Dormancy Breaking for Germination Testing

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ISTA/ISSS Joint Webinar
June 7, 2023
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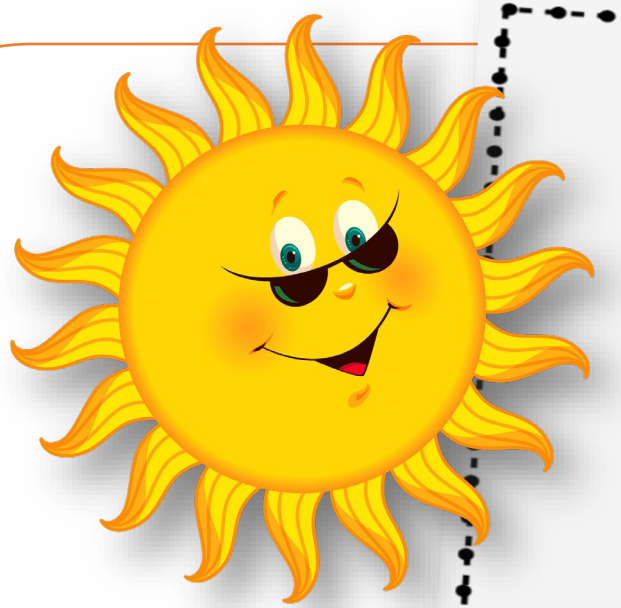
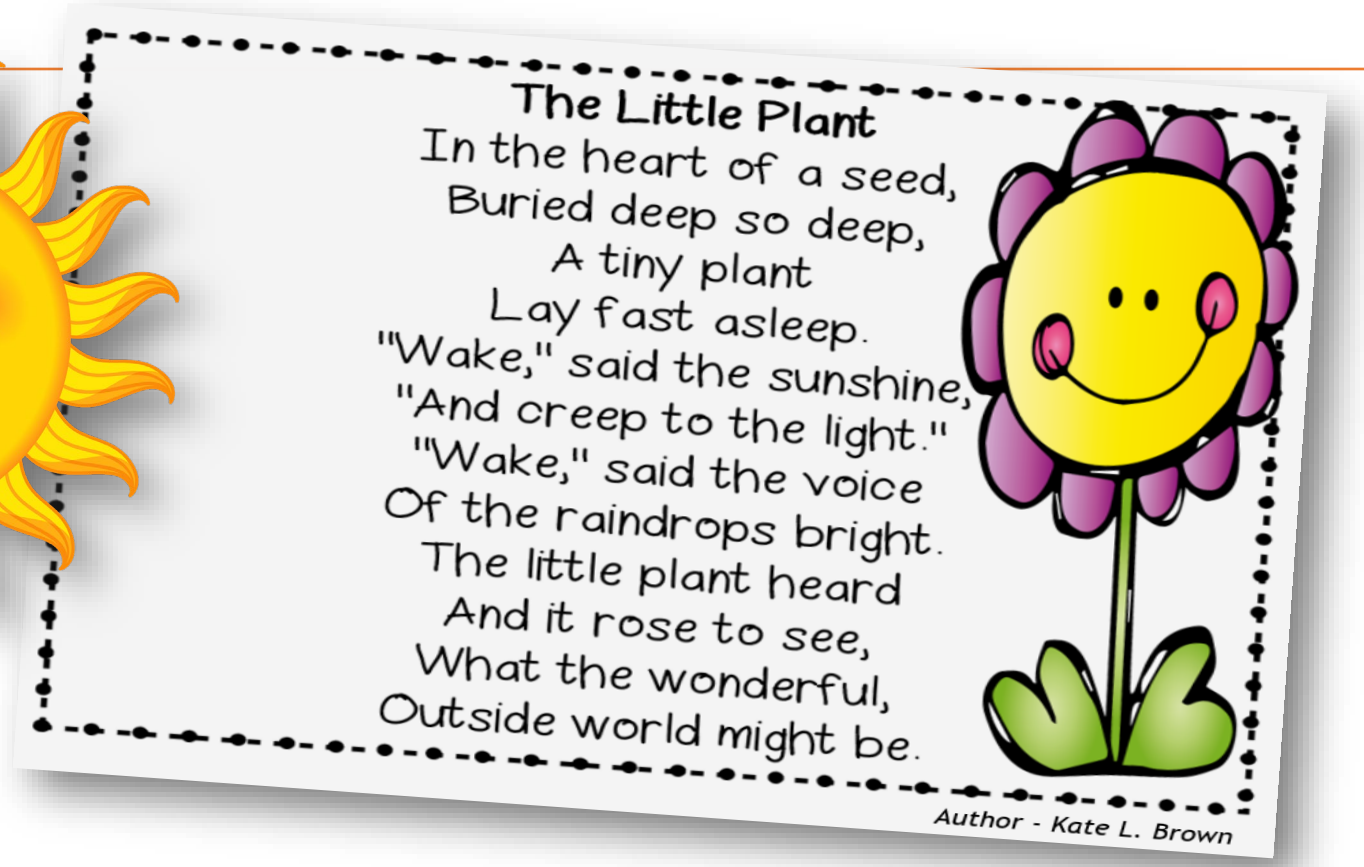
ISTA Seed Quality Assurance

isss international society for seed science

Professional Information & “Disclaimer”

- Accredited seed analyst by SCST (non-govt. org) and AOSA (govt. org) for several years
- Laboratory experience testing a wide range of species (e.g., agricultural, forage, turf, vegetable, flower, tree, shrub, native species, etc.)
- Worked in govt., seed cert., commercial, and international company (*ISTA accredited*) seed laboratories
- Experience using the ISTA Rules, AOSA Rules, USDA Rules, and Canadian Rules for testing seeds
- Conducted science-based validation studies using ISTA protocols
- Serve as the Vice-Chair of the ISTA Germination TCOM
- Serve as the SCST Co-Chair of the AOSA/SCST Germination and Dormancy Sub-Committee
- Not a seed physiologists…not a PhD…*but I have friends that are…*





But just like some of us, some seeds like to "sleep late" and are slow to "wake"…that is…to *GERMINATE*!!! In the world of seeds, this is commonly refereed to as "DORMANCY".

