SOYBEAN

SEEDLING EVALUATION

Seed Purity and Germination Unit
Central Seed Laboratory
Canadian Food Inspection Agency

Revised September 2001

(Ce document est disponible en français)

1. Introduction

Several problems may be encountered in the evaluation of soybean seedlings, eg. Judging severity of lesions; assessing missing or discoloured primary leaves; assessing severity of disease; evaluating small seedlings; assessing hypocotyl length on seedlings with no primary root. Lack of consistency in the evaluation of these factors may cause different labs to obtain different results. The purpose of this addendum to the *Canadian Methods and Procedures for Testing Seed* is to increase between-lab uniformity in the testing of soybean seed and is to be followed by all Canadian labs testing soybean for the purpose of grading seed.

The evaluations described here were discussed and agreed upon by members of most of the Canadian soybean testing laboratories during a joint CFIA-CSAAC workshop held in Ottawa on September 15, 2000.

2. Test Conditions

The appearance and health of seedlings at the time of seeding evaluation can be affected by the environmental conditions (light, temperature, substrate moisture, humidity) during the germination and growth period. Certain conditions may cause seedling defects which experienced analysts can recognize and judge to be test condition-induced, such as seedlings with decay apparently caused by secondary infection and seedlings with brown or missing primary leaves apparently caused by test conditions. While compensating for test conditions may give accurate results in most cases, it makes it impossible to detect certain real seedling defects when they do occur. In order to avoid this possibility, the analyst must be wary of over-compensating for inadequate test conditions. Unless there is direct evidence that damage has been caused by test conditions, all seedlings which have severely damaged or impaired essential structures must be classified as abnormal. If the analyst suspects that this damage has been caused by the test conditions, then the sample should be retested under different conditions. If the observed abnormalities caused by adverse test conditions persist, then the laboratory must identify the cause and correct it. The following descriptions are applicable to seedlings grown in a substrate such as sand.

Fig 2.1. Soybean grown under different test condition. Soil in high intensity light growth chamber, sand in low intensity light cabinet, soil in low intensity light cabinet.

3. Essential Structures

Essential structures are those structures which play a part in the continuing development of the seedling and plant. Included are: roots, hypocotyl, epicotyl and cotyledons (see Fig. 3.1). A seedling must be classified as abnormal if any of these structures are damaged to the extent that it can no longer function.

