

SEMIS: Germination Rules and Testing

Michael Stahr CSA,CGT

Iowa State University Seed Laboratory

Seed Enterprises Management Institute
University of Nairobi

The Germination Test

- (5.1) The object of the germination test is to determine the maximum germination potential of a seed lot, which can then be used to compare the quality of different lots and also estimate the field planting value.
- Testing under field conditions is normally unsatisfactory, as the results cannot be repeated with reliability.
- Lab methods have evolved in which the external conditions are controlled to give the most regular, rapid and complete germination for the majority of samples of a particular species.
- The conditions have been standardized to enable test results to be reproduced within limits as near as possible to those determined by random sample variation.

Necessity for Germination Testing

- Second of two critical parts of producing data for labeling (the other being purity testing).
- Ideal conditions for the seed/seedlings, but favorable for fungi and other organisms as well.
- Can have good correlation with field emergence.
- Some people question how lab tests using laboratory substrates can relate well to the growing conditions in the “real world”.

Seed Enterprises Management Institute
University of Nairobi

Source of seeds for germination ¹

Pure seed includes:

- seed that is damaged, but more than half still present
- Discolored, shriveled, fungal-infested, or having begun germinating.
- Smaller seeds within a lot.
- Insect damaged, as long as it isn't determined that less than half the seed is present.

¹ AOSA Rules for Testing Seed, Volume 1

Definitions

- Germination: emergence & development from the seed embryo of those *essential* structures which, for the kind of seed in question, are indicative of the *ability* to produce a *normal* plant under *favorable* conditions (**AOSA**).
- Germination of a seed in a lab test is the emergence and development of the seedling to a stage where the aspect of its essential structures indicates whether or not it is able to develop further into a satisfactory plant under favorable conditions in soil. (**ISTA**, 5.2.1)
- Favorable conditions for various species have been determined by research and referee testing.

Moisture and aeration

- Substrate must supply needed moisture without restricting aeration of the seeds.
- If water forms around finger when pressing substrate, there is excess water*.
- It is desirable have the relative humidity in the growth chamber at approximately 95%.
- Inspect tests at frequent intervals for moisture.
- For soil tests, water should be added to soil until it can be formed into a ball, but break freely.

AOSA

IOWA STATE UNIVERSITY

Seed Science Center

Substrate Options

AOSA

B = between blotters

TB= top of blotters

T = paper toweling (folded or rolled)

S = sand
medium

TS= top of sand

P = covered petri dishes with two

TC = top of crepe cellulose paper w/o a blotter. TS = top of sand

TCS = top of CCP w/o a blotter and covered
with 1/2 to 3/4 inch layer of sand.

ISTA

TP = top of Paper

BP = between paper

PP = pleated paper

O = organic growing

TO=top of organic medium

S = sand

Substrata Considerations

- Incoming substrata (paper towels, blotters, sand, soil, etc,) should be checked for suitability. Options include checking for toxicity by planting timothy, sending material to another lab to be analyzed, and determining water holding capability.
- When choosing a substrate to use, compare ease of planting and evaluation versus cost and efficiently using germinator space.

Temperature

- Single numerals: constant temperature in ° C.
- numeral-numeral: alternating temperatures (i.e. 20°-30° C)
 - 1st numeral: 16 hours without light
 - 2nd numeral: 8 hours with light
- If alternating temperatures not available on weekends, keep test at lower temperature.
- Temperatures should not vary more than one degree C. up or down due to chamber.

AOSA

Light

AOSA:

- Should be cool white fluorescent source.
- Illuminate at least 8 out of 24 hours.
- Up to 16 hours beneficial for some test conditions and lots, but continuous light should not be used unless it is known it doesn't inhibit germination.

ISTA:

- Illumination is generally recommended for the sake of better developed seedlings.
- In certain cases light is needed to promote germination of dormant samples.
- On the other hand, light may be inhibitory to germination and the substrates should be kept in darkness, this is indicated in the last column of Table 5A.

Duration (ISTA)

- Duration of treatment to break dormancy is not taken as part of the germination period.
- If it seems advisable ... the prescribed test period may be extended by seven days or for up to half the prescribed period for longer tests (AOSA: 2-5 days).
- Test may be ended when *maximum germination* of sample has been obtained.
- Time of first count is approximate, but must be sufficient to permit seedlings to reach a stage of development which allows for accurate evaluation.

Duration (ISTA)

- Times in table 5A refer to the highest temperatures. The first count may have to be postponed for lower temperatures.
- Intermediate counts should be kept to a minimum to reduce the risk of damaging seedlings which are not sufficiently developed.
- Intermediate counts to remove seedlings that are adequately developed are recommended to make counting easier and to prevent them from affecting development of other seedlings.