A common question is…."*Just how do you 'wake up' a seed?"*

Presentation Outline

- Brief overview of what dormancy is/is not
- Rudimentary requirements for germination
- Basic types of dormancy
- ISTA Handbook on Seedling Evaluation Introduction
- Purpose of Dormancy Breaking Procedures
- ISTA Rules Dormancy Breaking Procedures
- Function of Dormancy Breaking Procedures
- Reporting on ISTA Orange Certificate (OIC)

Dormancy – What is it?…What it is NOT?

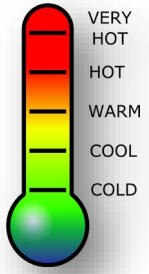
Seed Dormancy -

Refers to a situation in which **viable** seeds fail to germinate, even when provided with **normally favorable conditions** that initiate germination.

***Note**: Seeds must be **imbibed** and determined to be **viable** to be classified as "**dormant**". Also, "**dormant**" is **NOT** the same as "**hardseededness**" or "**state of quiescence**"…because in these two conditions seeds have not imbibed moisture.*

Basic conditions typically required for germination:

1) Appropriate **moisture**
2) Appropriate **temperature**
3) Adequate **air** exchange (*exceptions e.g., Oryza sativa/Rice*)
4) Exposure to **light** (*natural or artificial*) required by some species



Dormancy – Basic Types (1)

Primary/Enforced Dormancy –

These types of dormancies delay germination of the seed.

- **Physical** dormancy caused by the seed coat
- **Mechanical** dormancy caused by the seed coat
- **Chemical** dormancy
- **Morphological** dormancy
- **Physiological** or **Intermediate** dormancy
- **Deep** dormancy
- **Double** dormancy

Dormancy – Basic Types (2)

Primary/Enforced Dormancy –

- **Seed coat physical dormancy** prevents the seed from absorbing water
- **Seed coat mechanical dormancy** when the seed coat prevents the embryo from expanding and emerging from the seed coat
- **Chemical dormancy** is when internal chemicals inhibit/prevent the seed from germinating

Dormancy – Basic Types (3)

Primary/Enforced Dormancy –

- **Morphological dormancy** is when the embryo is immature when the seed separates from the mother plant
- **Physiological or Intermediate dormancy** occurs because a physiological condition is not being met
- **Deep dormancy** is when seeds remain dormant until favorable conditions are met
- **Double dormancy** is the combination of any of the previously mentioned factors