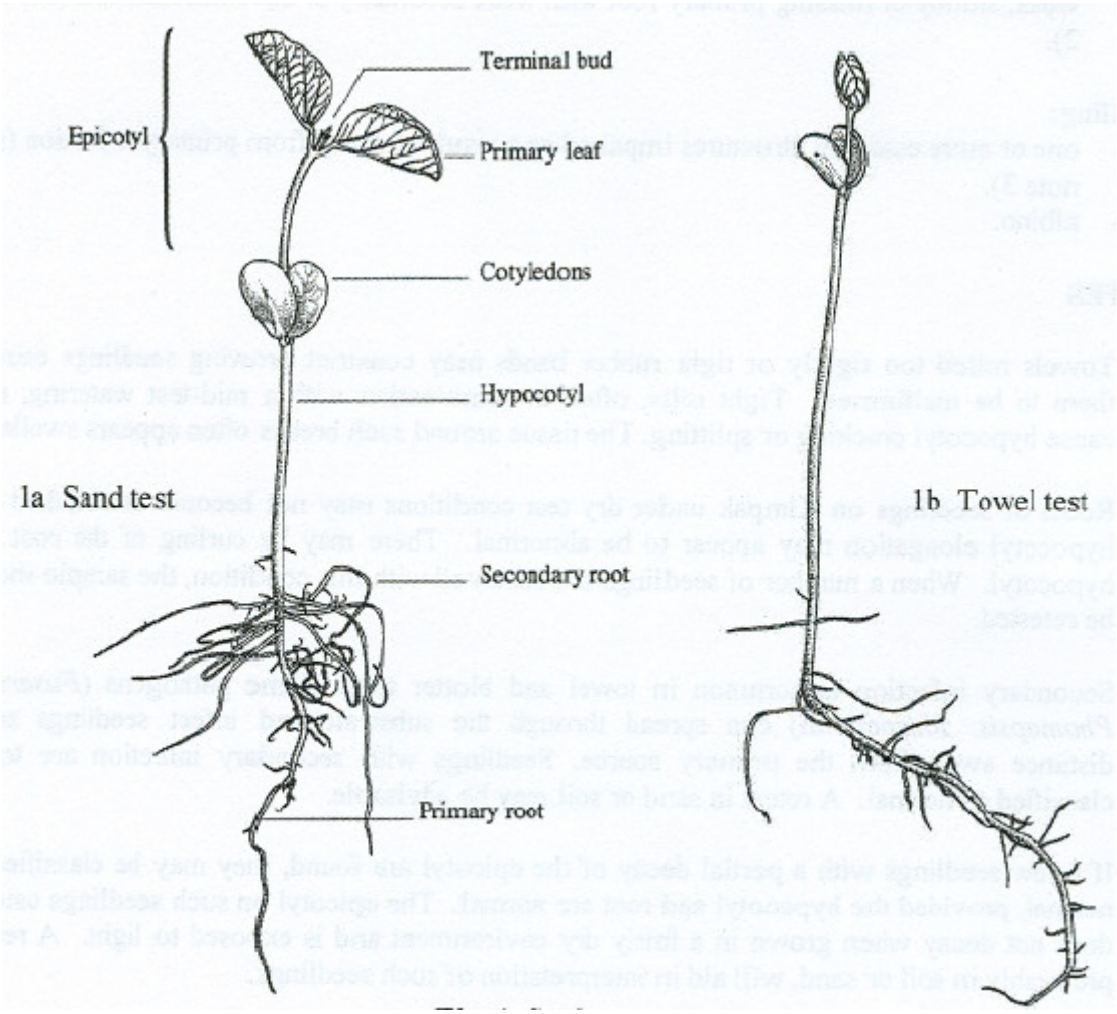

Fig 3.1. Essential Structures (From AOSA Seedling Evaluation Handbook)

3.1 Roots

The primary root develops from the radicle which forms the lower part of the embryonic axis. Depending on the degree of damage to the radicle, the primary root may vary in length from normal to completely missing, or it may have lesions or be diseased. Adventitious roots will develop from the base of the hypocotyl if the radicle has been completely destroyed. Such seedlings can be classified as normal provided these adventitious roots are strong and the hypocotyl has sufficient length (see section 7.2 - Damaged Hypocotyl).

3.2 Hypocotyl

The hypocotyl develops from the part of the axis lying between the cotyledons and the radicle. Damage to this area of the axis will result in lesions in the seedling. Adventitious roots may develop from breaks in the hypocotyl, and may from the principal root system when the radicle and lower hypocotyl have been completely severed. Seedlings which have a severely shortened hypocotyl will have difficulty emerging in the field. Short seedlings caused by damage should not be confused with slow seedlings. In the latter, the primary root is intact (see sections 7.2 - Damaged Hypocotyl and 7.3 - Late Germination).

3.3 Epicotyl

The epicotyl is the part of the axis above the cotyledons, and is made up of a stem, two primary leaves and a terminal bud. Growth of the epicotyl follows growth of the primary root and hypocotyl, and so in seedlings from late-germinating seeds, the epicotyl may be quite small. When judging the size of the leaves, they should be considered in relation to the size of the rest of the seedling (see section 5.1 - Small Primary Leaves).

3.4 Conducting Tissue

The root/shoot axis is made up of a central stele (or vascular cylinder), surrounded by the cortex and epidermis (See Fig. 3.2). The conducting tissue is contained in the outer layers of the stele, and serves to transport water and nutrients. The conducting tissue is not readily visible without magnification, but the analyst should be able to estimate its location. It is strongly recommended that analysts spend some time observing the structure of the seedling under the microscope. It is particularly useful to observe various types of lesions in cross section, in order to get a “feel” for the link between appearance and severity.