Definitions

- (5.2.3) The following structures are essential to a seedling's continued development into a satisfactory plant: root system, shoot axis, cotyledons, terminal buds, coleoptile.
- (5.2.4) Normal seedlings show the potential for continued development into satisfactory plants when grown in good quality soil and under favorable conditions of moisture, temperature and light.

To be classified as normal, a seedling must conform with one of the following:

- Intact seedlings: seedlings with all their essential structures well developed, complete, in proportion and healthy.
- Seedlings with slight defects: seedlings showing certain slight defects of their essential structures, provided they show an otherwise satisfactory and balanced development comparable to that of intact seedlings of the same test.
- Seedlings with secondary infection: seedlings which it is evident would have conformed with the above two bullet points, but which have been affected by fungi or bacteria from sources other than the parent seed.

Definitions

5.2.5 Abnormal seedlings do not show the potential to develop into a normal plant when grown ...

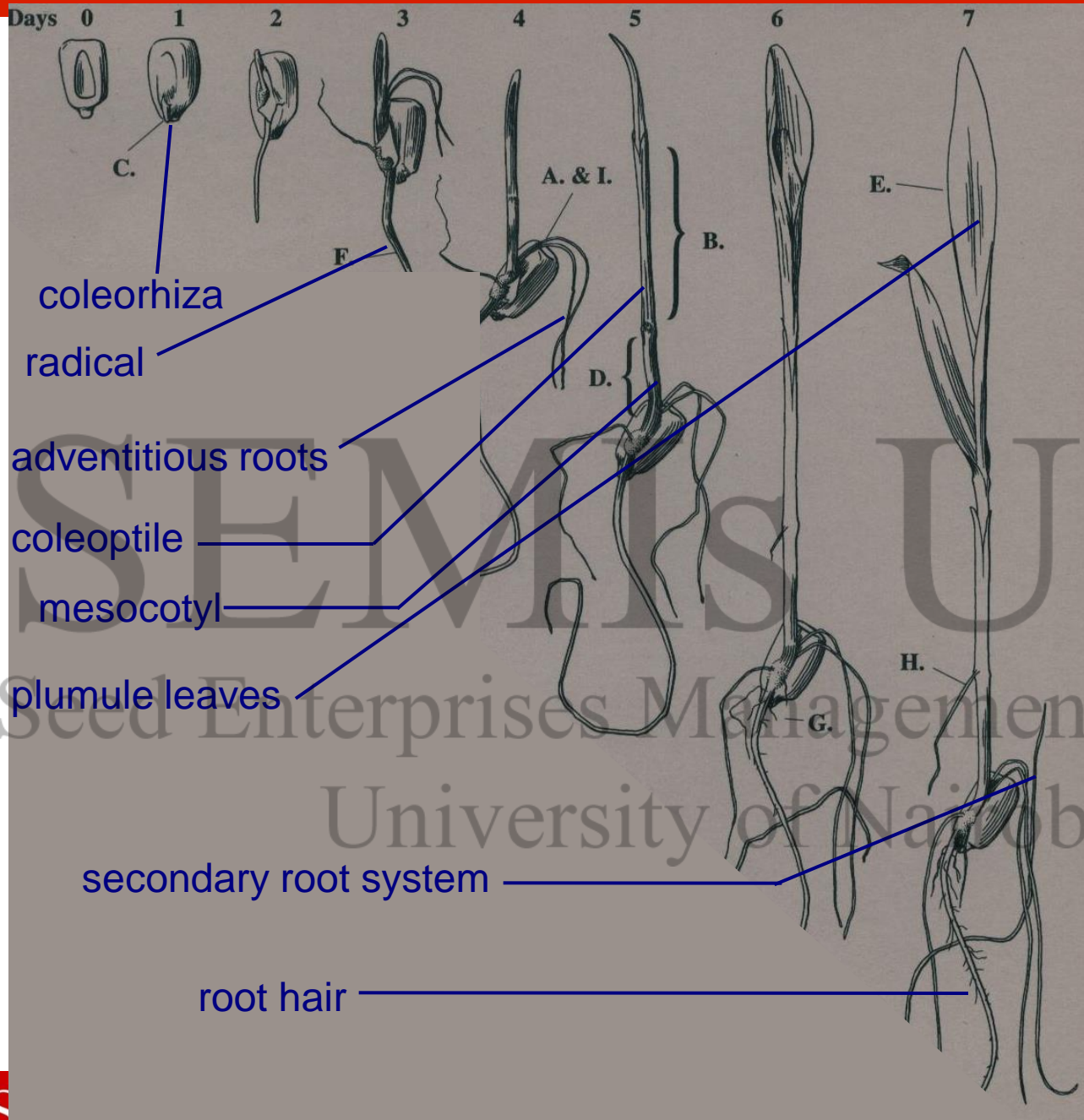
The following seedlings are classified as abnormal:

