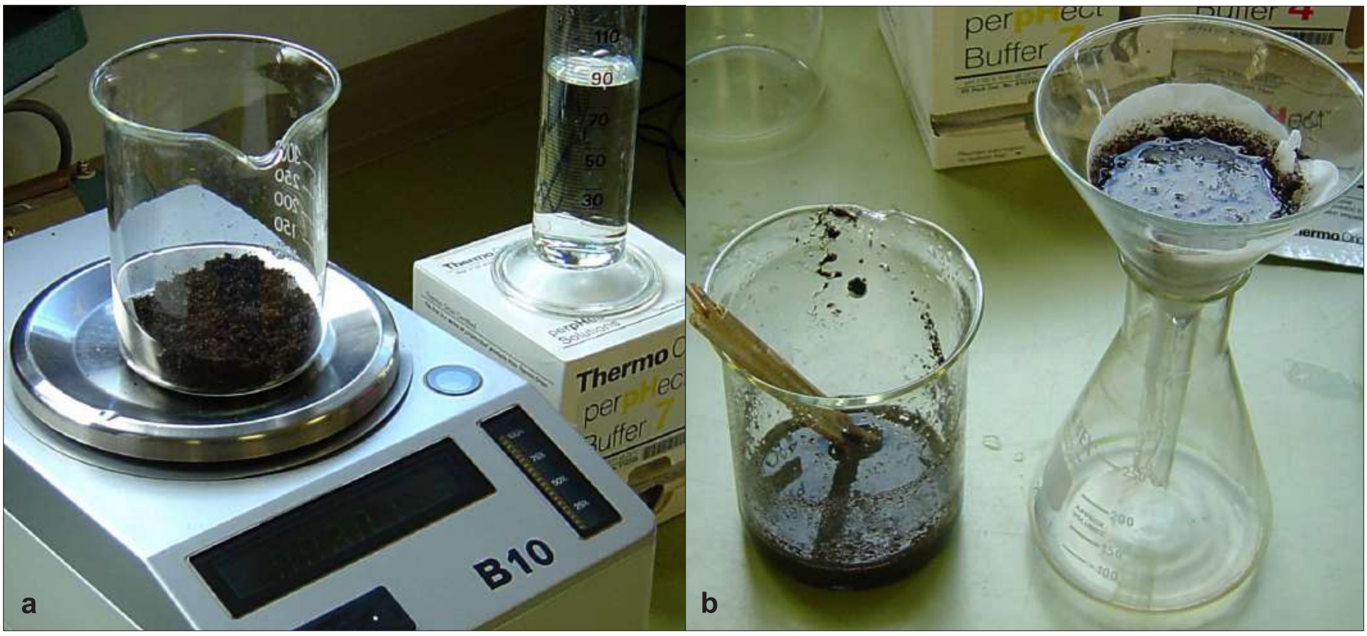


Figure A5.24 20 g of media are mixed with 100 ml of water, which is used for germination tests[7], at 20 °C ±1 °C (**a**). This is mixed and left for 30 min before filtering (**b**).

7 It is recommended that the conductivity of the water should be < 0.2 millisiemens/m and its pH should be > 5.6 at 25 °C.

Figure A5.25 The conductivity of the filtrate is measured using a calibrated conductivity meter with a dip cell.

A5.6 Germination procedures – growing media specification checks: cleanliness and innocuity

A5.6.1 Specification

The ISTA Rules give the specification for the cleanliness and innocuity of the growing media:

‘The growing media must be free from seeds, fungi, bacteria or toxic substances, which may interfere with the germination of seeds, the growth of seedlings or their evaluation.

‘A substrate which shows statistical evidence that the number of germinated seeds or normal seedlings is decreased compared to a reference substrate of known quality due to toxic materials in the substrate is said to be phytotoxic. Visual evidence of symptoms can be sufficient to declare a substrate phytotoxic. Phytotoxic media must not be used for germination tests.

‘The presence in the substrate of micro-organisms (saprophytes and/or pathogens) can affect the germination or the development of seedlings. A substrate which shows statistical evidence that the number of germinated seeds or normal seedlings is decreased compared to a reference substrate of known quality due to micro-organisms must not be used for germination tests unless the substrate is disinfected before use. Disinfection should be carried out in such a way that no chemical which might suppress or kill seed borne disease organisms remains within the growing media. In addition, disinfection must not render the media phytotoxic. Biological tests for phytotoxicity and micro-organisms should be carried out after disinfection.’

A5.6.2 Measurement principle

Cleanliness and innocuity of media are determined using biological tests.

A5.6.3 Procedure

A5.6.3.1 Tests for phytotoxicity

Germination tests are carried out: with the substrate being checked and a reference substrate (media acceptance test).

To verify that a bach of media is suitable for germination tests, seeds of certain species evaluated in the laboratory that are known to be sensitive to toxic substances, are used: *Agrostis gigantea*, *Allium cepa*, *Apium graveolens*, *Beta vulgaris*, *Brassica* sp., *Cichorium intybus*, *Eragrostis curvula*, *Festuca rubra*, *Hordeum vulgare*, *Lactuca sativa*, *Lepidium sativum*, *Petunia* sp., *Phaseolus vulgaris*, *Phleum pratense*, *Pisum sativum*, *Sesamum indicum*, *Solanum lycopersicum*, *Sorghum bicolor*, *Trifolium* sp., *Triticum aestivum*, *Zea mays*.

At least 400 seeds each of two sensitive species are tested on four samples of the media taken at random from the batch of media being evaluated.

Evaluating the tests requires the assessment of:

- the percentage of germinated seeds at an early stage (on or before the days specified in Table 5A of the ISTA Rules for first count of the species used for the test); and/or
- the occurence of normal and abnormal seedlings with symptoms due to toxic substances, non-germinated seeds after the final count.

Specific symptoms include: shortened roots, sometimes discoloured tips, roots raised from the substrate, root hairs ‘bunched’, short and/or thick hypocotyls. In Poaceae, symptoms also include coleoptiles that are flattened and shortened.

When the media is to be used for the testing of a limited number of species, e.g. *Hordeum vulgare* or *Zea mays*, the phytotoxicity tests can be carried out using these species rather than two sensitive species.

For those unfamiliar with phytotoxic symptoms a positive reference substrate can be set up. This is the reference substrate to which a herbicide, that is known to induce phytotoxic effects, is added; for example for *Petunia*, dichlobenyl is added at a concentration of 14 mg/litre.

Analysis of the results of the final count should be carried out using an appropriate statistical test.

Visual evidence of the absence of symptoms and statistical analysis, showing no significant difference between the substrate being tested and the reference substrate, are required to declare a substrate suitable for use.

A5.6.3.2 Tests for freedom from the negative effects due to micro-organisms

The biological test used to assess phytotoxicity can be used with the additional observation of the number of decayed seeds and/or seedlings with infection.

A5.6.4 Acceptable probabilities obtained when carrying out analysis of variance

As a general rule, when carrying out ANOVA on the results of media acceptance tests: if the new media gives a lower result than the reference media and the probability of obtaining such a result by chance is 5 % or lower (probability = 0.05 or lower), then the media should be rejected. It must not be used for germination tests, unless action can be taken to alleviate its deleterious effect on germination. In cases when the probability is close to the limit, e.g. between 5 % and 10 %, the decision whether to reject the media or not should be based on the experience of the analyst.

A5.6.5 Examples of analyses of results of biological tests for cleanliness and innocuity

Laboratories should analyse the results of innocuity tests statistically. Single factor Analysis of Variance (ANOVA) is an example of the type of analysis that can be undertaken.

Example 1 – Media is phytotoxic and has an adverse effect on the germination of indicator species

Observations

In test media, stunted root growth was observed as well as roots growing upwards from the germination media. (Photographs of examples of seedlings exhibiting phytotoxic effects as a result of harmful substances within the germination media, are given in Section A5.5.6.)

Results of germination tests

Table A5-C Results[8] of germination tests in four samples of each, the germination media and the reference media.

Sample	Results of germination tests in four samples of the batch of germination media being tested	Results of germination tests in four samples of the reference media
1	83.0	94.0
2	82.0	91.0
3	79.0	93.0
4	86.0	92.0
Mean	82.5	92.5

Table A5-D Single factor analysis of variance (ANOVA).