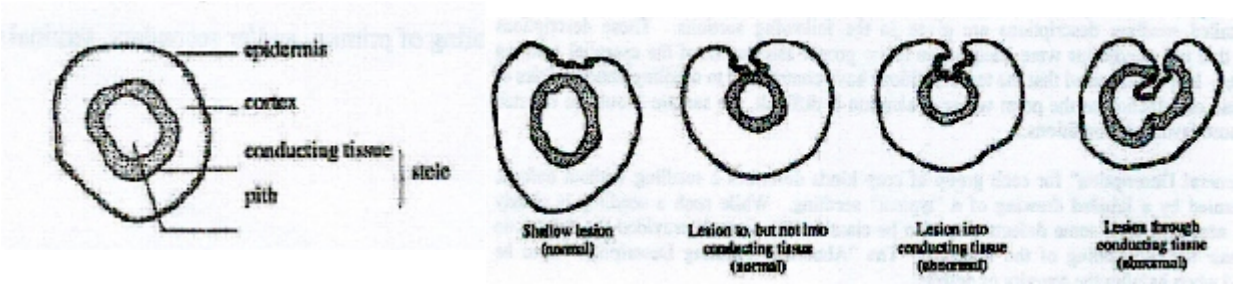

Fig. 3.2. Soybean hypocotyl cross-section. (From AOSA Seedling Evaluation Handbook).

4. Lesions

Lesions are most often found in the hypocotyl, but may also be found in the epicotyl or root. Lesions are considered to be a serious defect because they interference with the movement of water and nutrients through the affected area, and increase susceptibility of the seedling to micro-organism (disease) attack.

Two factors which are to be considered when assessing the severity of lesions are the depth of the lesion and the degree of healing.

4.1 Depth of Lesions in the Hypocotyl and/or Epicotyl

Seedlings with lesions extending into the conducting tissue are classified as abnormal. The analyst must therefore judge whether or not the lesion penetrates the outer layer of the central stele; the best way to do this is to break or cut the structure at the point where the lesion appears to be the deepest.

In general, four levels of depth occur.

4.1.1 Surface

Only the epidermal layer is affected. The seedling often appears as having been "skinned", and may have brown striations running across the lesion. When the affected area is observed in cross-section, there is no significant indentation into the inner tissues. The hypocotyl has retained its normal rounded shape in the affected area. These seedlings are to be classified as normal.

4.1.2 Severe "Deep"

These lesions go right through the vascular tissues, into or through the centre of the stele (into the pith), and are easily identified as being severe. These seedlings are to be classified as abnormal.

4.1.3 Severe "Shallow"

These lesions have an appearance similar to the "skinned" or surface lesion, in that when cut, there may not be a significant indentation visible, and they may have brown striations running across the lesion. These lesions are typically broad and appear healed. They can be distinguished from a surface lesion by the "lumpy" appearance and feel of the unaffected side of the hypocotyl. The hypocotyl is also somewhat flattened in the area of the lesion. The lesion in these seedlings has removed a significant portion of the vascular tissue and is to be classified as abnormal.

4.1.4 Intermediate

These are the most difficult to judge, since the depth in relation to the position of the central stele is not readily apparent. The seed analyst must cut or break the structure (ie. hypocotyl and/or epicotyl) at the most severe point of the lesion and make a judgement as to depth.

