Seed Health Com – Annual Update

Ruud Barnhoorn

Agenda

- Members
- Rules changes
- New Rule 7-035
- Method development and validation
- Special projects
  - Detection seed-born pathogen *Paracidovorax citrulli* by Multispectral Imaging (Xiulan Xu)
  - Application of High-Throughput Sequencing in the detection of Viruses in seed (Yanhong Qiu)
  - SHCOM accreditation 2.0
  - The Seed Health web-based platform
- Workshops & Seminars
- Intercross TCOM collaborations
- Other research and future steps

ISTA ANNUAL MEETING 2026

Members (15)

Rouke Bakker (NZL) *stepped down* - Angela Thüringer (AUT)

- Studied "Applied plant sciences" at the University of Natural Resources and Applied Life Sciences in Vienna, Master thesis topic: “Studies on the species spectrum of the fungal genus “*Diaporthe*” on soybeans from Austrian seed multiplication regions”.
- Working at AGES in Vienna (Austria) in the seed health testing laboratory for Austrian seed certification since December 2013.
- Specialized on testing on fungal seed pathogens such as *Tilletia spp*., *Microdochium nivale*, *Ustilago nuda*, *Phomopsis*-complex, *Ascochyta pisi*, *Alternaria linicola*, *Botrytis cinerea* and many more.

Isabelle Serandat (FR) *retired* - Jaiana Malabarba (FR)

- Studied “Biological Sciences” at the University of Vale do Rio dos Sinos (Brazil), Master degree in Molecular and Cellular Biology and Phd. degree in Molecular and Cellular Biology at the Federal University of Rio Grande do Sul (UFRGS) and Max Planck Institute for Chemical Ecology (Germany).
- Working at GEVES in Angers (France) since 2023 with the current position as Head of Pathology Laboratory at SNES (Station Nationale d’Essais de Semences).

- Two additional applicants of value shared their interest of becoming SHCOM members.
  - They are both accepted by the committee and a letter to the ECOM is addressed.

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Rules changes (1)

- Rules change proposal in chapter 1 and chapter 7

C.7.4 Reporting seed health tests

The ISTA Seed Health Committee proposes a modification to the rules governing the specification of results for seed health tests under the "Other Determinations" section of the OICs. The current two-option result scoring system, as defined in Chapter 1, Section 5.2.10, and Chapter 7, Section 6 (Reporting Results), is considered insufficiently descriptive. Information on the level of infection is regarded as highly valuable and should therefore be included as a mandatory element in OIC reports. This consideration has led to the following proposed changes.

The AOSA Rules do not have seed health tests specified so no there is no impact of the proposed rule changes..

This was noticed by an auditor and an editorial change proposed by the Rules Committee Chair and Vice-chair.

RUL Committee Votes	Yes:	14	No:	0	Abstain/Absent:	0	

CURRENT VERSION	PROPOSED VERSION
7.6 Reporting results The results of a test for seed health must be reported under 'Other determinations' as follows: • either qualitative or quantitative results, as specified in the individual methods; • ~~negative and positive results~~, as specified in the individual methods; •the scientific name of the pathogen detected; •the percentage of infected seeds;	**7.6 Reporting results** The results of a test for seed health must be reported under 'Other determinations' as follows: • either qualitative or quantitative results, as specified in the individual methods; • not detected and detected with the percentage/no. of infected seeds/subsamples out of the total tested between brackets, as specified in the individual methods; •the scientific name of the pathogen detected; •the percentage of infected seeds; ....

CURRENT VERSION	PROPOSED VERSION
1.5.2.10 Seed health test The results of a test for seed health must be reported under 'Other determinations' as follows: • either qualitative or quantitative results, as specified in the individual methods; • ~~negative and positive results~~, as specified in the individual methods; •the percentage of infected seeds;	**1.5.2.10 Seed health test** The results of a test for seed health must be reported under 'Other determinations' as follows: • either qualitative or quantitative results, as specified in the individual methods; • not detected and detected with the percentage/no. of infected seeds/subsamples out of the total tested between brackets, as specified in the individual methods; ....

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Rules changes (2)

- Rules 7019a and 7-019b: Changing the to specified temperature ranges into a more flexible temperature indication.