Dormancy – Basic Types

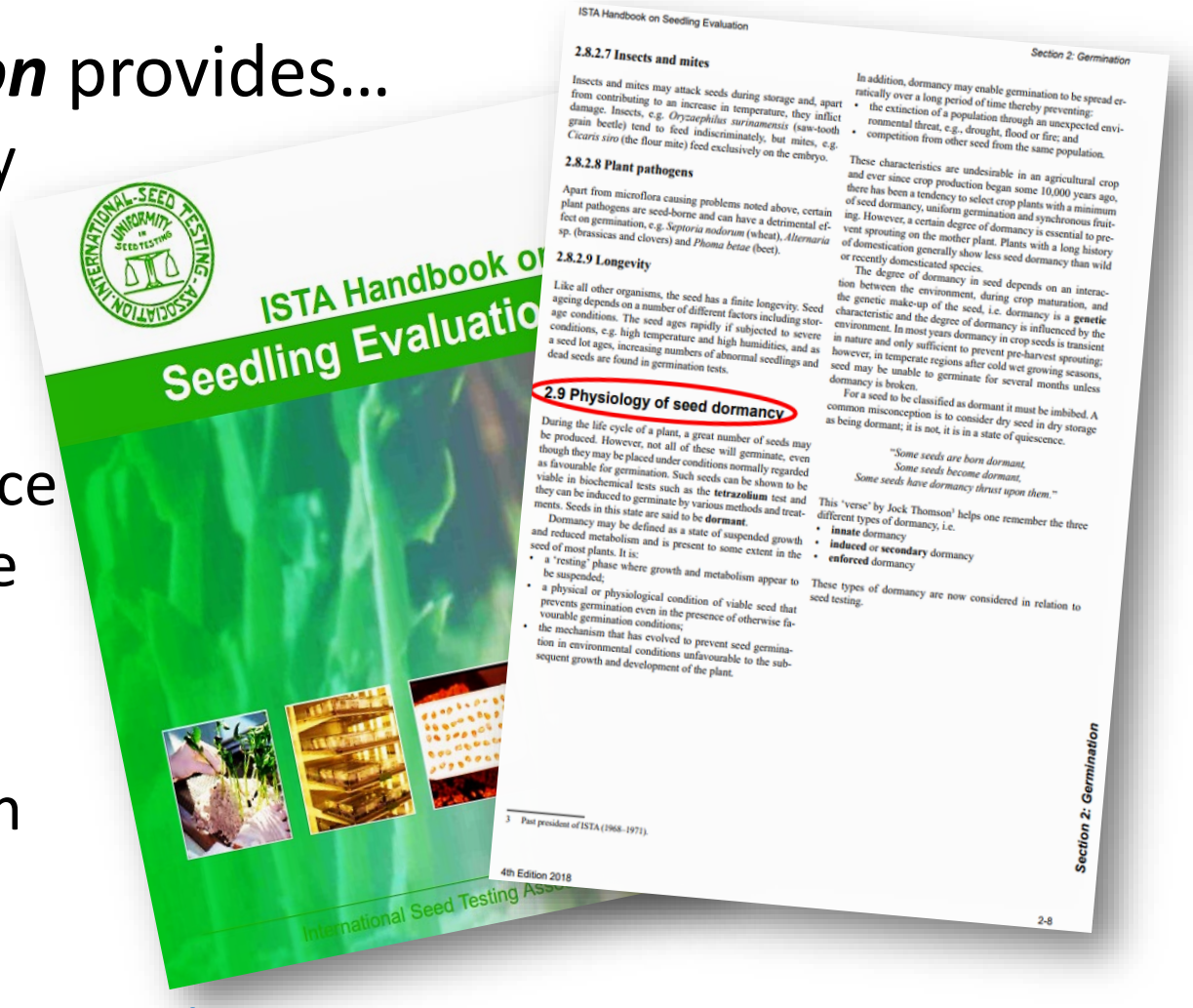
Secondary/Induced Dormancy –

- Caused by **temperature** extremes, too hot or too cold
- Caused by prolonged **darkness** or prolonged **light**
- Caused by **water** stress, too much or not enough
- Caused by lack of **oxygen/gas** exchange

Seed Dormancy Information: ISTA Hndbk on Seedling Evaluation

ISTA Handbook on Seedling Evaluation provides…

- Essential information on seed physiology (***Dormancy - Sec. 2.9***)
- Conditions for carrying out germination tests in laboratories
- Gives educational and illustrated guidance for the evaluation of seedlings for a large number of species
- Supplements and provides additional information on the methods described in the **ISTA Rules**



ISTA Publications https://www.seedtest.org/en/handbooks-calibration-samples/seedling-evaluation-4th-edition-2018-product-1016.html

Purpose of Procedures for Breaking Dormancy

Chapter 5: The germination test

5.1 Object

"The **object of the germination test** is to determine the **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**…**"

5.2.1 Germination

Germination of a seed in an ISTA 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 favourable conditions** in the field."

Purpose of Procedures for Breaking Dormancy

Chapter 5: The germination test

5.6.3 Procedures for promoting germination of dormant seed

"…If **dormancy** is suspected, **more complete germination** may be obtained by **retesting** after one or a combination of **dormancy-breaking procedures**…and all other procedures listed in 5.6.3.1, 5.6.3.2, and 5.6.3.3 can be used for any species without restriction…"

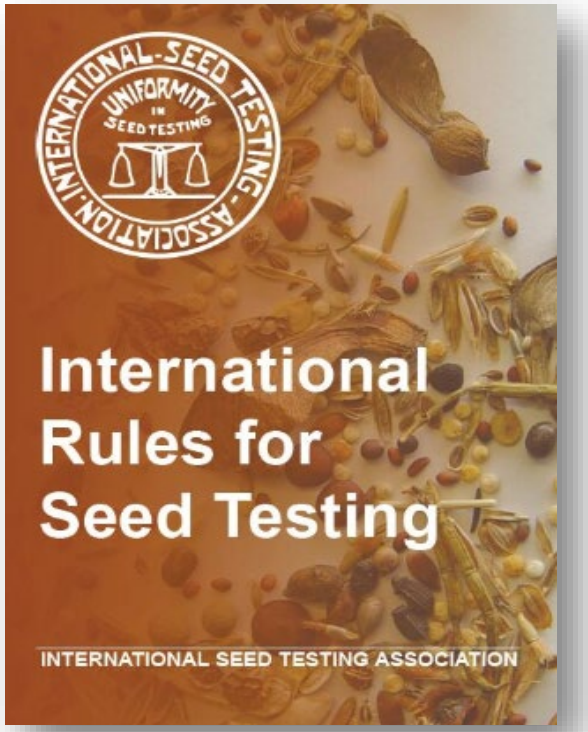
ISTA Rules - Procedures for Breaking Dormancy

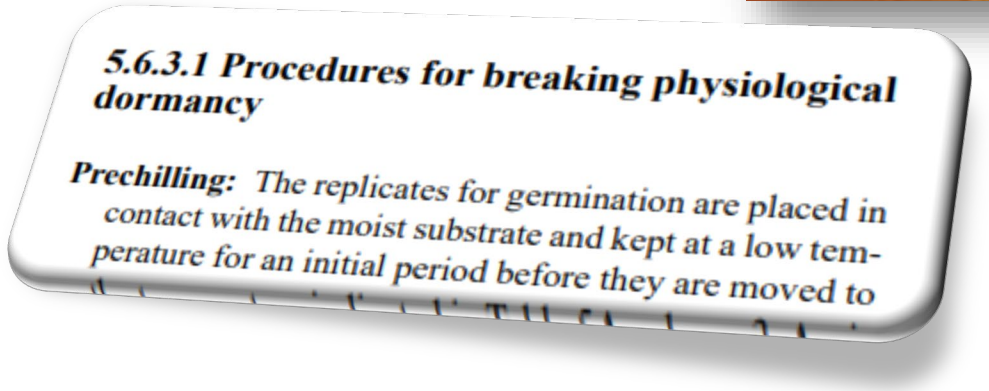
Dormancy breaking procedures listed in 5.6.3.1

- Prechilling
- Preheating
- Prestorage*

  (**This is actually "State of Quiescence", seeds are not imbibed.)*
- Light *(Minimum of 8 hours per 24-hour period.)*
- Sealed polyethylene envelopes/resealable plastic bags

  (*Increases ethylene gas and carbon dioxide.)*
- Gibberellic acid (GA3) solution
- Potassium nitrate (KNO3) solution
- Acid scarification
- Mechanical scarification