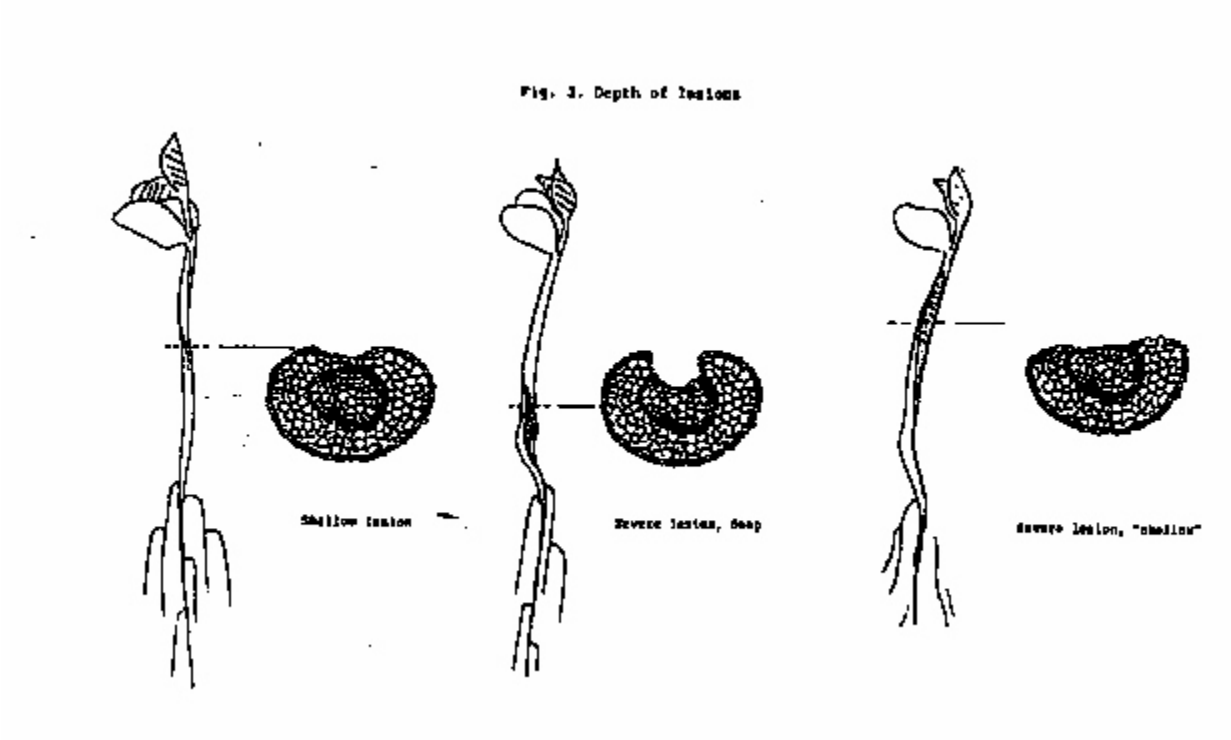

Fig. 4.1. Depth of lesions

4.2 Root Lesions

Lesions in the primary root, whether on the surface of the root or displayed as a split root, are not to be assessed as long as there is sufficient healthy secondary root development between the lesion and the hypocotyl. However, if the lesion or split in the primary root is large enough to breach the conducting tissue in the hypocotyl, then the seedling is to be classified abnormal.

4.3 Healing

Lesions should be considered as healed if the gap caused by the lesion has been filled in by the production of cells from the surrounding tissues. This may result in swelling of the affected area and may appear as a “knee” such as is often seen in field or garden beans. Such seedlings are classified as normal. If there is a gap which has not completely filed in and there is still an opening into the hypocotyl, then the lesion should be judged as any other lesion, ie. abnormal if deep, normal if not deep.

Before the growth of seedling structures such as the hypocotyl begins, any damaged or dead tissue in the embryo often turns brown. As growth of the healthy tissue occurs the damaged or dead tissue is stretched and breaks apart. This tissue shows up as brown spots or striations along the length of the lesion. These brown areas should not be interpreted as evidence of “healing”.

5. Epicotyl

There are three commonly observed epicotyl problems:

5.1 Small Primary Leaves

Leaf size is affected by the growth environment and/or the presence of damage. Since the seedling puts its energy into growth of the root and hypocotyl first (to ensure establishment and emergence of the seedling), small seedlings may have very small primary leaves. However, the size of the primary leaves may be an indication that there is damage to the epicotyl, at or near the base of the leaves. In such cases, assessment of the seedling must be based on the damage and not on the size of the primary leaves. The size of the primary leaf for any particular seedling should be estimated in relation to the size of that seedling; ie. The “normal” size of a leaf for a small seedling will be less than that for a larger seedling. In estimating the size of the primary leaf, the leaf area, not the leaf length is to be considered.

