



International Seed Testing Association

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Submitted Comments on the 2021 ISTA Rules proposals

Consolidated by: Ernest Allen, Rules Chair & Sue Alvarez, Vice Chair

Editorial corrections;

- **General comment:** The List from 2019 has all the species with the author abbreviations. The new proposals of the rules say to report in accordance with the List of stabilized plant names without the author abbreviation. Does this apply only on the certificates? Please clarify?
 - **Rules committee response:** see response to the next comment.
- **General comment:** In the editorial changes, edits for 1.5.2.2 and 3.7 omitted the authority. They are however, listed in table 2C. Please explain the difference in Table 2C and the examples listed in these sections.
 - **Rules committee response:** Historically, ISTA has not required scientific names to display authorities on OIC or BIC certificates. During the Stabilized List update in 2019, scientific names were updated throughout the rules. Examples used throughout the rules were also updated with the entire scientific name; including authorities. This was done in error as the scientific names in examples had previously never included authorities. This editorial change is correcting the error.

C.4.2 Reduced Test

Issues: ***Please see rules proposal document (OGM20-05) for updated proposal**

- **General comment:** There are doubts, whether the definition of a minimum sample size for a reduced test in the case of very expensive seeds meets the interest of ISTA's stakeholders. They likely prefer the old version without the minimum sample size. Do the regulations for reduced other seed determination affect 2.5.4.5 (the first bullet point)?
 - **Purity committee response:** Based on a majority vote of PUR, we revised the proposal as an editorial change, rather than a revision to be proposed. The decision is mainly to allow the committee more time to exam the revision.
- **General comment:** This proposal is not only about methodology. The changes proposed may cause severe problems for customers (i.e. Breeders and Seedbanks) that are dependent on OIC for exchange of their often very limited materials. The proposal may severely limit the possibilities for exporting seeds. Customers may not be willing to send their whole seed lots to testing labs due to the risks involved (lost by post, damage during transport, etc.), so it does not help to test the whole seed lot. In addition, it is often the case that countries explicitly want to see an amount of seed being examined as dictated by their import policy. What is ISF's position to this change? Laboratories and their customers need the ability to meet trade and import conditions in all parts of the world. This is possible with a reduced test. A suggested revision to the proposal is below.

- **Purity committee response:** Based on a majority vote of PUR, we revised the proposal as an editorial change, rather than a revision to be proposed. The decision is mainly to allow the committee more time to exam the revision.

The proposal was approved by majority vote in the Purity and EOC committees.

| CURRENT VERSION | PROPOSED VERSION |
|--|---|
| 4.2.4 Reduced test In a reduced test, less than the whole working sample seed weight is examined for all other seeds present except | 4.2.4 Reduced test A reduced test, where less than the whole working sample seed weight is examined for all other seeds present except for dust-like seeds (see 4.5.3.1). |

Rules Proposals for the International Rules for Seed Testing 2021 Edition

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|---|--|
| for Orobanchaceae species. In the case of very expensive seed (see 2.5.4.5), a reduced test can be performed. | In the case of very expensive seed (see 2.2.14), a reduced test can be performed using: <ul style="list-style-type: none"> a) A minimum of one fifth of the prescribed working sample weight. b) The whole seed lot when the seed lot weight is less than one fifth of the prescribed working sample weight. |
|---|--|

- **General Comment:** The reference used in C.4.2.4. is a definition defining small seed lots, not very expensive seed. Suggested change: change from “very expensive seed” in C.4.2 to “small seed lots.”
 - **Purity committee response:** Based on a majority vote of PUR, we revised the proposal as an editorial change, rather than a revision to be proposed. The decision is mainly to allow the committee more time to exam the revision.
- **General comment:** The reference to 4.5.1 in the Grey text box is misleading because this is a testing matter of the laboratory and has nothing to do with the availability of seeds from a special lot by the sender.

Are other TCOMS involved with this proposal? the term expensive/valuable seed should be discussed and agreed upon by more TCOM’s before a change of the rules (like this proposal) will be consistent.

- **Purity committee response:** Based on a majority vote of PUR, we revised the proposal as an editorial change, rather than a revision to be proposed. The decision is mainly to allow the committee more time to exam the revision.