5.6.3.1 Procedures for breaking physiological dormancy

Prechilling: The replicates for germination are placed in contact with the moist substrate and kept at a low temperature for an initial period before they are moved to

ISTA Rules - Procedures for Breaking Dormancy

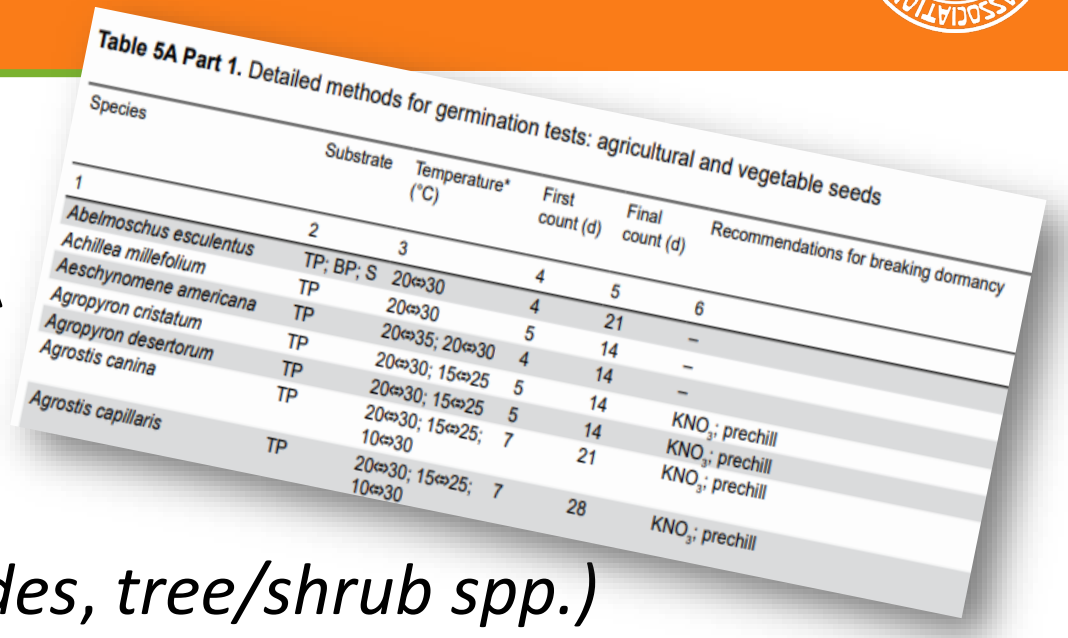


Table 5A Part 1. Detailed methods for germination tests: agricultural and vegetable seeds

Species	Substrate	Temperature* (°C)	First count (d)	Final count (d)	Recommendations for breaking dormancy
1	2	3	4	5	6
Abelmoschus esculentus	TP; BP; S	20⇔30	4	21	–
Achillea millefolium	TP	20⇔30	5	14	–
Aeschynomene americana	TP	20⇔35; 20⇔30	4	14	–
Agropyron cristatum	TP	20⇔30; 15⇔25	5	14	KNO_3; prechill
Agropyron desertorum	TP	20⇔30; 15⇔25; 10⇔30	7	14 21	KNO_3; prechill KNO_3; prechill
Agrostis canina	TP	20⇔30; 15⇔25; 10⇔30	7	28	KNO_3; prechill
Agrostis capillaris					

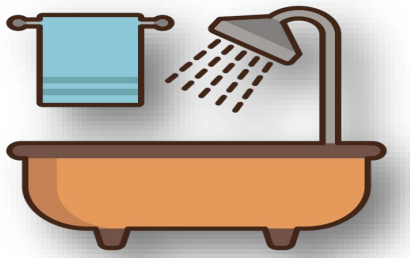
Dormancy breaking procedures NOT listed in 5.6.3.1, but found in Table5A

- Prewashing
- Presoaking
  (*e.g., Oryza sativa, Tetragonia tetragonoides, tree/shrub spp.)*
- Removal of enclosing structures (*e.g., pericarp, pod*)
- Incubation in moist substrate
- Alternating temperatures during the germination test period (*e.g., 20⇔30C*)
- Ethylene gas* (*No procedure provided.)*

(*Ethylene mentioned in ISTA Hndk. on Sdlg. Eval. 2.9.1.3.5 for *Helianthus/Oryza* but no procedure given.)

Treatments BEFORE the germination test

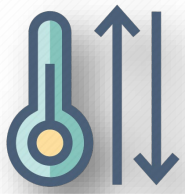
- Prestorage (Quiescence)
- Prechilling
- Incubation in moist substrate
- Preheating
- Prewashing
- Presoaking
- Removal of enclosing structures
- Mechanical scarification
- Acid scarification



ISTA Rules – Application of Procedures for Breaking Dormancy

Treatments <u>DURING</u> the germination test

- **Light**
- **Alternating temperatures** during the germination test period (*e.g., 20 ⇔30C*)



- Sealed **polythene/polyethylene envelopes** (*e.g., resealable plastic bags*)

- **Gibberellic acid** (GA3) solution applied to germination test substrate
- **Potassium nitrate** (KNO3) solution applied to germination test substrate

ISTA Rules - Table 5A Dormancy Breaking Info

Dormancy-breaking methods are found in the "*Recommendations for breaking dormancy*" column in Table 5A Part 1, 2, and 3 of the ISTA Rules.