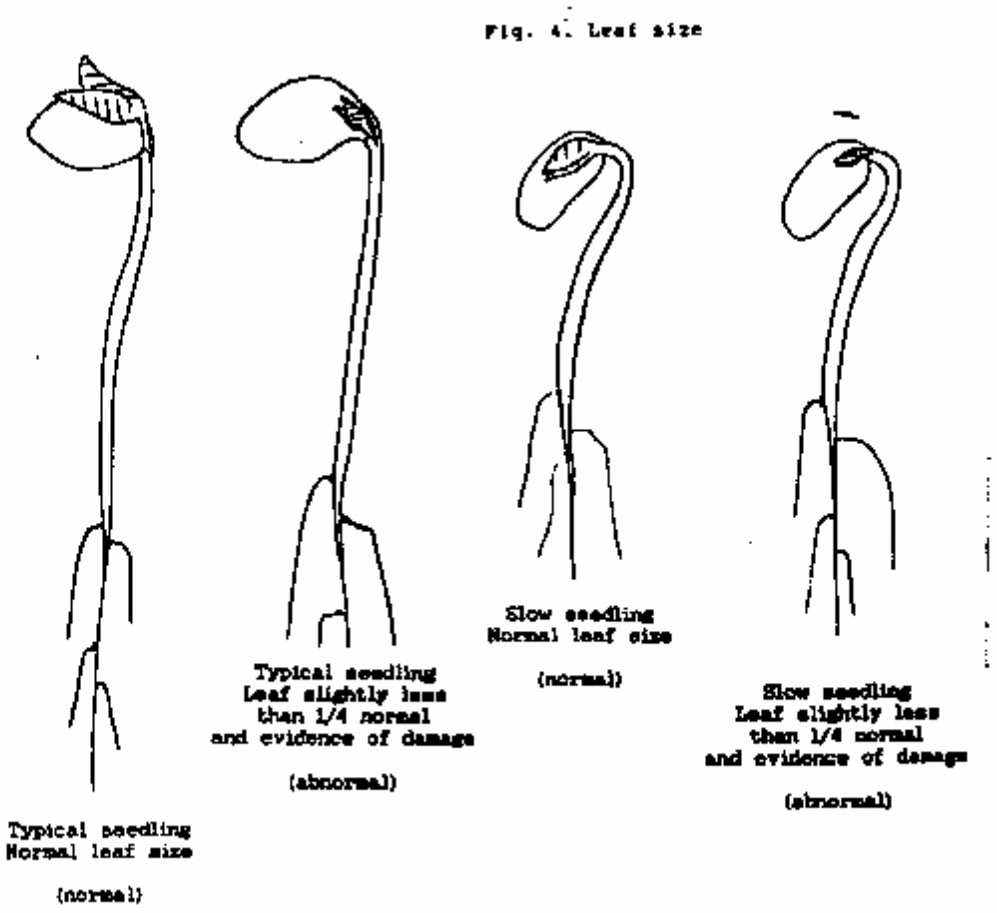

Fig. 5.1. Primary Leaf Size

5.2 Brown or Missing Leaves

A problem observed when tests are conducted under certain environmental conditions (it is not yet fully understood what these are), is that the primary leaves turn brown. In extreme cases these leaves shrivel and drop off, leaving a seeding with missing primary leaves. All seedlings with discoloured (brown and shrivelled) primary leaves, whether attached or detached (but still present), are to be classified as normal. However, should the detached primary leaves be missing, classify the seedling as abnormal.

If there is a sufficient number of these seedlings to affect the grade of the lot, then a retest should be made using alternate test conditions. If there was no disease problem observed with the sample, a rolled towel test should give a better result with respect to the brown leaf condition. If there was disease present, then a sand or soil test under higher light intensity and/or lower cabinet humidity should give better results.

