Scottish Agricultural Science Agency

Zurich Annual Meeting June 2006

ISTA Germination Committee Report

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The main aims of the Germination Committee are

- To update and contribute to improve the test methods in application of the increasing knowledge, the technical and technological progresses or the regulations requests as ISTA standard of accreditation, ISO 17025;
- To improve the rules in chapter 5 of the ISTA Rules;
- To create and improve training material e.g. Handbooks;
- To introduce method for species not covered by the rules (specific attention being paid to tropical and subtropical species); and
- To share knowledge among seed testing laboratories, facilitate the exchange of information and improve standardisation.

Progress

Since the 2005 ISTA meeting in Bangkok the main areas of activity have related to:

1. Proposals for changes to the ISTA Rules.

2. Revision of Seedling Evaluation Handbook.

3. Investigations aimed at improving Rules and uniformity.

4. Providing answers to questions on germination.

Proposals for changes to the ISTA Rules

- Introduction of New Species – *Crambe abyssinica*
- Introduction of New Species – *Triticum dicoccum*
- The use of $KN0_3$ for dormancy breaking in *Hordeum vulgare*
- Germination testing of *Betula pendula* and *B.pubescens* using 4 by 100 seed replicates
- Incorporating ISTA Seedling Evaluation Handbook abnormal seedling codes into the Rules

Revision of Seedling Evaluation Handbook

- To take account of changes in media definitions in the 2006 Rules
- To reflect Quality Assurance requirements, e.g. quality checks for germination media and temperature measurement in the Germination Laboratory
- Revision of the Seedling Evaluation Handbook guidance on *Lolium* seedling assessment to reflect evidence obtained in comparative test

Investigations aimed at improving Rules and uniformity

- Germination of Lettuce at different temperatures
- Germination of Sunflower in different media at different temperatures
- Use of KNO_3 in Brassica germinations as an aid to seedling assessment
- Use of Crepe Paper/Sand for Germination Tests

Providing answers to questions on germination

- From member laboratories
- From interested parties

RULES

Introduction of New Species – *Crambe abyssinica*

At the ordinary meeting in Bangkok Günter presented the results of a comparative trial involving 7 labs and 4 samples of Crambe