C.5.1. Germination method for *Brassica napus*

Issues:

- **General comment:** Rape seed has spring (15-20°C) and winter (20-30°C) types which require different temperatures for optimal germination. Removing either of the best suited temperatures for the type germinated may reduce optimal germination.

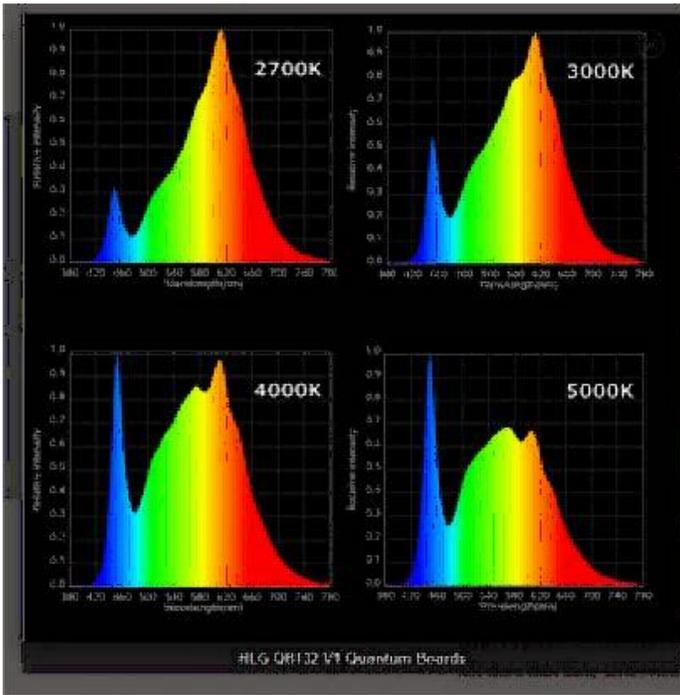
- **Germination committee response:** Current ISTA rules do not present as such, i.e. recommending 20-30°C for winter type and spring (15-20°C). Both temperatures are equivalent to a lab to choose in current rules.
- **Validation study:** The study used six lots. However, it did not specify which lots were spring and which were winter. Given that winter and spring have different optimal germination temperatures, this information could have played a significant role in the result outcome. Five of the lots produced results quite different from the sixth lot. Could this have been due to the sixth lot being the only different type?
 - **Germination committee response:** Lot 5 is the winter type, which has no difference from other lots in variation (i.e., repeatability and reproducibility was low at 20-30°C) responded to the tested 4 temperatures.
- **Validation study:** The study results concluded that there was not a significant difference between temperatures. However, there was a significant difference among ungerminated seeds. It appears that the proposed method was based on the number of ungerminated seeds and not a statistical difference in germination.
 - **Germination committee response:** Method performance according to ISO standards and ISTA validation requirements, the method repeatability, reproducibility, and accuracy are the parameters for its evaluation. The statistical significance is not the evaluation standards in multi-laboratories' comparison or a validation study, although it could be widely used in research.
- **General comment:** It is best to keep the 20-30°C germination temperature and add 15-20°C. While the validation study resulted in large Standard Deviations for 20-30°C it also generated similar mean germination rates and it worked for 5 of the 8 laboratories.
 - **Germination committee Response:** The Method Validation study was based on a solid experimental design the three important parameters were assessed; repeatability, reproducibility, and accuracy. There is clear evidence that 20<=>30 is the lowest performing temperature regime.
- **General comment:** The germination temperature of 15-20°C is not as common as 20-30°C for other species. This could cause capacity issues. Our country has always had good results using 20-30°C. We suggest keeping 20-30°C and adding 15-20°C as an alternate temperature.
 - **Germination committee response:** The Germination Committee wish to follow the results of the Method Validation Study and therefore remove 20<=>30 and include 15<=>25. The steady temperature of 20°C can also be used.
- **Validation Study:** This study only validated a change for *Brassica napus* var. *napus*. Other *B. napus* subspecies 'genetics' were not included and should have been part of the rule change proposal. They may be different. If a temperature change is to be made, a larger pool of genetics with all or most all *B. napus* subspecies/varieties should be included in the validation study (i.e. forage rape, Siberian kale, rutabaga, and other *Brasica napus* subspecies)
 - **Germination committee response:** The current ISTA rule uses the same temperature for all subspecies or varieties, although there could be genetic variations. In the ISTA approved document "ISTA Method Validation for Seed Testing", the requirement is to have "Preferably

six samples representing three levels of the quality component being assessed”. Seed quality is one of the biggest variations among other variables such as genetic differences. To validate a germination method, it is impossible to have all different breeds, varieties, types, and genetics incorporated into the multi-lab studies in six lots. This study was one of the studies considered more on varietal and type differences, but once again, it proved that the seed lot quality was impacted more by different temperatures (e.g., Lot 6 with low germination). However, we have noted this concern.