- Damaged seedlings: seedlings with any of the essential structures missing or so badly and irreparably damaged that balanced development cannot be expected.
- Deformed or unbalanced seedlings: seedlings with weak development or physiological disturbances or in which essential structures are deformed or out of proportion.
- Decayed seedlings: seedlings with any of their essential structures so diseased or decayed as a result of primary infection that normal development is prevented.

Detailed descriptions of seedlings in 5.2.3.A -5.2.5.A

5.2.7 Ungerminated seeds : not germinated by the end of the test period are classified as follows:

- Hard seeds: seeds which remain hard at the end of the test period because they have not absorbed water (ISTA). AOSA – because they have not absorbed water due to an impermeable seed coat.
- Fresh (dormant) seeds : seeds, other than hard seeds, which have failed to germinate ... but which remain clean and firm and have the potential to develop into a normal seedling.
- Dead seeds: neither hard or fresh nor have produced any part of a seedling.



coleorhiza

radical

adventitious roots

coleoptile

mesocotyl

plumule leaves

secondary root system

root hair

epicotyl

terminal bud

embryonic leaves

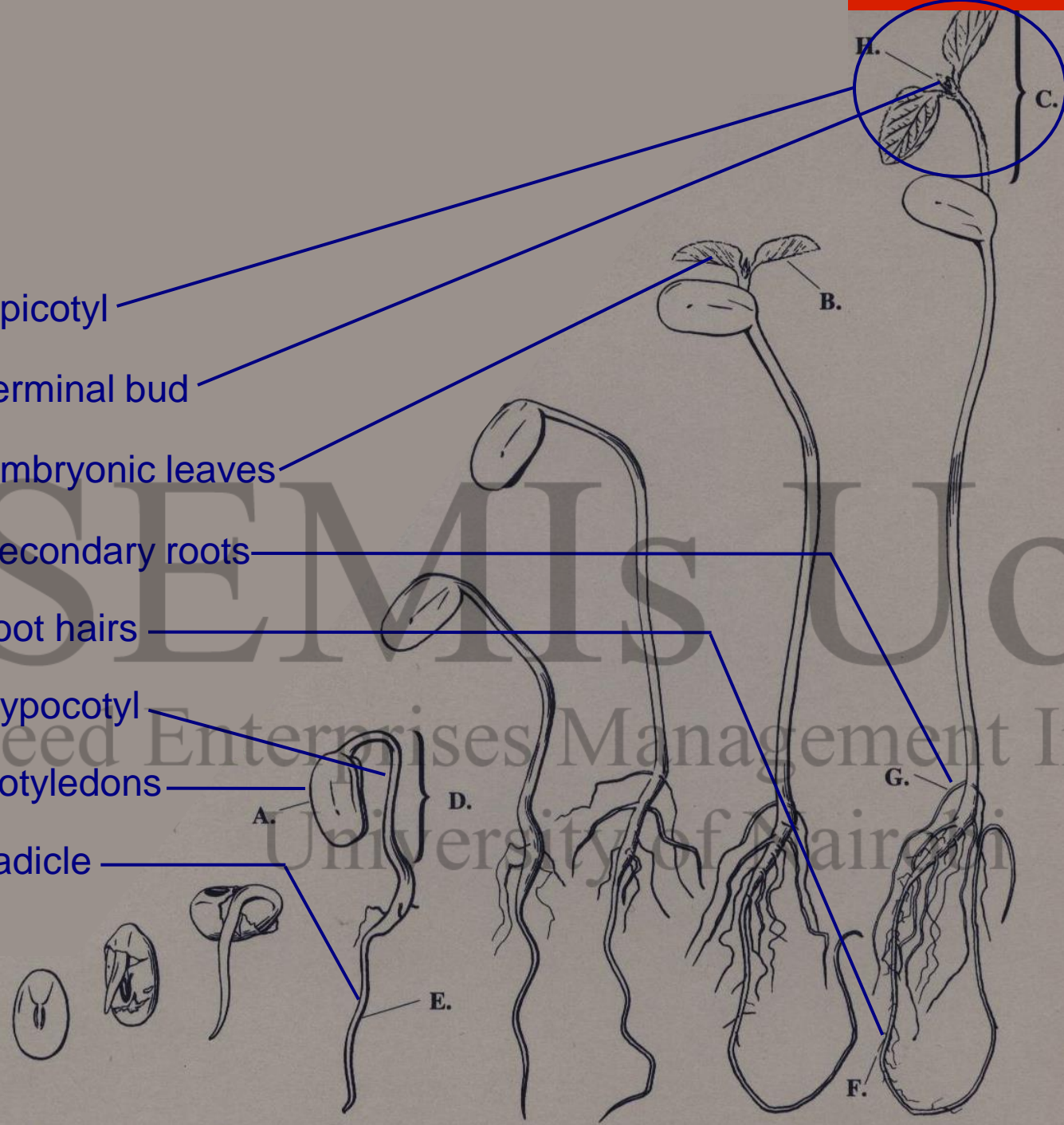
secondary roots

root hairs

hypocotyl

cotyledons

radicle



Uniformity of Evaluations

- Classification of seedlings can change due to volume of tests evaluated, the analyst's mental state, and the presence of many border-line seedlings.
- Within a lab, tests can be checked by other analysts or analysts can bring questionable seedlings around to all analysts to decrease the likelihood of evaluation changing.
- Referees (seed samples sent to multiple labs or virtual referees), visual study aids, and workshops can reduce variation.
- Virtual referees or proficiency tests must be well-done (clear images, obvious what "deficiency" is to be evaluated).

4.6 Retest when ...

- At final count, it is determined that a satisfactory germ has not been achieved.
- There is evidence the following distorting results:
 - a) improper test conditions
 - b) errors in seedling evaluations
 - c) presence of fungi or bacteria
 - d) inaccuracies in counting and recording results.
- There are problems due to chemical treatment, exposure to chemicals, or toxicity from any source.

Retest in soil or sand/soil mix and report results.

AOSA

Reasons for variation in results

- Sample improperly drawn from a bin, bulk bag, or pallets of bags. Primary samples not mixed well when combined.
- Sample not mixed before testing conducted at lab.
- Partial to extensive drying out of substrates.
- Fungal problems.
- Many border-line seedlings.
- Germination results in the 40% – 60% range.
- Improper “watering” of substrata before planting or after preliminary evaluation count.