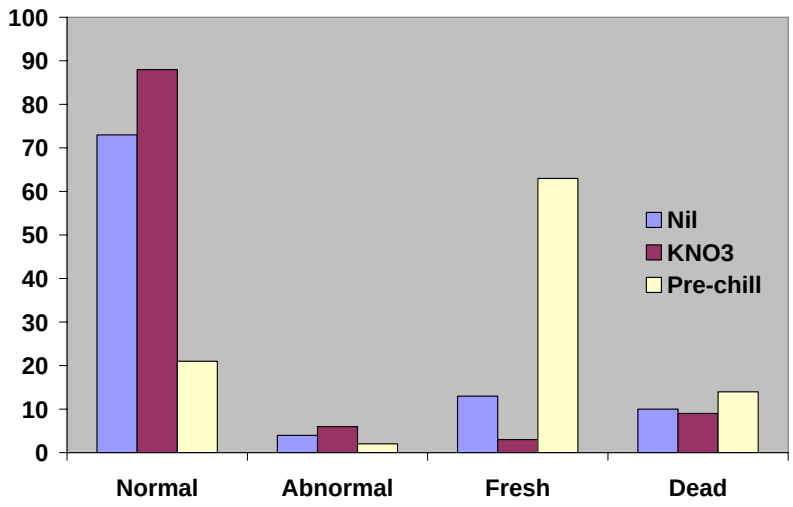


Figure 1: Mean Results when germinated at 20ºC

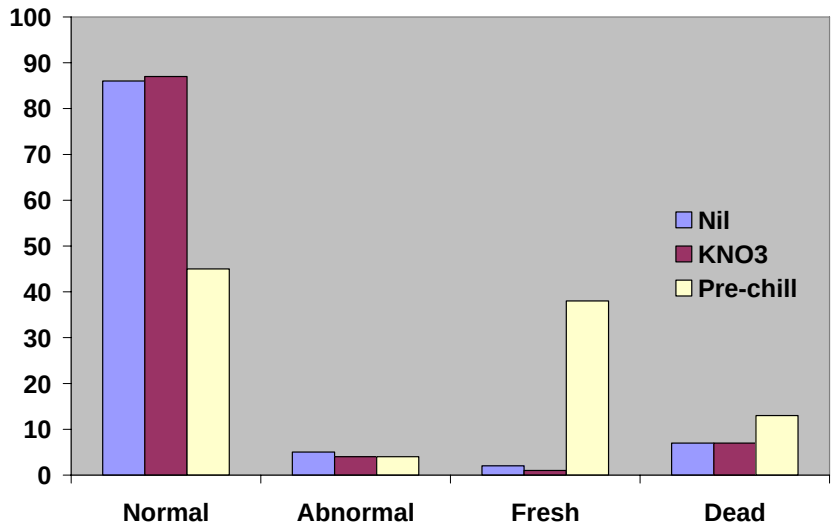


Figure 2: Mean Results when germinated at 20-30ºC

Introduction of New Species – *Crambe abyssinica*

As a result of the trial the following method is being recommended for inclusion in the Rules:

Table 5A, new entry for *Crambe abyssinica*

Species	Prescriptions for:				Additional directions including recommendations for breaking dormancy
	Substrate	Temperature °C	First count (days)	Final count (days)	
1	2	3	4	5	6
Crambe abyssinica Hochst. ex R. E. Fr.	TP; BP	20-30; 20	4	7	KNO_3

Introduction of New Species – *Triticum dicoccum*

The Germination Committee supports the application by the ENSRE laboratory in Italy to have *Triticum dicoccum* included in the rules. The germination method proposed is based on a comparative trial involving 4 ISTA laboratories and 4 samples of different quality.

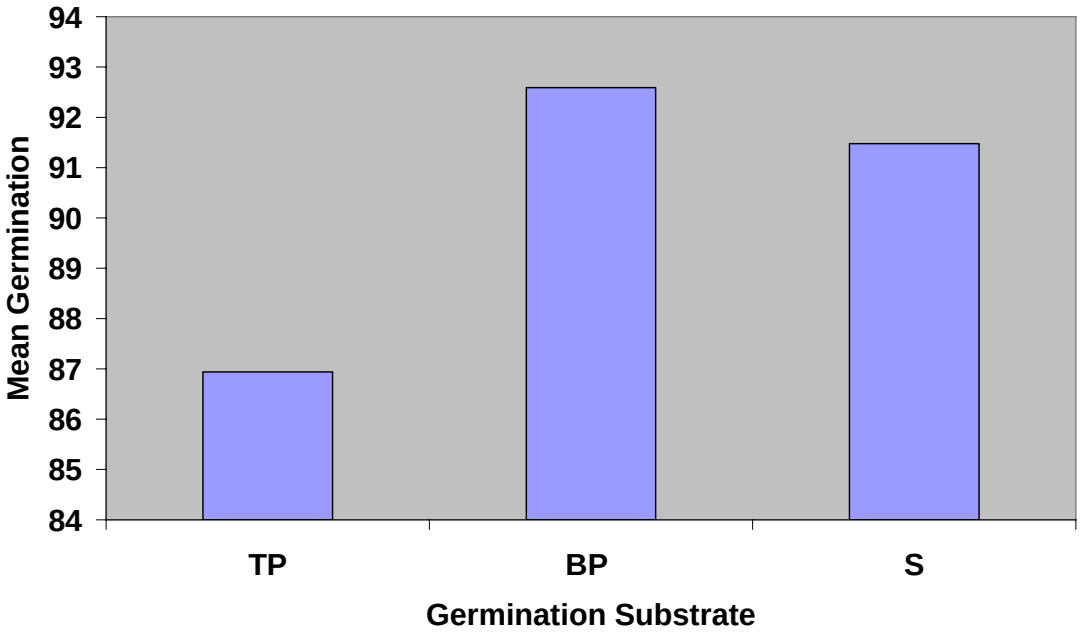


Figure 3: Mean germination of *Triticum dicoccum* in different substrates

Methods currently in the rules for other *Triticum* spp were examined.