Source of variance	Degrees of freedom	Sums of squares	Mean square	F-value	Probability
Total	7	230			
Media	1	200	200	40	0.00073
Error	6	30	5		

The germination of the indicator seed in the test media exhibits phytotoxic effects and the ANOVA shows that the mean germination of the four tests in the test media (82.5 %) is significantly lower than that obtained in the reference media (92.5 %). The probability of obtaining such a result by chance is 0.07 %. This batch of media should be rejected and must not be used for germination tests.

8 Germination results are the mean of one 4 × 100 seed test.

Example 2 – Media gives a lower germination of indicator species

Observations

In test media there were no signs of adverse effects on seedling growth or development.

Results of germination tests

Table A5-E Results[9] of germination tests in four samples of each, the germination media and the reference media.

Sample	Results of germination tests in four samples of the batch of germination media being tested	Results of germination tests in four samples of the reference media
1	91.0	94.0
2	88.0	91.0
3	89.0	93.0
4	86.0	92.0
Mean	88.5	92.5

Table A5-F Single factor analysis of variance (ANOVA).

Source of variance	Degrees of freedom	Sums of squares	Mean square	F-value	Probability
Total	7	50			
Media	1	32	32	10.67	0.017
Error	6	18	3		

Although there appears to be no adverse effect on seedling growth and development, the ANOVA shows that the mean germination of the four tests in the test media (88.5 %) is significantly lower than that obtained in the reference media (92.5 %). The probability of obtaining such a result by chance is 1.7 %. Further investigations need to be undertaken. What is the cause of the lower germination? Is there an increase in the number of ungerminated seed or abnormal seedlings in this batch of media? This batch of media should be rejected and must not be used for germination tests unless action can be taken to alleviate its deleterious effect on germination.

Example 3 – Media has no effect on the germination of indicator species

Observations

In test media there were no signs of an adverse effect on seedling growth or development.

Results of germination tests

Table A5-G Results[9] of germination tests in four samples of each, the germination media and the reference media.

Sample	Results of germination tests in four samples of the batch of germination media being tested	Results of germination tests in four samples of the reference media
1	95.0	94.0
2	88.0	91.0
3	94.0	93.0
4	91.0	92.0
Mean	92.0	92.5

9 Germination results are the mean of one 4 × 100 seed test.

Table A5-H Single factor analysis of variance (ANOVA).

Source of variance	Degrees of freedom	Sums of squares	Mean square	F-value	Probability
Total	7	35.5			
Media	1	0.5	0.50	0.086	0.779
Error	6	35.0	5.83		

There appears to be no adverse effect on seedling growth and development the ANOVA shows that the mean germination of the four tests in the test media (92 %) is not significantly different to that obtained in the reference media (92.5 %). This batch of media can be accepted and used for germination tests provided a similar result is obtained using another test species.

A5.6.6 Photographs of seedlings on media with phytotoxic effects

Figure A5.26 *Hordeum* seedlings: those on the left are abnormal due the phytotoxic effect of 2,4 D in the germination medium. Normal seedlings are on the right hand side.

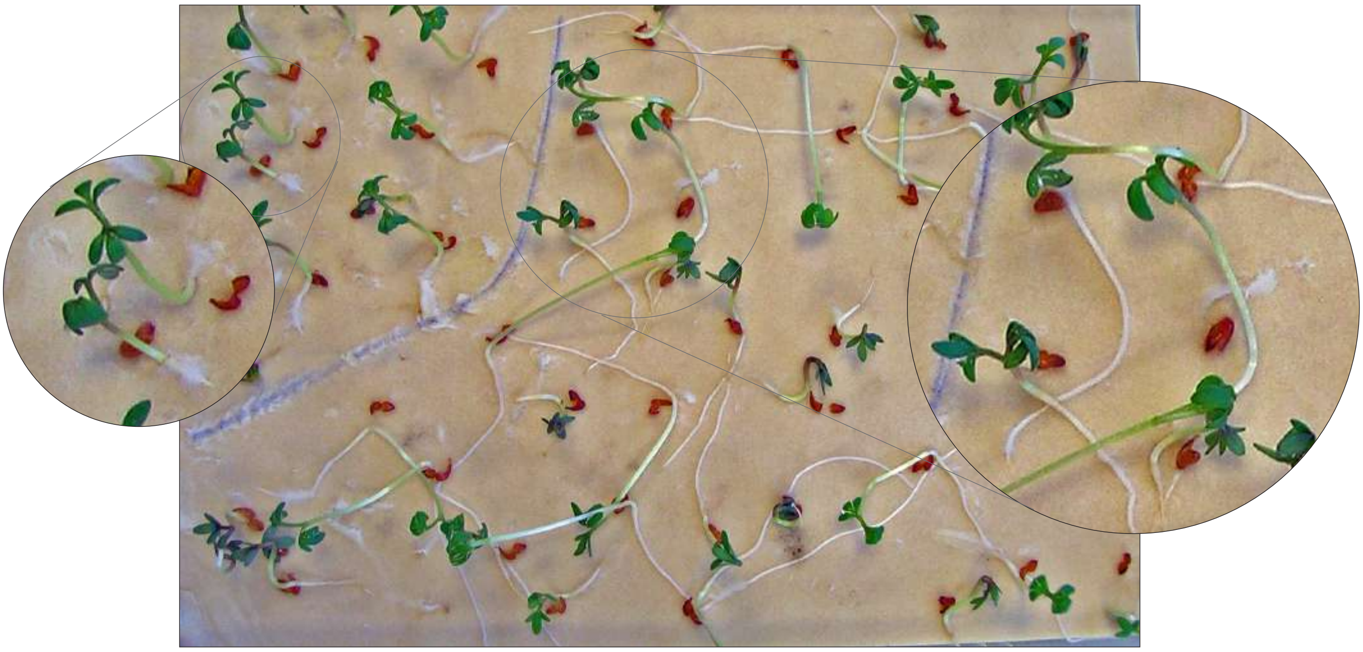


Figure A5.27 *Lepidium* seedlings affected by phytotoxic material in the top left hand side of the blotter pad. Seedlings affected by the phytotoxic material have short roots compared to normal seedlings.

Figure A5.28 *Lepidium* seedlings: those on the right are normal whilst those on the left are abnormal, having been affected by high levels of salinity in the germination medium.

Appendix 6: 50 % rule for evaluation of foliated cotyledons – guidelines relating to damaged, necrotic, decayed and discoloured tissue

A6.1 Principles

The 50 % rule should be applied in the evaluation of **foliated cotyledons**.[1] Seedlings are considered to be normal as long as half or more of the total cotyledon tissue is functional, but abnormal when more than half is missing, necrotic, decayed or discoloured.[2]

The 50 % rule is not valid if the point of attachment to the seedling axis or the tissues around the terminal bud itself are necrotic, decayed or albino, irrespective of whether half or more of the total cotyledon tissue is functional, because these defects affect the flow of sap. Seedlings with such damage are considered abnormal. If, however, the damage or defect at the point of attachment to the seedling axis is superficial and does not affect the conductive tissue, the seedling can be considered normal if half or more of the total cotyledon tissue is functional.

Discoloration caused by yellowing, colour degradation or chlorophyll deficiency is not considered to be as serious a defect as albinism. Seedlings are not considered abnormal even when such discoloration is found at the point of attachment to the seedling axis, provided that half or more of the total cotyledon tissue is functional. However, seedlings are considered to be abnormal when more than half of the total cotyledon tissue is discoloured in such a manner.

NOTE: Unless otherwise stated, all photographs of *Lactuca sativa* seedlings in Appendix 6 are to the same scale and may thus be used for size comparison purposes.

A6.2 Application of the 50 % rule

A6.2.1 Expansion of cotyledons

The ISTA Rules state that the 50 % rule should be applied when assessing whether defects to cotyledons renders a seedling abnormal. The seedling is abnormal if the cotyledons are defective to such an extent that less than 50 % of the original/estimated tissue (see A6.2.3 and A6.2.4) is functioning normally. It is therefore very important that the cotyledons are fully expanded when the assessment of seedling is made using the 50 % rule. To facilitate this, bell jars should be removed from tests grown on Copenhagen tanks and the lids can be removed from boxes containing pleated paper and Top of Paper (TP) tests several hours before seedling evaluation. Light should also be provided during this period. When the bell jars and lids are removed, care must be taken to prevent the tests from drying out.

It should be noted that delay in casting the seed coat in TP tests and lack of light can result in cotyledons that are not fully expanded and in spindly growth. Care should be taken when such seedlings are assessed using the 50 % rule.