- Improper counting. Improper classification of seedlings.



Preparing Planting Substrates

- Methods: rolling pin, C-clamps, add specified amount of water.
- Specified water:
 - 225 mL/100 g of paper towels*
- Can store left over substrate for several days to a week in plastic bags.





Planting Small-Seeded Samples

- Most small-seeded species are planted using a vacuum planter.
- The planter has a foot switch to turn the station on or off and also a valve for controlling the amount of vacuum.
- Some species are too small or too large planting with the vacuum planter.
- Planting heads vary from large rectangular ones used for cereals to small round ones used for some grasses and flowers.
-



Germinators

- Free-standing units have fine temperature control.
- Changes temperature quickly for alternating temperatures.
- Conviron units provide moisture.



Smaller “Walk-in” Germinators

- Heater and cooling units.
- Heat is taken from fluorescent lights.
- 10' X `12' (3.1 X 3.7 meters)



Evaluation of Small-Seeded Samples

- Humidity is controlled within the “bucket”, hinged box, or sandwich box. Germinators that supply humidity tend to dry or saturate samples in open containers (our units don't supply moisture).
- Species that have one or more intermediate evaluation counts have normal and dead seeds removed and the substrate re-moistened one a week.
- Intermediate results are recorded on hard-copy work cards.
- Final results are recorded on cards and also entered into the computer.

Large-Seed Planting



- Maize is planted using acrylic planting boards. Holes in boards vary in size from #4 (popcorn) to #8 (large flat kernels). Holes can be round or oval.
- Soybean seeds are planted with a large vacuum planting head. This insures that seeds stay in place (they tend to move somewhat with trip boards) and that there is less chance of fungi moving from seed to seed.
- Maize seeds are almost always treated and therefore don't stay in place well on the vacuum planter.



Large Seed Evaluations

- Some samples can have a fair amount of fungi (which can produce air-borne spores) or bacteria (which with fungi can give off unpleasant odors).
- To deal with this, the Seed Lab has used several configurations of ventilations ducts. The current system vents to the outside and allow for the amount of air movement to be regulated.
- Most tests on large-seeded species have a single evaluation count.



- Left: Seeds after planting. Upper tray has moist sand over CCP.
- Middle: Cold test cart just before evaluation.
- Right: Germination trays of inbred maize

References

- Rules for Testing Seeds, Volume 1.
Association of Official Seed Analysts
- International Rules for Seed Testing
International Seed Testing Association

Seed Enterprises Management Institute
University of Nairobi