Reproducibility and Repeatability was good using BP and S but there was significant variation between laboratories when TP was used.

This is similar to what is found in *Triticum spelta*.

Introduction of New Species – *Triticum dicoccum*

As a result of their Trial ENSE, supported by the Germination Committee, is recommending the inclusion of the following method in the Rules:

Table 5A, new entry for *Triticum dicoccum* Schrank ex Schubl.

Species	Prescriptions for:				Additional directions including recommendations for breaking dormancy
	Substrate	Temperature °C	First count (days)	Final count (days)	
1	2	3	4	5	6
Triticum dicoccum	BP; S	20	4	8	Prechill; GA_3; Preheat (30-35°C)

The use of KNO_3 for dormancy breaking in *Hordeum vulgare*

In Bangkok Günter presented the results of a comparative trial involving 11 laboratories using different dormancy breaking treatments on 6 lots of barley.

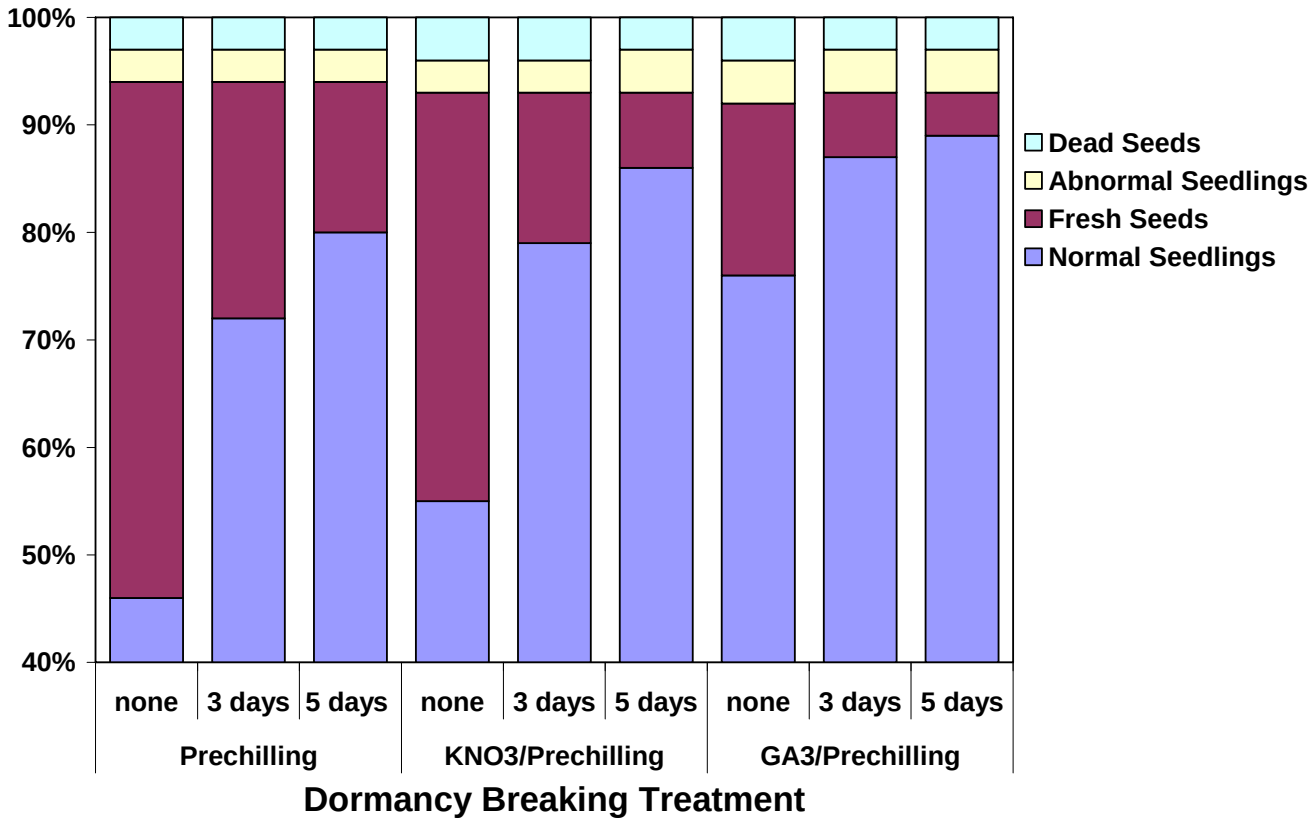


Figure 4: Mean germination of six barley seed lots using different dormancy breaking treatments

Introduction of KNO_3 as a dormancy breaking recommendation for Barley

As a result of the comparative test the Germination Committee, is recommending a modified entry in the Rules for Barley:

Table 5A, modified entry for *Hordeum vulgare*

Species	Prescriptions for:				Additional directions including recommendations for breaking dormancy
	Substrate	Temperature °C	First count (days)	Final count (days)	
1	2	3	4	5	6
Hordeum vulgare	BP; S	20	4	7	Preheat (30-35°C); Prechill; GA_3; KNO_3

Testing *Betula pendula* and *B.pubescens*

The Germination Committee support the proposal by the Forest Tree and Shrub Seed Committee to allow the possibility of testing the germination of *Betula pendula* and *B.pubescens* using counted (4 x 100 seeds) replicates as well as by weighted replicate. We recommend an amendment to Table 5A Part 2 Tree and Scrub Seeds

Amend entries in Table 5A. Part 2. Tree and shrub seeds

Species	Prescriptions for:		First count (days)	Final count (days)	Additional directions including recommendations for breaking dormancy