Fig. 5.2 Brown Leaves (seedling on right)

5.3 Epicotyl "Bent-Over"

In soybean, the epicotyl is naturally bent over, resting between the cotyledons, until elongation pulls the primary leaves free. A bent-over epicotyl should be considered abnormal if there is evidence of damage which would have caused the condition. Typically there is a lesion at the base of the epicotyl or at the point of attachment of the cotyledons. This damage results in unequal growth of tissues around the epicotyl, causing the epicotyl to bend. Sometimes the bending is accompanied by a failure of one of the primary leaves to develop at the same rate as the other. This is further evidence that there has been damage. As a guideline, if the epicotyl is bent 90 degrees or more, as the result of damage, then it should be classified as abnormal. Damage occurring below the point of attachment of the cotyledons may cause a lesion of the hypocotyl which extends either into the epicotyl, or into one of the cotyledons. A lesion which extends into the epicotyl may cause bending as described above, and such a seeding is abnormal. A lesion which extends up into the cotyledon may cause some bending of the upper hypocotyl, but usually does not cause bending of the epicotyl itself. This lesion should be judged as any other lesion, ie. abnormal if deep, normal if not deep.

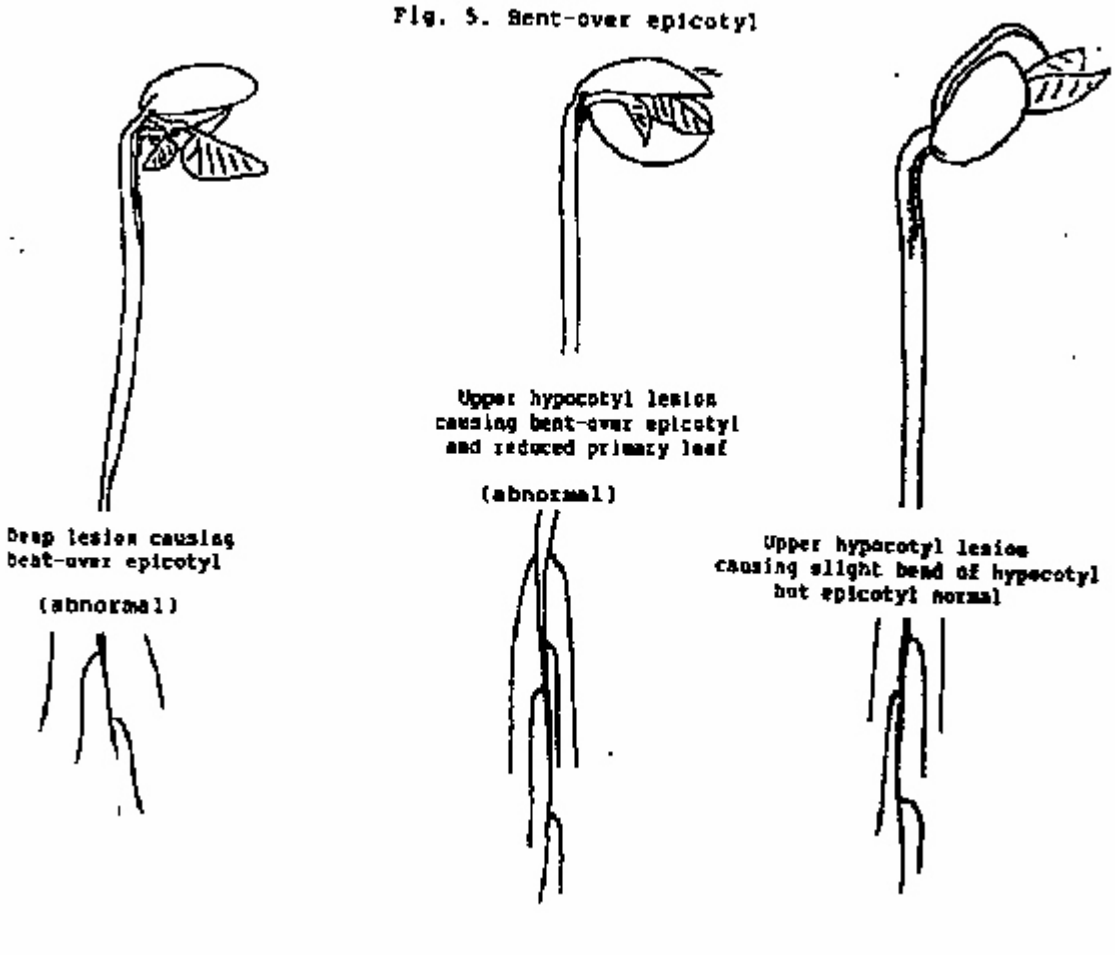

Fig. 5.3. Bent-over epicotyl

6. Diseased Seedlings

Infection is the entrance and spread of disease organisms in living material (eg. seedling structures) often causing disease symptoms and decay. Two modes of infection are observed in seed testing:

Primary Infection: the disease organism is present and active in the seed and/or seedling itself.

Secondary Infection: the disease organism has spread from adjacent seeds or seedlings.

Unless there is clear evidence that the infection is secondary (ie. the analyst can directly observe that the source of infection is a neighbouring seed or seedling), it must be assumed that the infection is primary. If there is an excessive amount of secondary infection, such that it would be difficult to identify primary infection, then the sample should be retested using an alternate germination procedure (such as increasing the seed spacing).

Seedlings with primary infection are to be assessed as follows:

6.1 Cotyledons

Follow the 50% rule, ie. if more than 50% of the cotyledonary tissue is infected, the seedling is classified as abnormal. It may be necessary to break open the cotyledons to determine the degree of penetration of infection. Remember to assess the total volume of the cotyledons not just the surface (see Fig. 6.1).

Fig. 6.1 Surface infection of cotyledons

6.2 Epicotyl, Hypocotyl, Roots