A6.2.2 Variation in cotyledon size

It should be noted that the cotyledon size of normal seedlings in a given test can vary by 50 % or more, particularly where lots of mixed varieties are tested. If a seedling is balanced, with the various structures in proportion to one another, it is considered to be normal, irrespective of the absolute sizes of the individual parts (Figure A6.1).

1 Cotyledons which during seed germination and seedling development expand, turn green and photosynthesise.
2 Discoloured includes white albino tissue as well as areas showing signs of yellowing and colour degradation or chlorophyll deficiency.

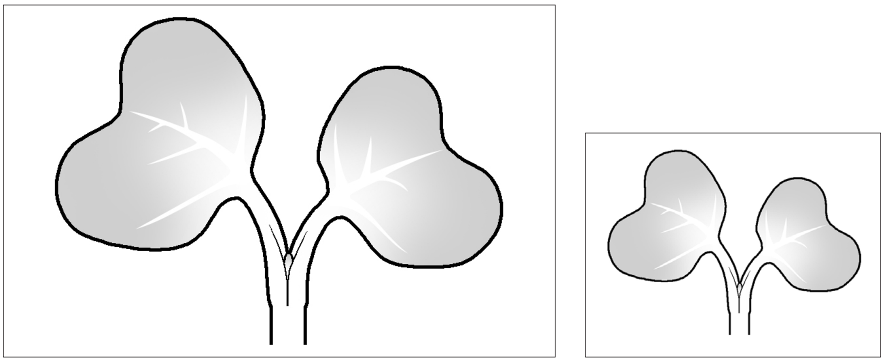


Figure A6.1 Intact cotyledons of two seedlings from the same germination test. The seedling with the small cotyledons is considered to be normal, provided that the seedling is balanced. Care must be taken to select a comparable standard seedling when defective seedlings are assessed according to the 50 % rule.

A6.2.3 Evaluation based on original tissue

If the cotyledons have necrosis or other defects, but nevertheless develop and expand normally, they must be evaluated on the basis of the original tissue. That is, they are evaluated according to the actual areas of defective and normal tissue in the cotyledons (Figures A6.2, A6.3). If 50 % or more of the total cotyledon tissue is normal, the seedling is assessed as normal, providing that there is no evidence of damage or decay to the shoot apex or surrounding tissue.

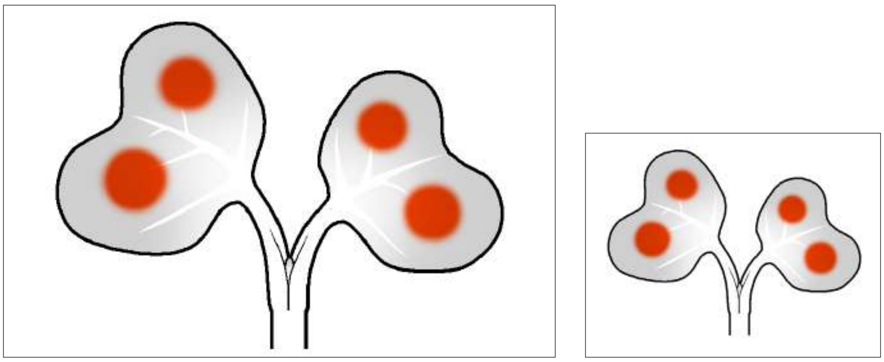


Figure A6.2 Evaluation of original tissue: normal seedlings. The total damaged area is smaller than half of the original tissue, and more than 50 % of the original tissue is functioning normally.

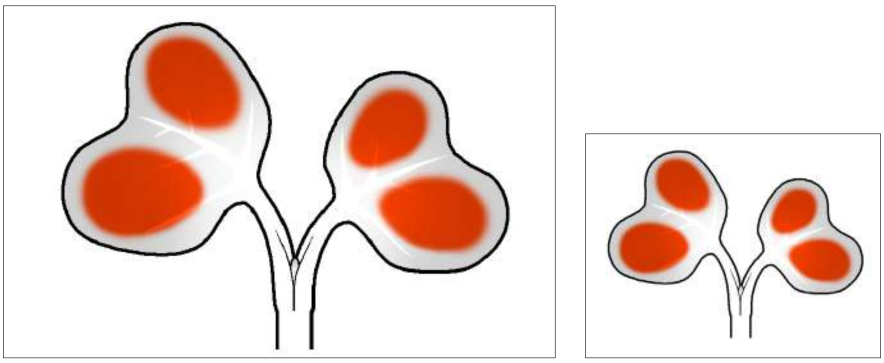


Figure A6.3 Evaluation of original tissue: abnormal seedlings. The total damaged area is larger than half of the original tissue, and less than 50 % of the original tissue is functioning normally.

● = Necrotic, decayed or discoloured tissue

A6.2.4 Evaluation based on estimated tissue

However, if the cotyledons have necrosis or other defects and do not develop or expand normally as a result of the defect, then the seedling shall be evaluated on the basis of the estimated tissue that would be present if the necrosis/defect were not present, and the cotyledons had developed and expanded normally (Figures A6.4–6).

Intact seedlings from the same test must be taken as a standard and used to assess the defective seedling according to the 50 % rule. The selection of such intact seedlings is based on the judgement of the analyst. In tests where seedlings have a range of different sizes of cotyledons, several seedlings will be required as standards (see A.6.2.2).

When only one cotyledon is defective and does not develop or expand normally, the estimated tissue of the defective cotyledon must be assessed with reference to the normally developed cotyledon (Figure A6.4).

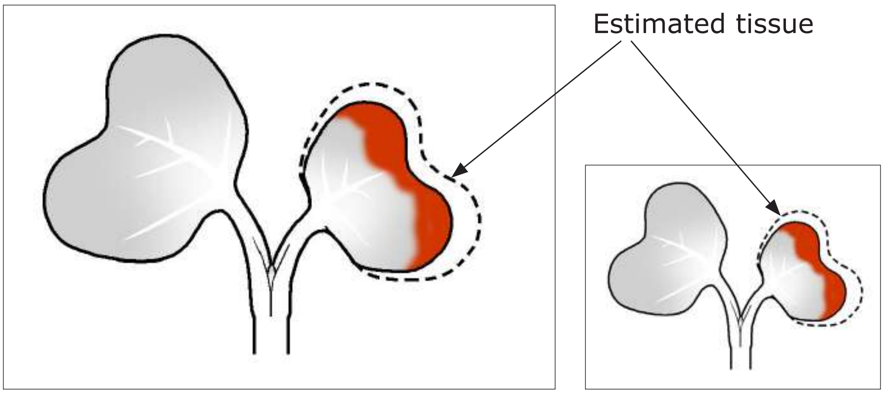


Figure A6.4 Evaluation of estimated tissue: normal seedlings. One cotyledon is defective and does not develop or expand normally. The estimated tissue size of the defective cotyledon is deduced with reference to the other normal cotyledon.

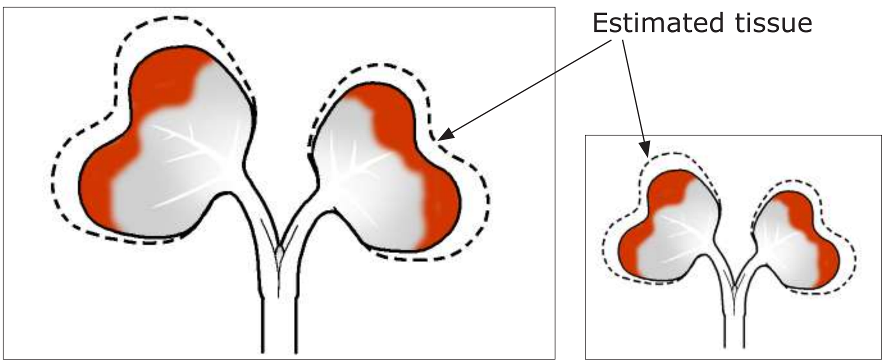


Figure A6.5 Evaluation of estimated tissue: normal seedlings. The damaged areas are smaller than half of the original tissue, and more than 50 % of the estimated tissue is functioning normally.