	Substrate	Temperature °C			
1	2	3	4	5	6
Betula pendula	TP	20-30	7	21	No prechill and prechill for 21 days at 4 °C. Double tests.
Betula pubescens	TP	20-30	7	21	

SEEDLING EVALUATION HANDBOOK

Revision of Substrate Definitions in line with European Standards definitions for Growing Media

The 2006 ISTA Rules contain definitions of substrates based on International Standards. "*Growing Media"* as a generic term for all substrates – paper, sand and other media such as the organic mixtures of peat, sand, perlite, etc. Mixtures of peat, sand perlite, etc are referred to as *"Organic Growing Media"* rather than Compost. In addition the Rules give general definitions for the main paramaters to be taken into account for all the media

Germination Media Parameters

The 2006 ISTA Rules contain definitions of substrates based on International Standards. *"Growing Media"* as a generic term for all substrates – paper, sand and other media such as the organic mixtures of peat, sand, perlite, etc. Mixtures of peat, sand perlite, etc are referred to as *"Organic Growing Media"* rather than Compost.

In addition the Rules give general definitions for the main paramaters to be taken into account for all the media

- Water retention;
- pH;
- Conductivity;
- Cleanliness and Innocuity

In the last year the committee have drawn up revisions to the Seedling Evaluation Handbook to reflect the changes in the Rules and Quality Assurance requirements.

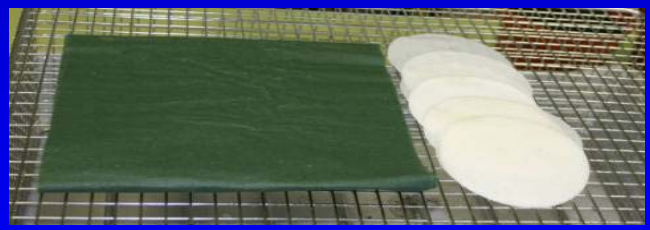
QA Procedures for evaluating substrates and measuring temperature

To assist laboratories in ensuring that the germination media they use in germination tests meet the specifications given in the ISTA Rules the Handbook now contains demonstration Standard Operating Procedures for the measurement of media parameters. There is also a demonstration SOP for the measurement of temperature in the germination laboratory. All the SOPs will contain flow charts with photographs.

Example Flowchart from Demonstration SOP

[1]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.