Table 5A Part 1. Detailed methods for germination tests: agricultural and vegetable seeds

Species	Substrate	Temperature* (°C)	First count (d)	Final count (d)	Recommendations for breaking dormancy	Additional directions	Additional advice
1	2	3	4	5	6	7	8
Abelmoschus esculentus	TP; BP; S	20⇔30	4	21	–	–	–
Achillea millefolium	TP	20⇔30	5	14	–	–	–
Aeschynomene americana	TP	20⇔35; 20⇔30	4	14	–	–	–
Agropyron cristatum	TP	20⇔30; 15⇔25	5	14	KNO_3; prechill	–	–
Agropyron desertorum	TP	20⇔30; 15⇔25	5	14	KNO_3; prechill	–	–
Agrostis canina	TP	20⇔30; 15⇔25;	7	21	KNO_3; prechill	–	–

Table 5A Part 2. Detailed methods for germination tests: tree and shrub seeds (continued)

Species	Substrate	Temperature* (°C)	First count (d)	Final count (d)	Recommendation for breaking dormancy	Additional directions	Additional advice
1	2	3	4	5	6	7	8
Acer rubrum, Acer saccharinum	S; (TP)	20	7	21	–	–	–
Aesculus hippocastanum	TS; (S)	20⇔30; (20)	7	21	Soak in water for 48 h; cut off scar end of seed. Do not remove testa from sown portion. Fresh nuts may require prechill	–	–

Chapter 5: The germination test

Table 5A Part 3. Detailed methods for germination tests: flower, spice, herb and medicinal species

Species	Substrate	Temperature* (°C)	First count (d)	Final count (d)	Recommendations for breaking dormancy
1	2	3	4	5	6
Abutilon ×hybridum	TP; BP	20⇔30; 20	5–7	21	–
Achillea clavennae	TP; BP	20⇔30; 20	5	14	Light
Achillea filipendulina	TP; BP	20⇔30; 20	5	14	Light
Achillea ptarmica	TP; BP	20⇔30; 20	5	14	Light
Achillea umbellata	TP; BP	20⇔30; 20	5	14	Light

What is the function of dormancy breaking procedures?

- **Simulates** cooler/cold winter conditions that often break dormancy
- Artificially **decrease inhibitors** and **increase promoters** inside of seeds
- **Disrupt the seed coat** to allow for imbibition and gaseous exchange
- **Stimulate germination** with exposure of the seed to light
- **Simulate** diurnal fluctuation of temperatures of **day and night cycle**
- **Stimulate internal activities** of the seed to encourage germination
- **Increase** natural concentrations of **ethylene** and **carbon dioxide**
- **Leach away** germination **inhibitors** in the seed
- **Removal of seed structures** that physically inhibit germination

(More details in the ISTA Handbook on Seedling Evaluation Sec. 2.9)

ISTA Rules – Dead or Dormant/Fresh???

******Caution: Always be aware!!!******

Adverse germination **test conditions** can cause **secondary dormancy** *(e.g., media too dry/too wet, inadequate gaseous exchange, no light during germination test).*

This phenomenon can cause **inaccurate test results** for **all** or **some** of the **replicates**. This induced dormancy can also be **greatly inconsistent** and can vary by species, seed lot, age of seed lot, test method, test replicates, etc.

******Just because a seed does not germinate during the germination test does not automatically mean it is dead!!!******

Biochemical Viability Determination of Ungerminated Seeds

Chapter 6: Biochemical test for viability: the topographical tetrazolium test

6.1 Object

The objects of biochemical tests are:

a. To make a quick estimate of the viability of seed samples in general and those showing dormancy in particular.

b. In the case of particular samples which at the end of a germination test reveal a high percentage of dormant seeds (5.6.5), to determine the viability of individual dormant seeds or the viability of a working sample.

How do you determine if a seed is DEAD or ALIVE?!?!?

Basic Process:

The **tetrazolium test** is the method typically used to determine the **viability of ungerminated seeds**, remaining at the end of the germination test.

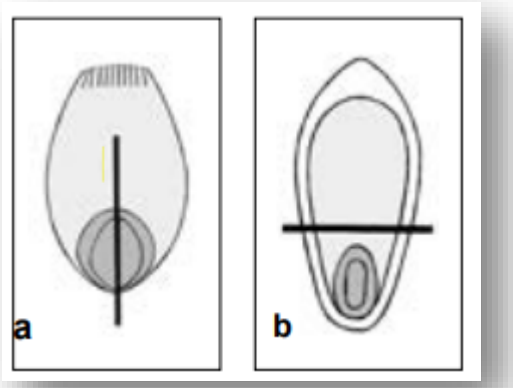
Imbibed seeds are carefully **cut**, allowing the embryo to be exposed to the **tetrazolium solution**. Respiring living seed tissue will **stain red** in color.

After an appropriate time soaking in the tetrazolium solution, the **staining patterns** and soundness of the tissue are **evaluated**. Seeds are then classified as "**viable**" or "**non-viable**". Viable seeds are reported on the ISTA certificate as "**fresh seeds**" and non-viable seeds are reported as "**dead seeds**".

Biochemical Viability Determination of Ungerminated Seeds

Example for Cereals:

Cut imbibed seeds remaining at the end of the germination test



a. **Longitudinal bisection cut through embryo**
b. **Transverse cut above embryo**