● = Necrotic, decayed or discoloured tissue

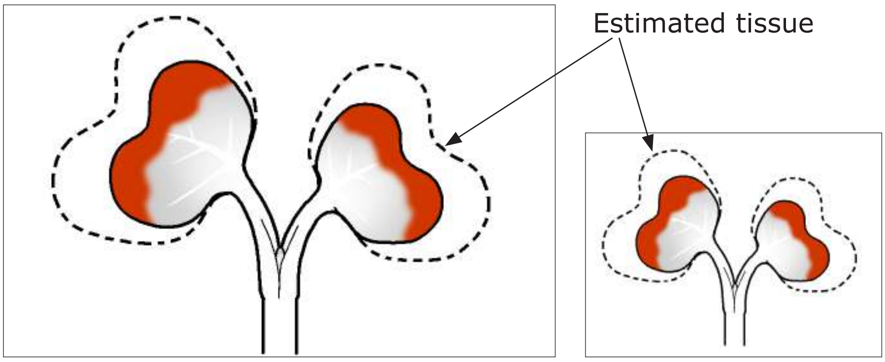


Figure A6.6 Evaluation of estimated tissue: abnormal seedlings. The damaged areas are smaller than half of the original tissue, but less than 50 % of the estimated tissue is functioning normally.

NOTE: In all cases, the estimated tissue shall be deduced with reference to the size of other seedling structures.

A6.3 Normal seedlings

A6.3.1 Intact cotyledons

Intact cotyledons are well developed and show no defects (Figure A6.7).

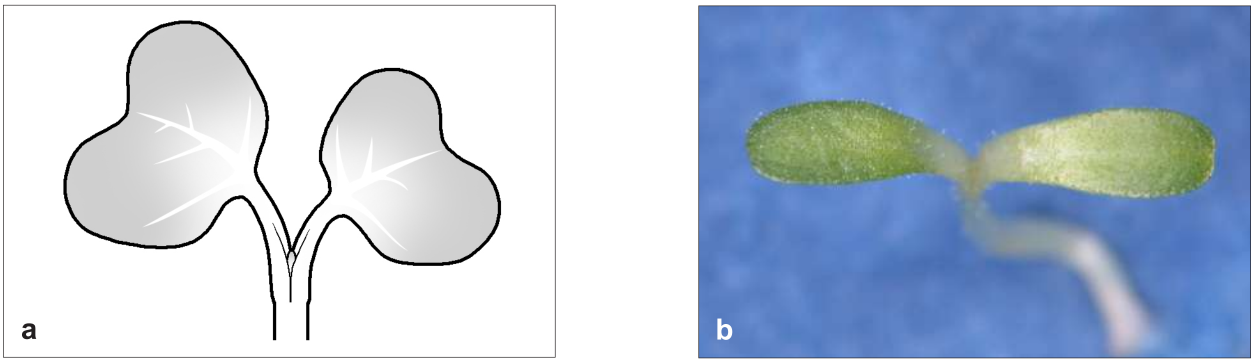


Figure A6.7 Normal seedling with intact cotyledons. **a** Diagrammatic representation. **b** *Lactuca sativa* cotyledons.

A6.3.2 Cotyledons with slight defects

The following defects are considered to be slight. Seedlings with such defects are considered to be normal, provided that 50 % or more of the total cotyledon tissue is functional and the seedlings are otherwise intact or have slight defects.

- The damaged areas are smaller than half of the total cotyledon tissue (Figure A6.8).
- Only one of the points of attachment to the seedling axis is damaged (Figure A6.9).
- The damaged areas are on the midribs, but some distance from the points of attachment to the seedling axis (Figure A6.10).
- The damaged areas are near the points of attachment to the seedling axis, but not on the midribs (Figure A6.11).
- Both points of attachment to the seedling axis are damaged, but only superficially (Figure A6.12).

● = Necrotic, decayed or discoloured tissue

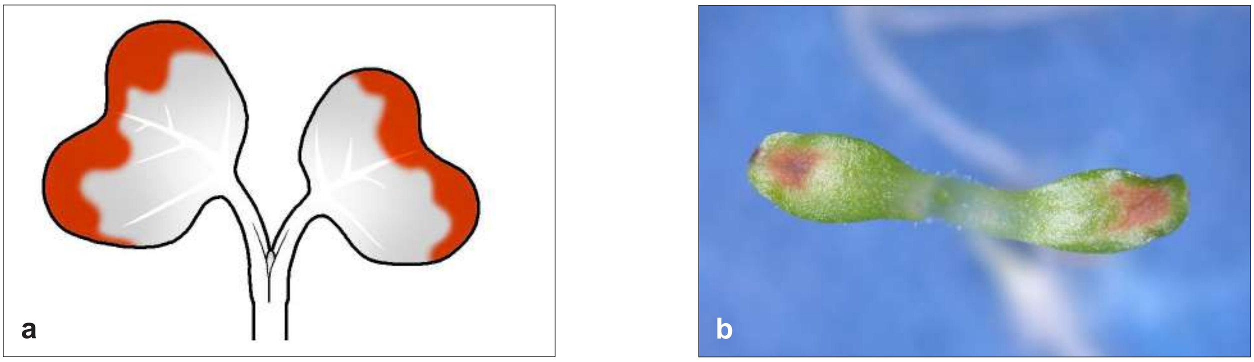


Figure A6.8 Normal seedling. The total damaged area is smaller than half of the total cotyledon area, and more than half of the total cotyledon tissue is functional. **a** Diagrammatic representation. **b** *Lactuca sativa* cotyledons.

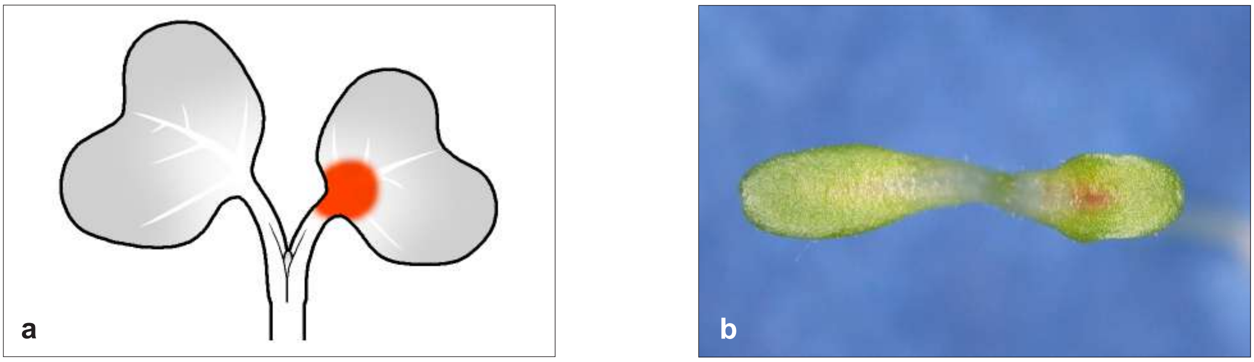


Figure A6.9 Normal seedling. One of the points of attachment to the seedling axis is damaged, but the other cotyledon is intact. **a** Diagrammatic representation. **b** *Lactuca sativa* cotyledons.

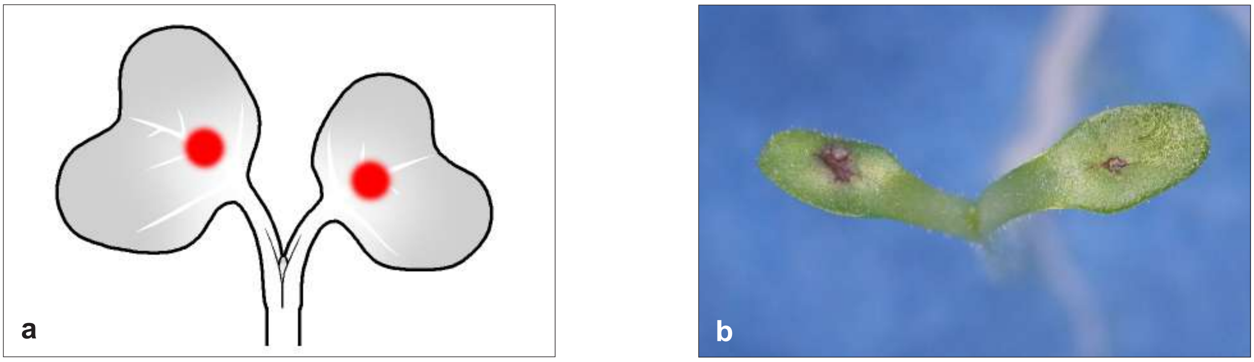


Figure A6.10 Normal seedling. The damaged areas are on the midribs, but some distance from the seedling axis. More than half of the total cotyledon tissue is functional. **a** Diagrammatic representation. **b** *Lactuca sativa* cotyledons.