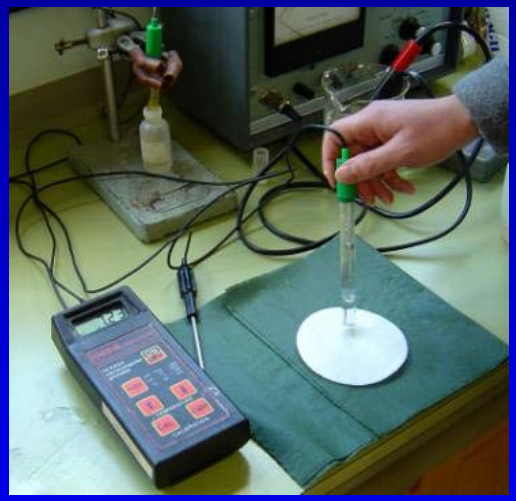
For Sand and Organic Growing Media, one volume of media are mixed with 5 volumes of water that is to be used for germination tests[1]. The mixture is stirred for 5 min and then allowed to stand for a minimum of 2 hours and a maximum of 24 hours. After standing the mixture is stirred and the stabilised pH value of the suspension solution measured.



For Paper Media samples are moistened, with water that is to be used for germination tests[1] and the pH is measured on the surface of the paper.

The pH is measured using a calibrated pH meter or pH paper

Using pH paper to measure the pH of paper germination media



For paper media when using a pH meter a specific probe manufactured for measuring the pH on the surface of paper must be used.

Surface (left) and dip (right) probes for pH meter

Surface probe for measuring the pH of paper

Guidance on the evaluation of primary roots of grasses (Section 12: Seedling type D – Seedling Group A-1-2-3-1 (*Lolium*)

A comparative test using photographs of grass seedlings demonstrated that laboratories did not appear to evaluate defects of the primary root in the same manner. Guidance has therefore been included in the handbook on the evaluation of primary roots and the extent to which secondary roots can be taken into account.

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 but when it is retarded
secondary roots may be taken into account in seedling evaluation

Representative genus: *Lolium*

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 absent or not sufficiently developed, secondary roots may be taken into account in seedling evaluation.

Development of seedling during the test

Secondary roots frequently develop during the test period. The appearance of the primary root is followed by elongation of the coleoptile with the first leaf developing inside.

Differentiation of normal and abnormal seedlings

Normal seedlings

root system	
the primary root	is intact (the length of the primary root should be at least 50% of the shoot size in case secondary roots are absent) or shows **acceptable defects** • retarded down to 30% of the shoot size provided secondary roots are developed. The combined length of the primary root and secondary roots should be at least 60% of the shoot size with a minimum length of primary root being 30% that of the shoot. • discoloured or necrotic spots • healed cracks and splits • superficial cracks and splits

Abnormal seedlings

root system	
the primary root	is **defective** if it • stunted or stubby • is retarded[1] • 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

[1]The primary root is abnormal if :
- in the absence of secondary roots, the primary root is less than 50% of the shoot size
- when secondary roots are present, the combined length of the primary and secondary roots are less than 60% of the shoot size with a minimum length of the primary root being 30% that of the shoot

Supplementary remarks

Evaluation of the root system

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




INVESTIGATIONS AND COMPARATIVE TESTS

Germination of Lettuce at different temperatures

There is some anecdotal evidence that symptoms of necrosis in lettuce can be reduced by germinating at 15ºC rather than 20ºC. A comparative test was set up to see if any evidence could be obtained to substantiate this. Unfortunately the seed lots included in the experiment were of too high a quality with an average germination of about 98%.

Germination of Sunflower in different media at different temperatures

This is a continuation of the study that led to the inclusion of organic growing media as a primary substrate. Sunflower seed lots have been germinated in Sand, Paper and Organic Growing Media at 20^{o}C, 25^{o}C and 20-30^{o}C alternating temperature.