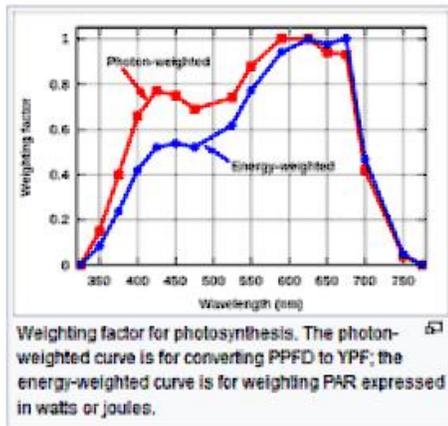
C.5.2. Precision on light for germination tests

Issues:

- **General comment:** This proposal may become an issue for laboratories which cannot easily meet the light requirements.
 - **Germination committee response:** The proposal provides guidance only as “should” is used rather than “must”.
- **General comment:** Is the light temperature (K) and the visible spectrum (nm) able to be readily measured by seed testing laboratories? Will ISTA routinely measure these as part of the ISTA audit?
 - **Germination committee response:**
 1. There is no need to measure the light, the Kelvin unit is an indication given from the lamp or LED supplier. This should give assurances to have the correct lighting ranges for germination and seedling growth.
 2. The auditors consider this proposal to be guidance for the laboratories and not a requirement.
 3. The laboratories during the ISTA audit will not be asked to provide records for the light output measurements.
- **General comment:** The ranges of sunlight are 3000k to 5000K. It appears that the recommendation of 3000K to 4000K are too narrow. Is there evidence to support this narrow range?
 - **Germination committee response:** The chart below is an example of the light spectra for 3000K, 4000K, and 5000K. According to two online sources (two sites cited) warm LED spectrums (3000K to 4000K) have the ideal amount of blue light and the optimal amount of yellow light for growth. At levels higher than 5000k, much of the red light is left out of the spectrum and plants will grow slowly and without much vigour.



Below, is an efficiency curve to calculate the PAR by cumulating the wavelengths. It shows that wavelengths at 600 and 700nm are important. 5000K lights lose a lot of spectrum in this range compared to 3000K or 4000K which brings a maximum of efficient light.



Please note, the Germination committee uses “Should” and not “Must” in the proposal.

C.5.3. Reporting Results

Issues:

- **General comment:** The word “specific” should be replaced by the word “specified” or “predetermined” since the applicant should specify the number of days or should at least agree with on a suitable number of days proposed by the laboratory.
 - **Germination committee response:** The Germination Committee has reconsidered this statement and will remove “or after a specific number of days” from the proposal.

- **General comment:** This rule proposal may inadvertently cause disbalance among ISTA labs by creating a competitive situation whereby laboratories are pressured to generate faster results to remain competitive. Removing the newly proposed language will keep ISTA germination tests uniform.
 - **Germination Committee response:** The Germination Committee has reconsidered this statement and will remove “or after a specific number of days” from the proposal.

C.5.4. Change in the germination evaluation of roots for *Helianthus annuus* to allow secondary roots

Issues:

- **General comment:** Hypocotyls are highly light sensitive, and roots are not. The two organs are not correlated and due to the varying light conditions within ISTA laboratories, it would be difficult to gain uniformity in testing using the hypocotyl as a point of measure to determine root sufficiency. Proposal: Remove current proposed wording and add “at least three secondary roots which do not show any defect listed for the primary should be present.”
 - **Germination Committee response:** The Germination Committee considered this proposal carefully but agreed this was the best evaluation. Previously this was agreed for *Glycine max*.
- **General comment:** To get better harmonization among ISTA, M&P, and AOSA rules, future good proposals like this one can/should be applied to all three testing rules.
 - **Germination committee response:** The Germination Committee has noted the above comment.