● = Necrotic, decayed or discoloured tissue

Appendix 6: 50% rule for evaluation of foliated cotyledons

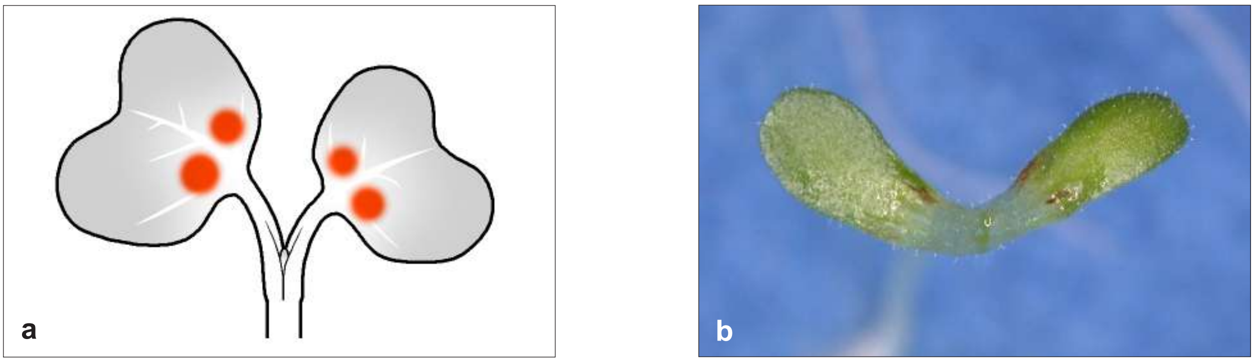


Figure A6.11 Normal seedling. The damaged areas are near the points of attachment to the seedling axis, but not on the mid-ribs. More than half of the total cotyledon tissue is functional. **a** Diagrammatic representation. **b** *Lactuca sativa* cotyledons.

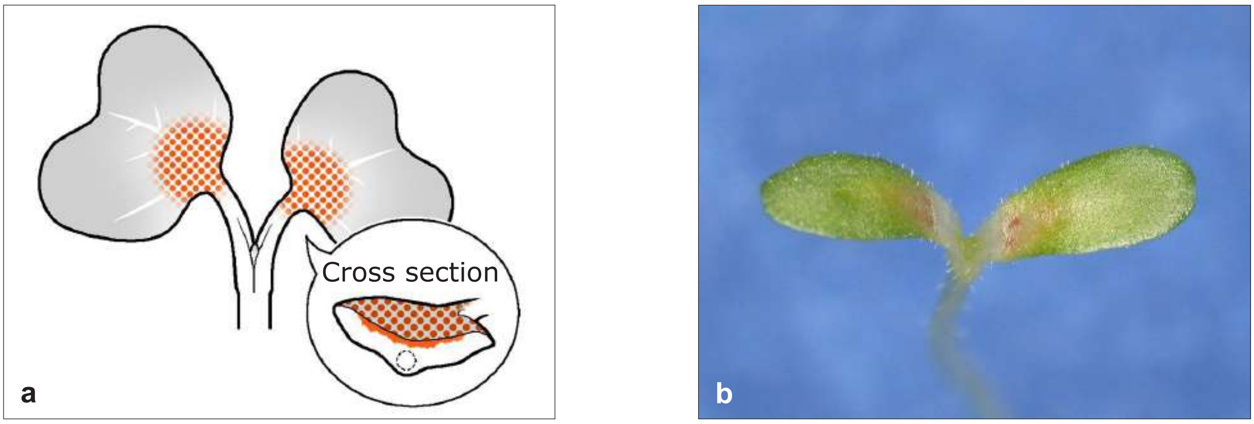


Figure A6.12 Normal seedling. The points of attachment to the seedling axis are only superficially damaged, and more than half of the total cotyledon tissue is functional. **a** Diagrammatic representation. **b** *Lactuca sativa* cotyledons.

Appendix 6: 50% rule for evaluation of foliated cotyledons

● = Necrotic, decayed or discoloured tissue

A6.3.3 Cotyledons with slight defects of chlorophyll deficiency

The following chlorophyll deficiency defects are considered to be slight. Seedlings with such defects are considered to be normal, provided that 50 % or more of the total cotyledon tissue is functional and seedlings are otherwise intact or have slight defects.

- The area of chlorophyll deficiency is smaller than half of the total cotyledon tissue, and more than half of the total cotyledon tissue is functional (Figure A6.13).
- Both points of attachment to the seedling axis are affected by chlorophyll deficiency, but more than half of the total cotyledon tissue is functional (Figure A6.14).

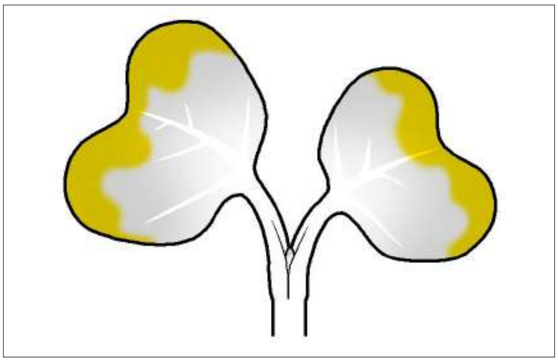


Figure A6.13 Normal seedling. The total range of chlorophyll deficiency is smaller than half of the total cotyledon area, and more than half of the total cotyledon tissue is functional. Diagrammatic representation.

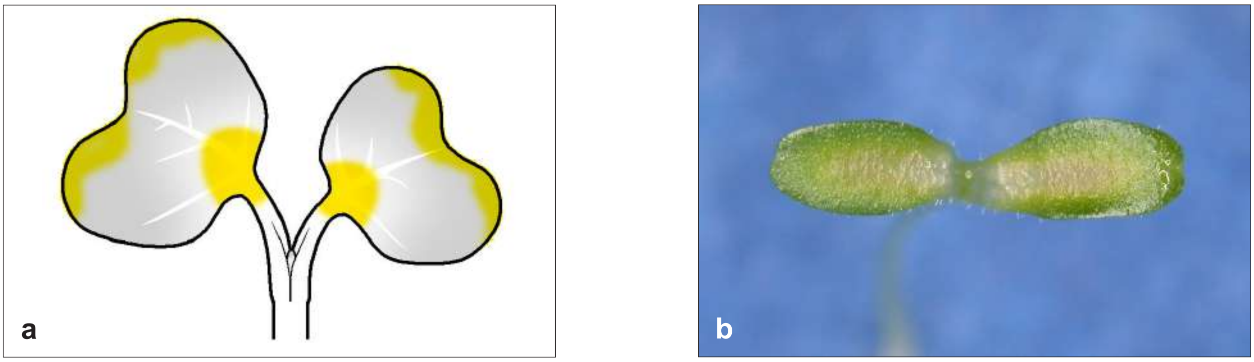


Figure A6.14 Normal seedling. Both points of attachment to the seedling axis are affected by chlorophyll deficiency, but more than half of the total cotyledon tissue is functional. **a** Diagrammatic representation. **b** *Lactuca sativa* cotyledons.

Appendix 6: 50% rule for evaluation of foliated cotyledons

● = Chlorophyll deficiency defects

A6.4 Necrosis of the conductive tissue

When there are necrotic spots near to the points of attachment to the seedling axis, it is essential to evaluate whether they are affecting the conductive tissue. If this is difficult to judge by external appearance, then the affected area should be cut and the cross section examined (Figures A6.15–21).

In the case of the seedlings in Figures A6.19–21, it is obvious that less than 50 % of the total cotyledon tissue is functional, so cutting was not required to assess these seedlings as abnormal. Cutting is also not required when the necrotic spot extends to both adaxial and abaxial sides of the basal points, since in such cases the conductive tissue is affected. Cutting is therefore only necessary when necrosis is found on only one side of the surface near or at the point of attachment, and more than half of the total or total estimated cotyledon tissue appears to be functional.