Use of KNO_3 in Brassica germinations as an aid to seedling assessment

The results of this study which is led by Anders Lomholt are being analysed at the moment. Preliminary analysis suggests that:

- KNO_3 improves germination
- KNO_3 gives more normal seedling and fewer abnormals
- This effect is most clear at the first count
- Pre-chilling treatment is also beneficial to germination of Brassicas

Use of Crepe Paper/Sand for Germination Tests

Crepe paper is the media of choice in many American laboratories. The ISTA rules permits the use of crepe paper provided it meets the quality criteria set out in the Rules. Many labs also use crepe paper with sand as a media. At the present time this is not permitted by the Rules. Kari Fiedler is going to lead a Comparative test with the aim of having crepe paper/sand included as an ISTA germination media.

Questions to the Committee

The Committee has had a busy year answering over 30 questions from different sources:

Origin	Question
1. Argentina	Rounding Procedure – Check my calculations
2. Netherlands	Testing seeds of rootstocks of Tomato and Cucumber
3. Nepal	Testing Papaya seed
4. Japan	Evaluation of lettuce with necrosis like symptoms
5. ECOM	Testing Seed Mixtures
6. Canada	Innocuity tests for germination media
7. Latvia	Testing procedures for beets seed
8. India	Testing procedures for coriander seed
9. USA	Copenhagen tank germinators`
10. Proficiency Committee	Problems with *Cynodon dactylon* and *Capsicum* proficiency test results
11. UK	Induced (secondary) dormancy in *Brassica* spp.
12. ISTA Auditors	Timing of Germination Counts
13. USA	Seed nomenclature
14. Czech Republic	Testing fewer than 400 seeds
15. ISTA auditors	Need for TZ accreditation when determining fresh seed?
16. UK	Comparative testing of wild flower species
17. Japan	Evaluation of Lettuce with necrosis
18. Japan	Evaluation of cotyledon damage
19. Norway	Calculation of speed of Germination
20. Netherlands	Action to be taken if pH of water is outwith ISTA limits
21. Switzerland	Dormancy breaking methods limited to Table 5A column 6?
22. Australia	Test conditions of *Trifolium subterraneum* inducing abnormal seedlings
23. Greece	Retest procedure when replicates of first an second test are out of tolerance
24. Netherlands	When to report 100% germination and tolerances for 0% and 100%
25. Netherlands	Completion of germination tests once a preset germination figure is obtained
26. Morocco	Germination protocols for *Beta* spp. and *Muscari comosum*
27. USA	Testing blends of different varieties of one species
28. ISTA Auditors	Applying fungicide prior to testing the germination of untreated seed
29. Germany	Vacuum counter use
30. UK/Angola	Equipment requirements for an ISTA laboratory and field laboratories
31. USA	Use of crepe paper and crepe paper & sand for ISTA germination tests
32. Indonesia	Testing true shallot seed
33. India	Germination protocol for coriander seed

Some questions are easy to answer but some give rise to debate and may eventually require changes to be made in the Seedling Evaluation Handbook or ISTA Rules.

Some examples of the more interesting questions include some from Japan, that were accompanied by excellent diagrams and photographs, and questions from the Proficiency Committee on problems with the *Cynodon* and *Capsicum* proficiency test results.