Evaluate seed staining patterns

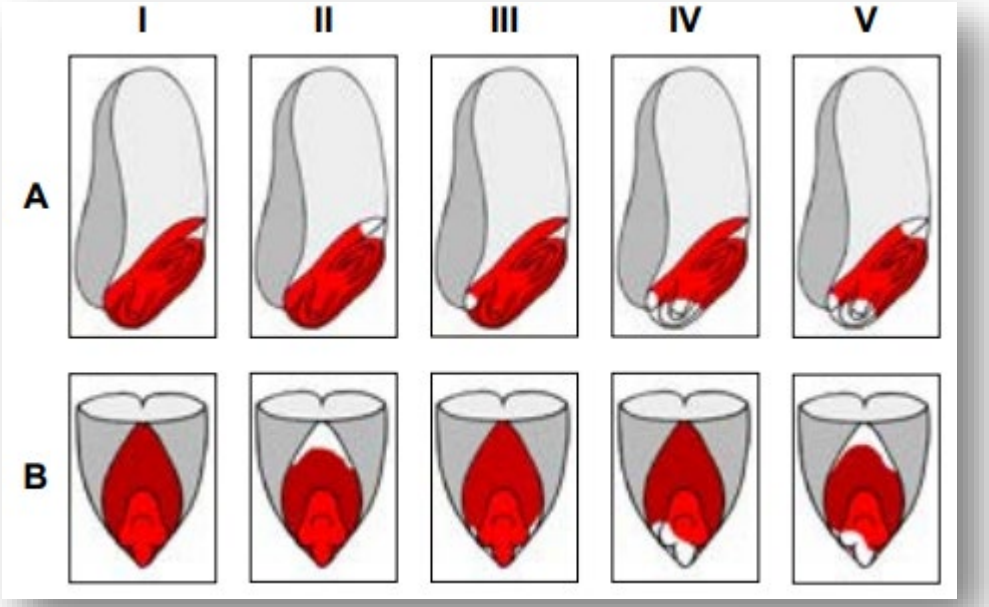


Figure 6.3a. Evaluation guide for cereals: viable seeds.

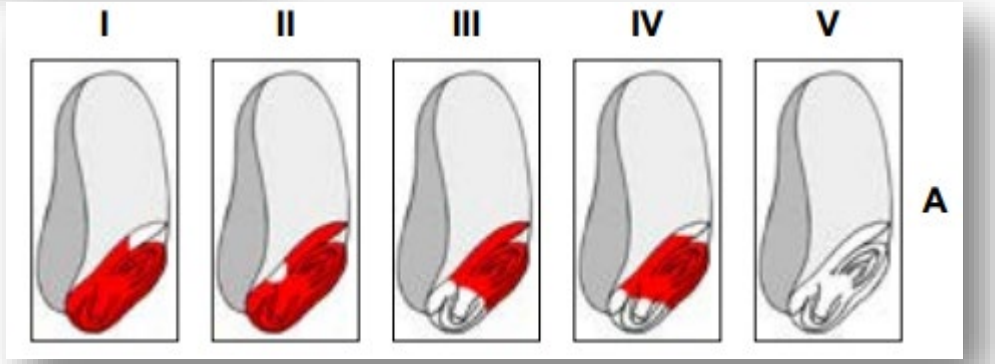
The figures in column **I** are all completely stained and viable. Columns **II–V** show the maximum area of unstained, flaccid or necrotic tissue permitted in viable seeds.

A The figures are representative for *Triticum*, ×*Triticosecale*, *Secale*, *Hordeum* and *Avena* when prepared by bisection or bisected for evaluation

B *Avena* prepared by transverse cutting

Figure 6.3b. Evaluation guide for cereals: non-viable seeds.

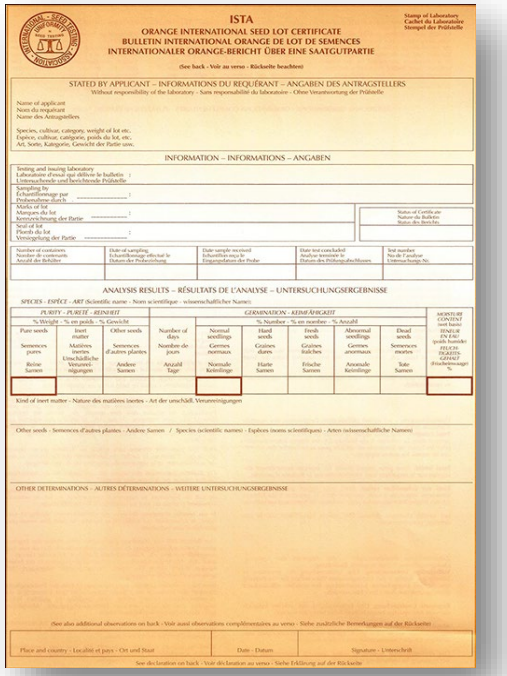
A Figures are representative for *Triticum*, ×*Triticosecale*, *Secale*, *Hordeum* and *Avena* when prepared by bisection or bisected for evaluation



Reporting info on the OIC

1) ISTA Rules 1.5.2.6 and 5.6.3 REQUIRES any "**special treatment and/or methods used to promote germination**" MUST be stated on the OIC under "**Other determinations**". This informs the person reading the OIC if any methods were used to break dormancy.