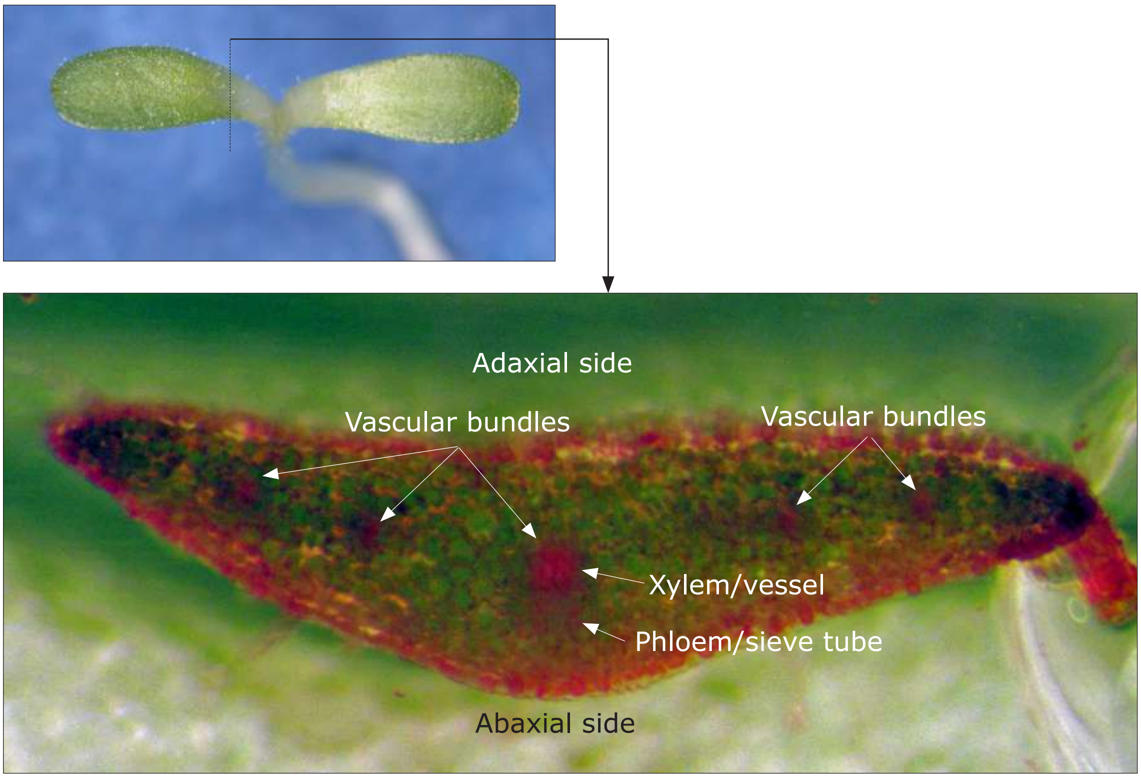


Figure A6.15 Cross section (below, enlarged) of an intact *Lactuca sativa* cotyledon (above), stained with safranin and fast green to show the position of the conductive tissue.

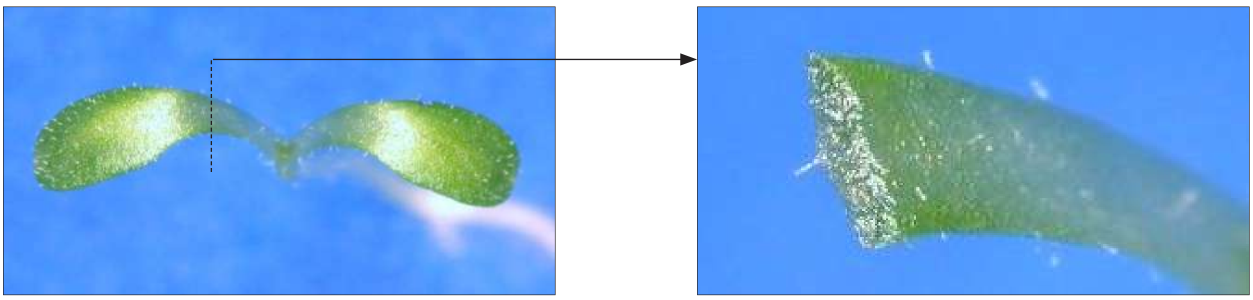


Figure A6.16 Normal seedling with intact cotyledons (right: enlarged cross section).

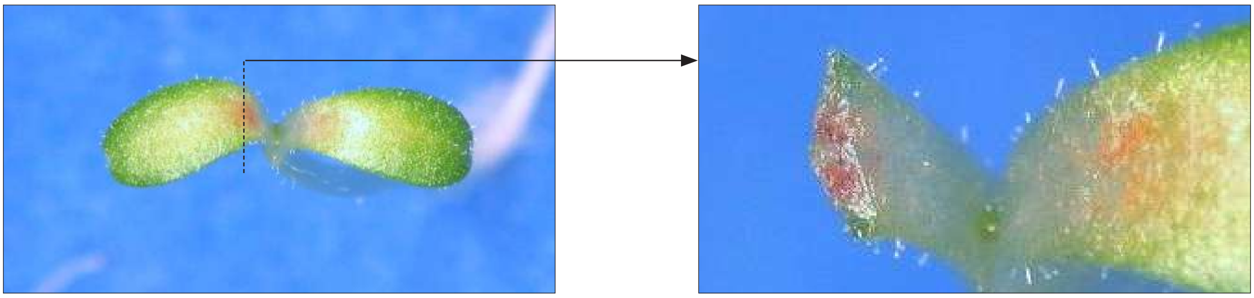


Figure A6.17 Normal seedling. A few small necrotic spots can be observed, but the conductive tissue is not damaged (right: enlarged cross section).

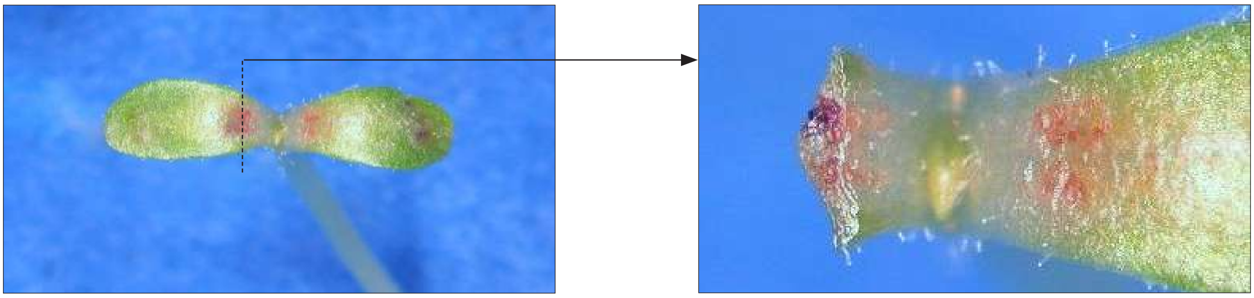


Figure A6.18 Normal seedling. Necrotic spots are clearly visible. However, the conductive tissue is not seriously damaged, and more than half of the total cotyledon tissue is functional (right: enlarged cross section).

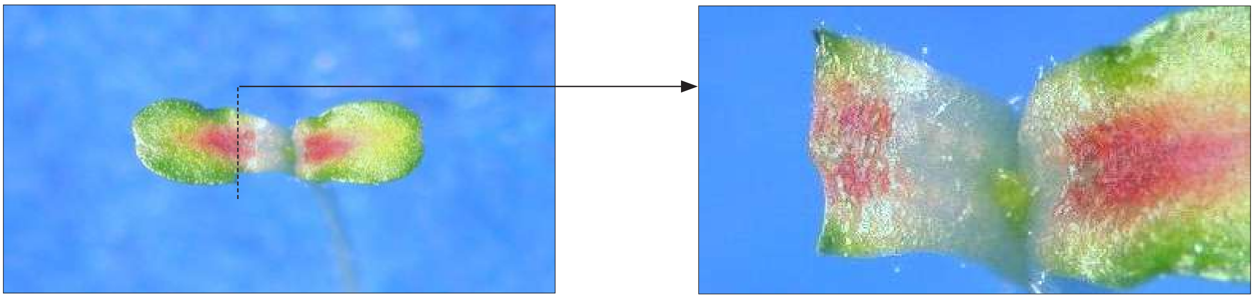


Figure A6.19 Abnormal seedling. Necrotic spots have invaded the conductive tissue. The cotyledons have neither developed nor expanded normally, and less than half of the total cotyledon tissue is functional (right: enlarged cross section).