Normal Seedlings

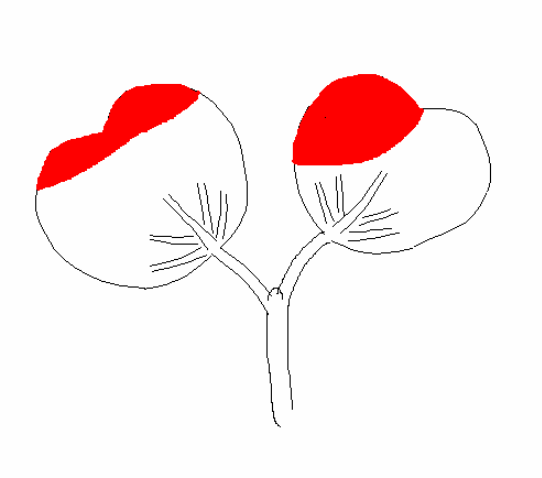


Figure 1: More than half of the total cotyledon tissue is functional

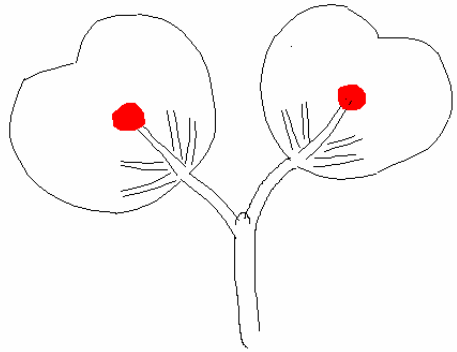


Figure 3: Damages are on the main veins, but far from the point of attachment

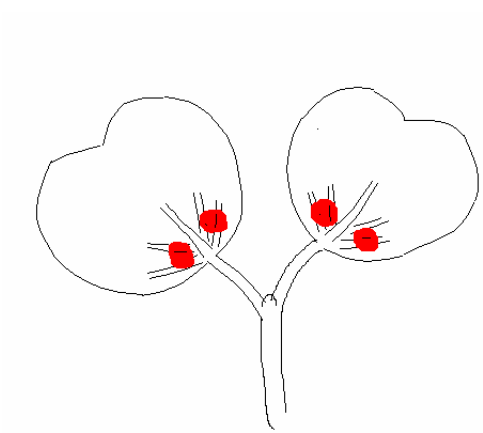


Figure 4: Damages are near from the point of attachment but not on the main veins

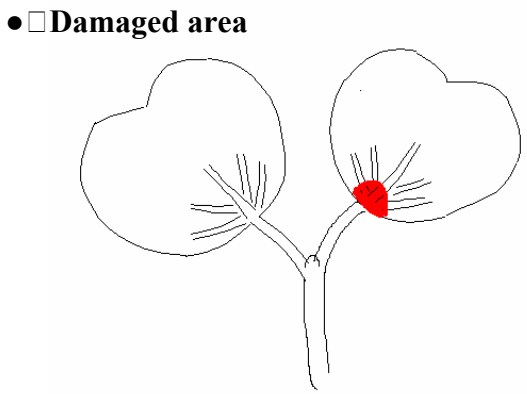


Figure 2: One of the points of attachment to the seedling axis is damaged

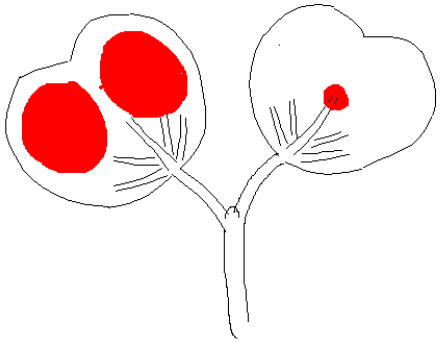


Figure 5: One of the main veins is damaged at the point of far from the attachment and more than half of the total cotyledon tissue is functional

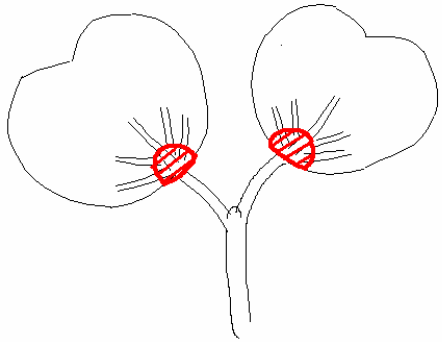


Figure 6: At the points of attachment to the seedling axis is damaged that are only superficial

Abnormal Seedlings

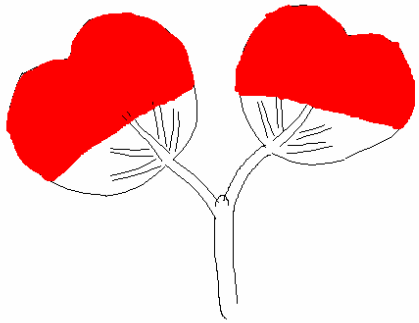


Figure 1: More than half of the total cotyledon tissue is not functional

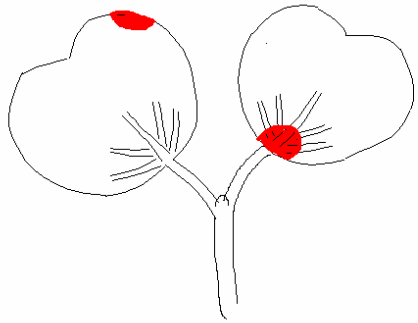


Figure 3: One of the points of attachment to the seedling axis is damaged and the other cotyledon is not intact