Once it has been determined that the infection is primary, the analyst must judge whether the infection has caused the structure to be impaired to the extent that it is unable to function. If the infection is superficial, ie. it does not extend deep enough to affect the central stele the seedling is classified as normal. If the infection penetrates deeply into the central stele (ie. further development of the structure has been impaired) then the seedling is classified as abnormal. Therefore, when assessing diseased epicotyls and hypocotyls, the same criteria used for depth of lesions should be used for assessing the degree of penetration of infection.

Disease on the primary root is not to be assessed as long as there is sufficient healthy secondary root development between the decay and the hypocotyl. If there are not sufficient healthy roots, then the disease is to be assessed as described above for the epicotyl and hypocotyl.

7. Small Seedlings

Fig. 7.1. Small seedlings. a. Normal; b. Small without defects (possibly late germinating), normal; c. Missing primary root and shortened hypocotyl due to mechanical damage, see 7.2 for evaluation.

7.1 Low Vigour or Weak Seedlings

These seedlings are weak, spindly, and small. They may have other defects, such as damaged cotyledons or hypocotyl lesions. If there are only a few (1-3) weak seedlings in your test, classify them as abnormal. However, if all the seedlings in the sample appear weak, classify the normal "weak" seedlings as normal and make a note of the samples appearance in the remarks of your certificate of analysis.

It is important not to confuse these seedlings with those that are late germinating (see section 7.3 - Late Germination).

7.2 Damaged Hypocotyl

This type of small seedling is usually the result of mechanical damage caused by harvesting and/or seed cleaning procedures. It is usually manifested in the seedlings as a missing primary root and significantly shortened hypocotyl, or as fractures at the base of the cotyledons with very little root development. These seedlings are to be classified as abnormal if they do not meet both of the following requirements:

1. The seedling's hypocotyl length is equal to or greater than the length of one of its own cotyledons and,

2. There are sufficient secondary or adventitious roots.

Fig. 7.2. Judging hypocotyl length. Seedlings to the left of the vertical line are normal, those to the right are abnormal. The marker indicates the length of the seedling's cotyledon, with hypocotyls shorter than this have been classified abnormal.

7.3 Late Germination

Some seeds (eg. hard seeds) germinate more slowly than others, and will produce seedlings which appear smaller than average for the test (see Fig. 7.1.b). Provided sufficient growth has occurred to enable observation of all essential structures, there is no apparent damage, and they are not low vigour or weak seedlings, these should be classified as normal.