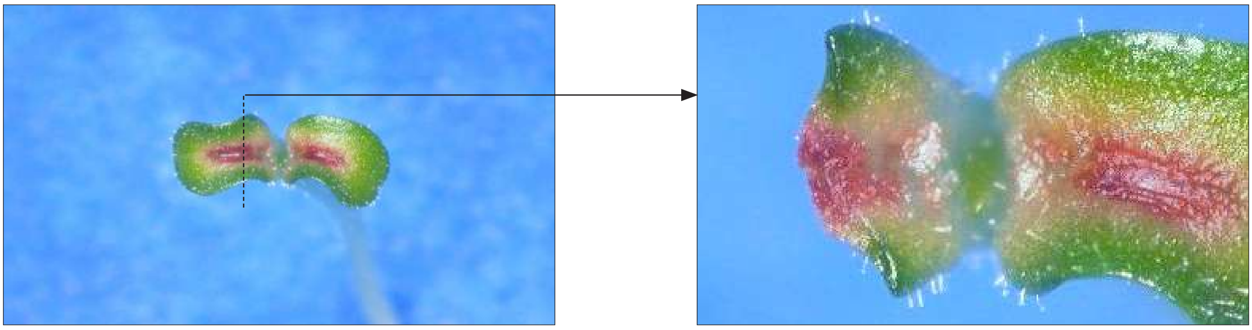


Figure A6.20 Abnormal seedling. Necrotic spots have invaded the conductive tissue completely. The cotyledons have neither developed nor expanded normally and are deformed (right: enlarged cross section).

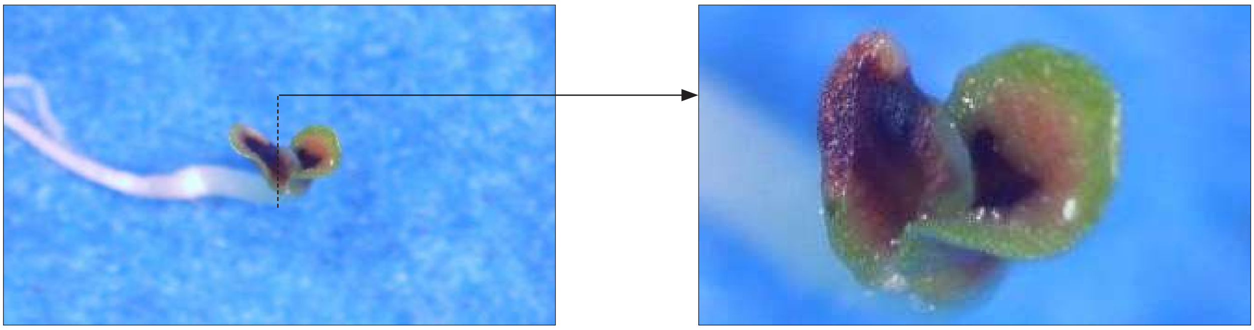


Figure A6.21 Abnormal seedling. Necrotic spots have invaded the conductive tissue completely (right: enlarged cross section). The cotyledons have neither developed nor expanded normally and are severely deformed.

A6.5 Abnormal seedlings

A6.5.1 Cotyledons with necrotic, decayed or discoloured tissue

Seedlings with the following defects are considered to be abnormal:

- damaged areas are larger than half of the total cotyledon tissue; more than half of the total cotyledon tissue is not functional (Figure A6.22);
- both of the points of attachment to the seedling axis are damaged (Figure A6.23);
- one of the points of attachment to the seedling axis is damaged, and the other cotyledon is not intact (Figure A6.24);
- the cotyledons are stunted, and more than half of the total estimated cotyledon tissue is not functional (Figures A6.25–27).

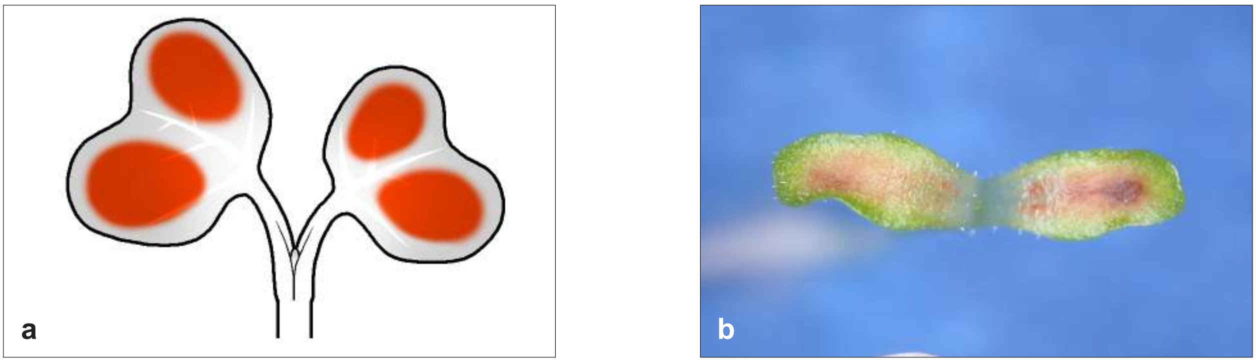


Figure A6.22 Abnormal seedling. The damaged areas are larger than half of the total cotyledon tissue. **a** Diagrammatic representation. **b** *Lactuca sativa* cotyledons.

● = Necrotic, decayed or discoloured tissue

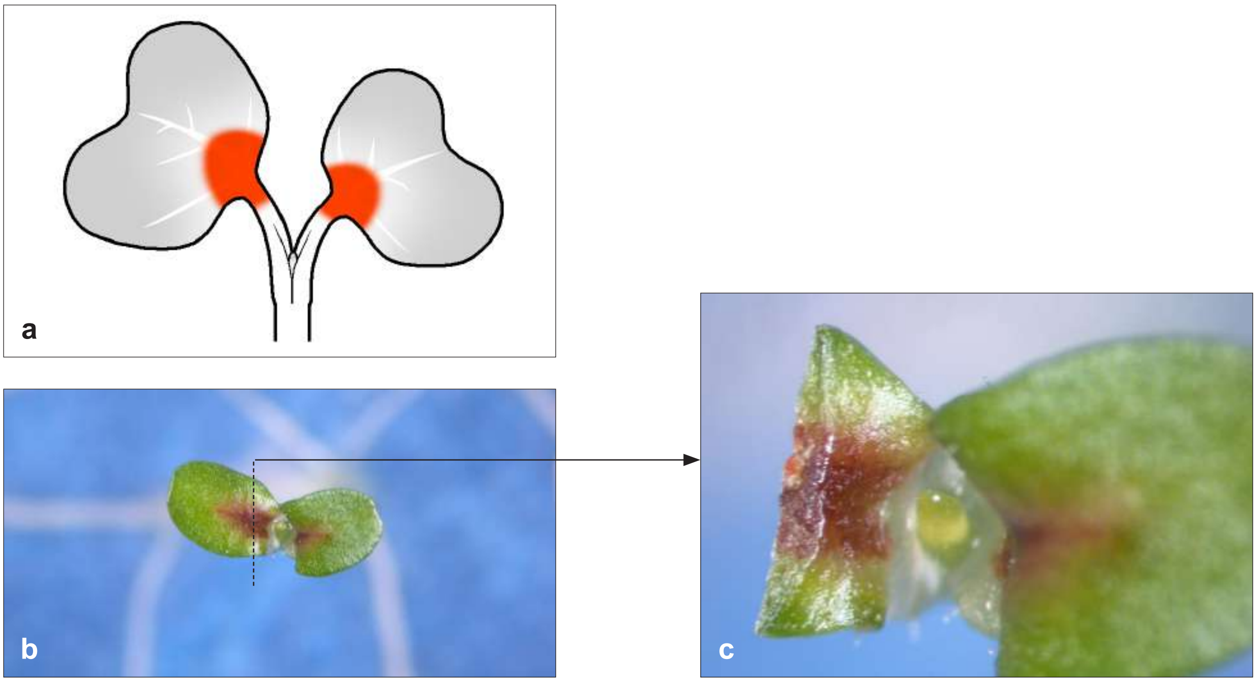


Figure A6.23 Abnormal seedling. Both points of attachment to the seedling axis are damaged, and the conductive tissue has been invaded by necrosis. **a** Diagrammatic representation. **b** *Lactuca sativa* cotyledons. **c** Enlarged cross section of *Lactuca sativa* cotyledon.