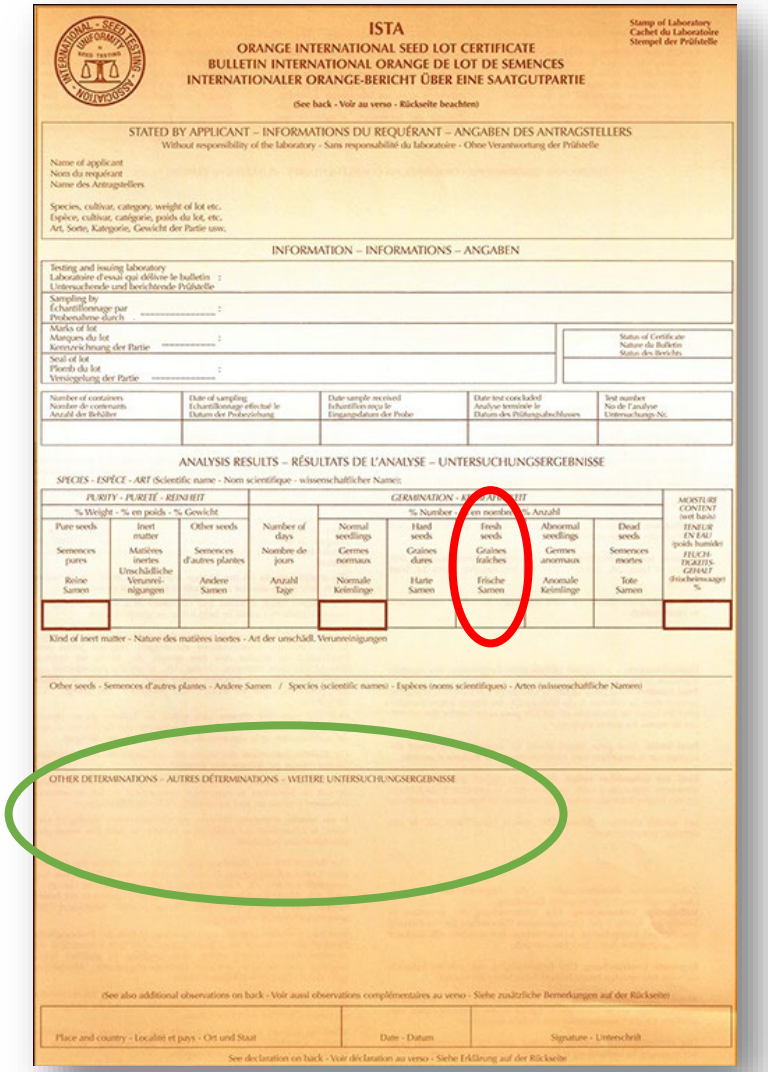
2) If there are **5% or more suspected fresh seeds** found in the germination test, their **viability must be determined** and reported on the OIC. There is a required process to determine the viability of the ungerminated seeds to assess if they are "fresh seeds" or "dead seeds".


ISTA
ORANGE INTERNATIONAL SEED LOT CERTIFICATE
BULLETIN INTERNATIONAL ORANGE DE LOT DE SEMENCES
INTERNATIONALER ORANGE-BERICHT ÜBER EINE SAATGUTPARTIE

3) Labs have the option to conduct "**Parallel Tests**", ISTA Rules 5.2.3, with one test using **germination promoting procedures/methods** and one test **without**. Both test results can be reported on the OIC.

****The important issue is that the percentage of fresh/dormant seed is determined in a seed lot before it is sold and planted. This info should be provided to the end user that is going to sow/plant this seed lot. To most growers, **"fresh seeds" are considered "dead seeds"** because the seed will typically not germinate within the grower's desired time frame. There will be no uniform emergence of the seedlings when the seed lot has a significant percent of fresh/dormant seeds.****

Reporting info on the OIC



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ORANGE INTERNATIONAL SEED LOT CERTIFICATE
BULLETIN INTERNATIONAL ORANGE DE LOT DE SEMENCES
INTERNATIONALER ORANGE-BERICHT ÜBER EINE SAATGUTPARTIE

Stamp of Laboratory
Cachet du Laboratoire
Stempel der Prüfstelle

(See back - Voir au verso - Rückseite beachten)

STATED BY APPLICANT – INFORMATIONS DU REQUÉRANT – ANGABEN DES ANTRAGSTELLERS
Without responsibility of the laboratory - Sans responsabilité du laboratoire - Ohne Verantwortung der Prüfstelle

Name of applicant
Nom du requérant
Name des Antragstellers

Species, cultivar, category, weight of lot etc.
Espèce, cultivar, catégorie, poids du lot, etc.
Art, Sorte, Kategorie, Gewicht der Partie usw.

INFORMATION – INFORMATIONS – ANGABEN

Testing and issuing laboratory
Laboratoire d'essai qui délivre le bulletin
Untersuchende und berichtende Prüfstelle

Sampling by
Echantillonnage par
Probenahme durch

Marks of lot
Marques du lot
Kennzeichnung der Partie

Seal of lot
Plomb du lot
Versiegelung der Partie

Status of Certificate
Nature du Bulletin
Status des Berichts

Number of containers Nombre de contenants Anzahl der Behälter	Date of sampling Echantillonnage effectué le Datum der Probeziehung	Date sample received Echantillon reçu le Eingangsdatum der Probe	Date test concluded Analyse terminée le Datum des Prüfungsabschlusses	Test number No de l'analyse Untersuchungs-Nr.

ANALYSIS RESULTS – RÉSULTATS DE L'ANALYSE – UNTERSUCHUNGSERGEBNISSE

SPECIES - ESPÈCE - ART (Scientific name - Nom scientifique - wissenschaftlicher Name):

PURITY - PURETÉ - REINHEIT				GERMINATION - KEIMFÄHIGKEIT					MOISTURE CONTENT (wet basis) TENEUR EN EAU (poids humide) FEUCHTIGKEITSGEHALT (Frischeinwaage) %
% Weight - % en poids - % Gewicht				% Number - % en nombre - % Anzahl					
Pure seeds Semences pures Reine Samen	Inert matter Matières inertes Unschädliche Verunreinigungen	Other seeds Semences d'autres plantes Andere Samen	Number of days Nombre de jours Anzahl Tage	Normal seedlings Germes normaux Normale Keimlinge	Hard seeds Graines dures Harte Samen	Fresh seeds Graines fraîches Frische Samen	Abnormal seedlings Germes anormaux Anomale Keimlinge	Dead seeds Semences mortes Tote Samen	

Kind of inert matter - Nature des matières inertes - Art der unschädl. Verunreinigungen

Other seeds - Semences d'autres plantes - Andere Samen / Species (scientific names) - Espèces (noms scientifiques) - Arten (wissenschaftliche Namen)