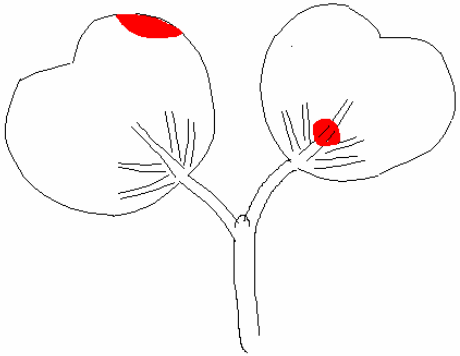


Figure 4: One of the main veins is damaged at the point of near from the attachment and the other cotyledon is not intact

● Damaged area

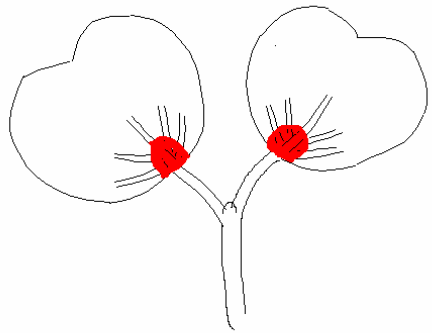


Figure 2: Both of the points of attachment to the seedling axis are damaged

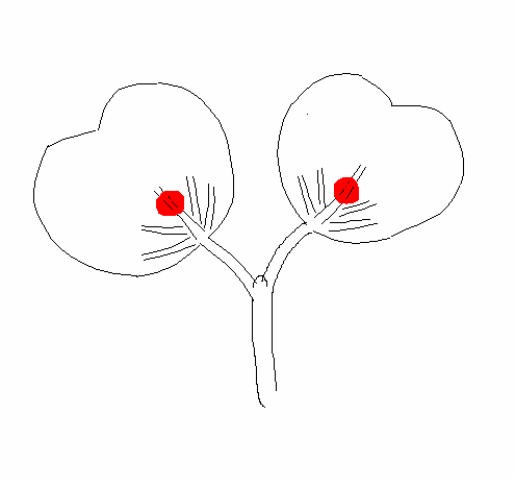


Figure 5 Damages are on the main veins that are near from the point of attachment

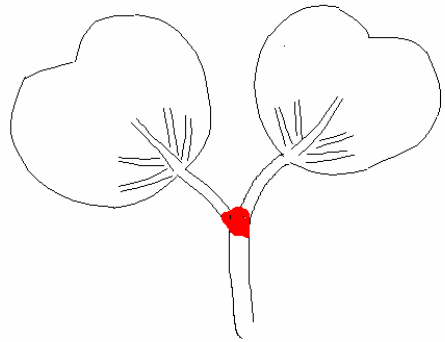


Figure 6: The terminal bud is damaged

Level of necrosis (lettuce)

Level 1: Minute spots on the surface (usually not visible)

Level 2: Many small spots on the surface but not affect to the conductive tissue (visible)

Level 3: Necrosis is reached to the conductive tissue

Level 4: Necrosis is reached to the conductive tissue and another part is affected also

Level 1 Sectioned: Conductive tissue is not affected

Level 3 Sectioned: Conductive tissue is affected

Germination results of 2 laboratories in *Capsicum annum* proficiency test

Lot 1

Laboratory	PP	Artificial Compost
A	70	Did not retest
B	69	72

Lot 2

Laboratory	PP	Artificial Compost
A	67	68
B	68	74

Lot 3

Laboratory	PP	Artificial Compost
A	46	81
B	65	81

Thanks for listening ….

… any questions ?