C.7.1. Sample and subsample size

Issues:

- **General Comment:** Why is the sample size reduced from 1000 seeds to 400 in the proposal?
 - **SHC Committee Response:** This reduction is only for method 7-004. For other methods, only editorial modification for harmonization of wording are done.

400 seeds allow a detection at the 1% level. This number is consistent with all the other methods for detection of fungi on blotter or media. In fact, this request came from the International Seed Health Initiative (ISHI) to decrease from 1000 to 400 seeds for method 7-004. Mainly because of vegetable Brassicas. Growth conditions and incidence of the disease on vegetable species allow a detection level at 1% by testing 400 seeds. A lower detection level is not required. The validation studies showed that if an infected seed is present in the sample tested, it is detected as each seed is tested individually.

The 1000 seed sample in the ISHI protocol was based on what was written in the ISTA method. This issue is one of the reasons that ISHI has independently published their methods: so that they can dictate their own sample sizes. In order to have uniformity in seed testing, we should try to have equivalent sample sizes. Understandably, field crops like Brassica napus/rapa may require a higher number of seeds, which the current proposal does not prohibit (it sets a minimum sample size), thus a lab can test more seeds if needed.

- **General comment:** The detection limit relevant for the size of subsamples is not the number of pathogen propagules **per seed** which can be detected, but the number of pathogen propagules which can be detected **in a sample**. Please consider the proposal below to change the wording in the following way (to be adapted to the specific tests):

“The sizes of the total working sample and subsamples in terms of number of seeds depend on the desired tolerance standard (maximum acceptable percentage of seeds infested) and detection limit (minimum number of pathogen propagules which can be detected in a sample). The minimum sample size should be xxx seeds and the maximum subsample size should be xxx seeds.”

- **SHC Committee Response:** At this point, the SHC does enough time to modify this sentence in all the methods, as it requires discussion on the term propagules if associated with sample. Here the sentence was just copied from methods where it existed to other methods to harmonize. We propose to postpone this modification to next 1st Nov rules proposals, to let time to the committee to discuss and come with a consensus sentence.

C.7.3. Safety precautions

Issues:

- **General comment:** The proposal specifies protective eyewear when working with ethidium bromide. It does not specify protective eyewear when working with UV light which can cause damage to the eyes. A new proposed wording is below:

“Ultraviolet UV light must not be used without appropriate precautions. Ensure that UV protective eyewear is utilised also when working with ethidium bromide.”

- **SHC Committee Response:** The sentence suggested is already included in the proposal. Here the UV are only used with Ethidium bromide.
“Ultraviolet UV light must not be used without appropriate precautions. Ensure that UV protective eyewear is utilised when working with ethidium bromide.”

C.9.1 Changes to the use of “must”, “should”, and “may”

Issue:

- **General comment:** The committee is encouraged to make sure all the “must” that will be changed to “should” is well reasoned and does not influence a critical step in the testing process such as the proposed changed in 9.2.5.7.
 - **Moisture committee response:** The Committee decided to conduct a review of the proposal. Based on this second review, several proposed changes were withdrawn to reduce the possibility of unintended consequences on critical steps in the testing process.

C.15.1 Clarification of the assessment of the radicle emergence for *Zea mays* and *Triticum aestivum* subsp. *aestivum* in the radicle emergence test

Issue:

- **General comment:** It seems too restrictive to say “should be made by eye” to assess all radical emergence. For example, it will exclude using computer vision and image analysis. It might be more clear to say “should be made by the evaluation on the radical length without magnification”
 - **Vigour Committee Response-** This test was developed using a ‘by eye’ method and was validated on this basis. There has not been a comparative test to assess whether computer vision or image analysis gives the same results as ‘by eye’. When we do have the data from a comparative test available, the term ‘by eye’ may be omitted. However, any image analysis technique will have to be validated through the Method Validation programme, using ‘by eye’ as the accepted basis. The Vigour Committee is currently participating in an ISTA Special Project in which the potential for two image analysis systems to be used for assessment of radicle emergence in vegetable Brassica species is being evaluated.