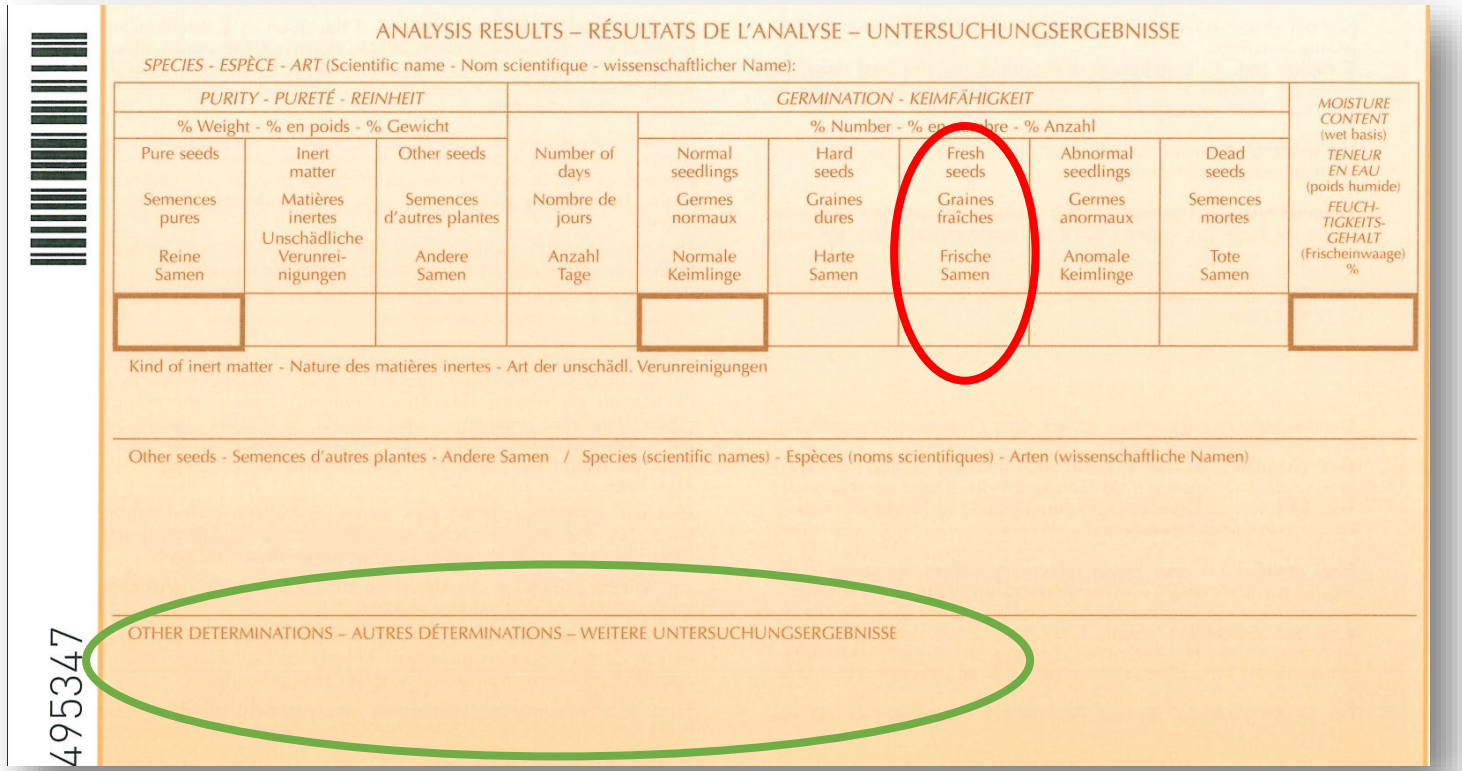
OTHER DETERMINATIONS – AUTRES DÉTERMINATIONS – WEITERE UNTERSUCHUNGSERGEBNISSE

(See also additional observations on back - Voir aussi observations complémentaires au verso - Siehe zusätzliche Bemerkungen auf der Rückseite)

Place and country - Localité et pays - Ort und Staat	Date - Datum	Signature - Unterschrift

See declaration on back - Voir déclaration au verso - Siehe Erklärung auf der Rückseite

MUST report dormancy breaking procedures used in "**Other determinations**" and the percent "**Fresh seeds**" determined by appropriate procedures.



ANALYSIS RESULTS – RÉSULTATS DE L'ANALYSE – UNTERSUCHUNGSERGEBNISSE

SPECIES - ESPÈCE - ART (Scientific name - Nom scientifique - wissenschaftlicher Name):

PURITY - PURETÉ - REINHEIT				GERMINATION - KEIMFÄHIGKEIT					MOISTURE CONTENT (wet basis) TENEUR EN EAU (poids humide) FEUCHTIGKEITSGEHALT (Frischeinwaage) %
% Weight - % en poids - % Gewicht				% Number - % en nombre - % Anzahl					
Pure seeds Semences pures Reine Samen	Inert matter Matières inertes Unschädliche Verunreinigungen	Other seeds Semences d'autres plantes Andere Samen	Number of days Nombre de jours Anzahl Tage	Normal seedlings Germes normaux Normale Keimlinge	Hard seeds Graines dures Harte Samen	Fresh seeds Graines fraîches Frische Samen	Abnormal seedlings Germes anormaux Anomale Keimlinge	Dead seeds Semences mortes Tote Samen	

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OTHER DETERMINATIONS – AUTRES DÉTERMINATIONS – WEITERE UNTERSUCHUNGSERGEBNISSE

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References

- **ISTA Rules**
- **ISTA Handbook on Seedling Evaluations**
- **Seed Dormancy -** Drew Mutlick, Erin Milligan, and Mark Mullin

  Link https://www.powershow.com/view/b54c3-NjU5Y/Seed_Dormancy_powerpoint_ppt_presentation

Thank you!

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