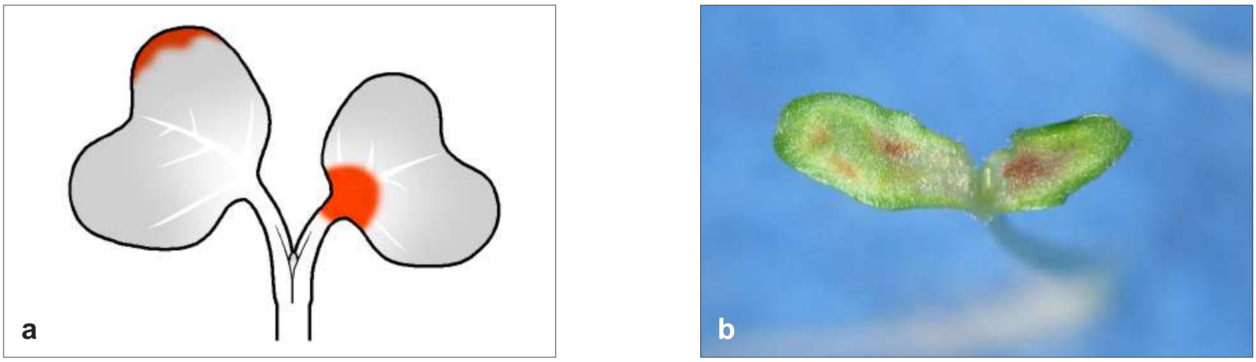


Figure A6.24 Abnormal seedling. One of the points of attachment to the seedling axis is damaged, and the other cotyledon is not intact. **a** Diagrammatic representation. **b** *Lactuca sativa* cotyledons.

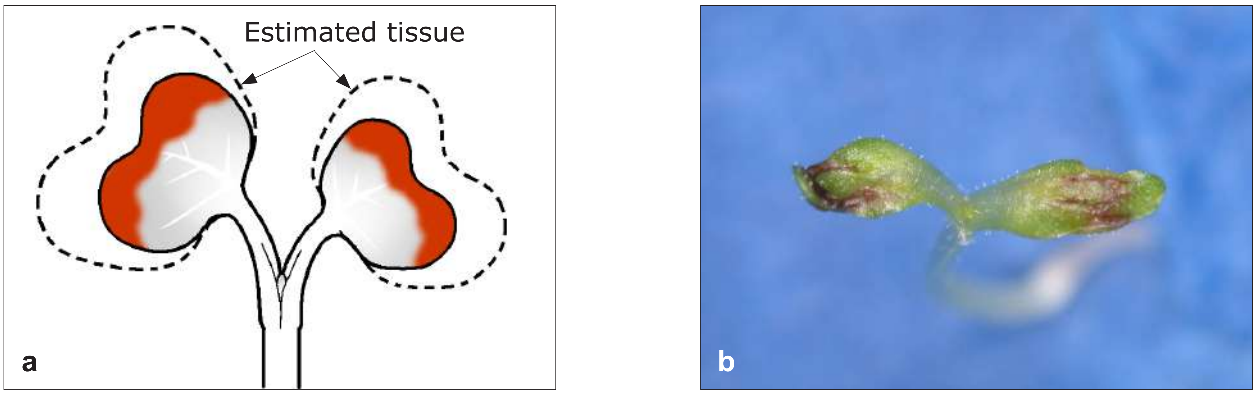


Figure A6.25 Abnormal seedling. The cotyledons are stunted, and more than half of the total estimated cotyledon tissue is not functional. **a** Diagrammatic representation. **b** *Lactuca sativa* cotyledons.

● = Necrotic, decayed or discoloured tissue

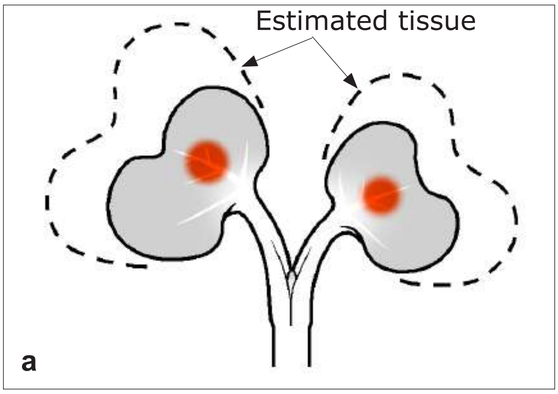


Figure A6.26 Abnormal seedling. There are damaged areas on the midribs, so that the cotyledons do not develop or expand normally. Less than half of the total estimated cotyledon tissue is functional. **a** Diagrammatic representation. **b** *Lactuca sativa* cotyledons.

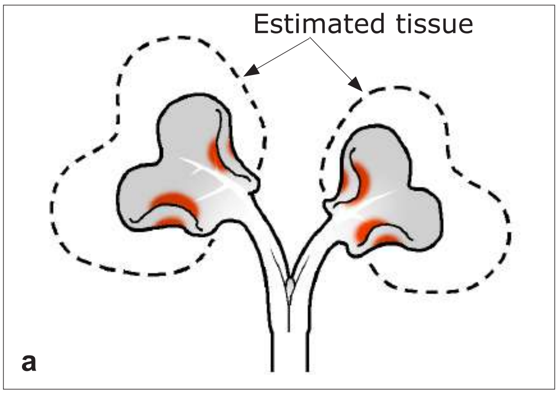


Figure A6.27 Abnormal seedling. There are damaged areas on the cotyledons, which do not develop or expand normally. Less than half of the total estimated cotyledon tissue is functional. **a** Diagrammatic representation. **b** *Lactuca sativa* cotyledons.

● = Necrotic, decayed or discoloured tissue

A6.5.2 Cotyledons with defects of chlorophyll deficiency

Seedlings with the following chlorophyll deficiency defects are considered to be abnormal:

- more than half of the total cotyledon tissue is affected by chlorophyll deficiency and is not functional (Figure A6.28);
- the surrounding tissue is deformed by chlorophyll deficiency, and more than half of the total cotyledon tissue is not functional (Figure A6.29).

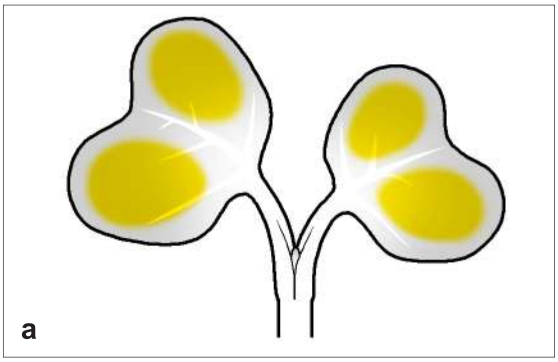


Figure A6.28 Abnormal seedling. More than half of the total cotyledon tissue is affected by chlorophyll deficiency and is not functional. **a** Diagrammatic representation. **b** *Lactuca sativa* cotyledons.

Figure A6.29 Abnormal deformed *Lactuca sativa* seedling, of which more than half of the total cotyledon tissue is not functional due to chlorophyll deficiency.

(yellow dot) = Chlorophyll deficiency defects

A6.5.3 Defects of the terminal bud and surrounding tissues

If the terminal bud and surrounding tissues are damaged, necrotic, decayed or discoloured, the seedling is considered to be abnormal, and the 50 % rule is not applied (Figure A6.30).

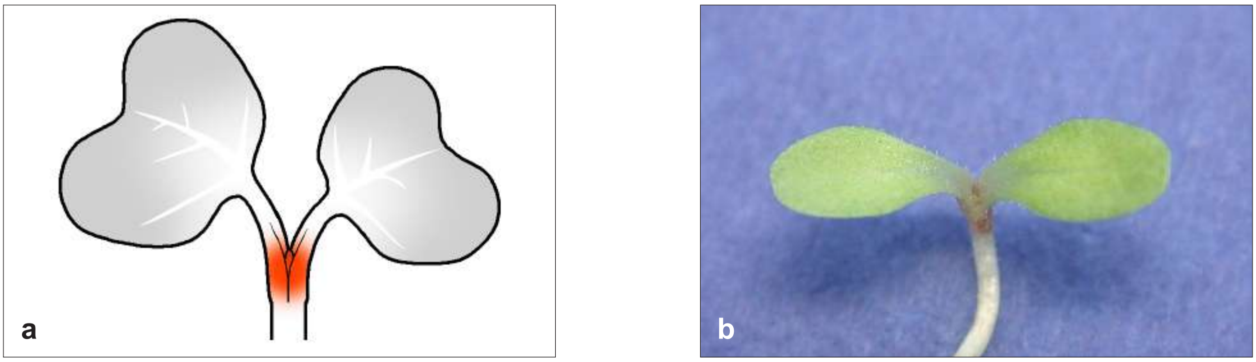


Figure A6.30 Abnormal seedling. The cotyledons are normal, but the terminal bud and surrounding tissues are damaged. **a** Diagrammatic representation. **b** *Lactuca sativa* cotyledons.

● = Necrotic, decayed or discoloured tissue