R7-019a

CURRENT VERSION	PROPOSED VERSION
6.5	**6.6**
Incubator: operating at 28–~~30~~ °C	**Incubator:** operating at 28 ±2 °C
3.1.3 incubate for 48h at 28 °C	3.1.3 incubate for 48h at 28 ±2 °C
4.3 Incubate plates at 28–~~30~~ °C and examine after 3–4 d.	4.3 Incubate plates at 28 ±2 °C and examine after 3–4 d.
5.3 Incubate sectored plates for 25-48 h at 28–~~30 °C~~	5.3 Incubate sectored plates for 25-48 h at 28 ±2 °C
6.1 Grow seedlings of a Brassica cultivar known to be susceptible to all races of Xcc/Xcr (e.g. cabbage 'Wirosa', see Vicente et al., 2001) in small pots or modules until at least 3–~~4~~ true leaf stage.	6.1 Grow seedlings of a Brassica cultivar known to be susceptible to all races of Xcc/Xcr (e.g. 'Wirosa'; see Vicente et al., 2001) at 20–30 °C (±2 °C) in small pots or modules until at least 2–3 true leaf stage.
6.6 Grow on plants at 20–~~25 °C~~.	6.6 Grow on plants at 20–30 °C (±2 °C).

R7-019b

CURRENT VERSION	PROPOSED VERSION
1.5	**1.6**
Incubator: operating at 28–~~30 °C)~~	**Incubator:** operating at 28 ±2 °C
2.7 Incubate plates at 28–~~30~~ °C upside down and examine after 4–6 days (CCP).	2.7 Incubate plates at 28 ±2 °C upside down and examine after 4–6 days (CCP).
6.3 Incubate sectored plates for 3–4 d at 28–~~30~~ °C.	6.3 Incubate sectored plates for 3–4 d at 28 ±2 °C.
7.6 Grow on plants at 20–30 °C	7.6 Grow on plants at 20–30 °C (±2 °C)

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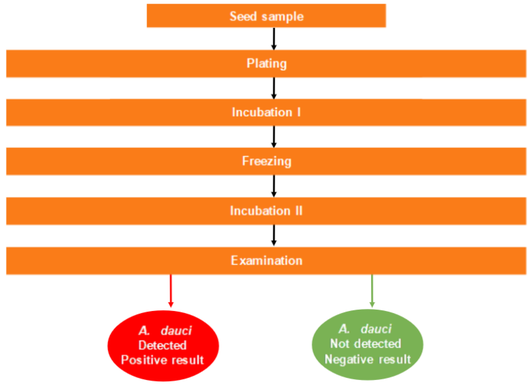
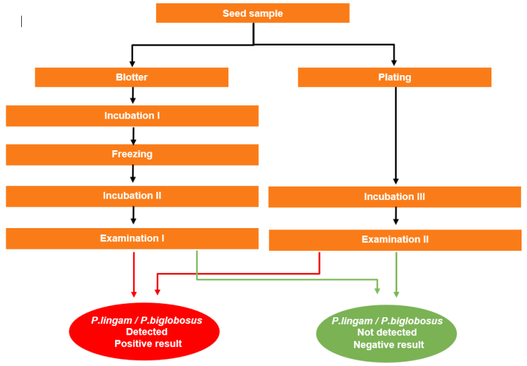
Rules changes (3)

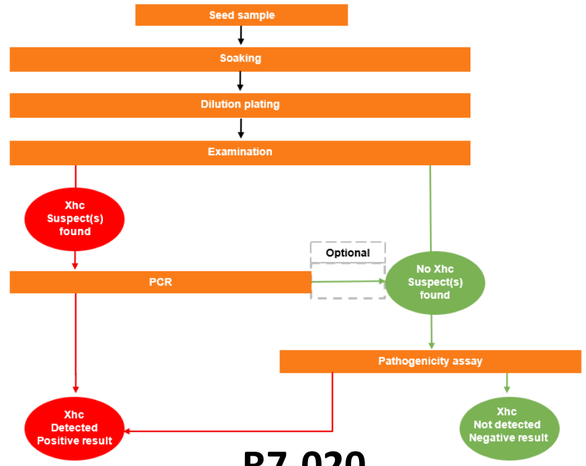
- Inclusion of test execution flow diagrams to all Chapter 7 rules.



R7-001a



R7-004



R7-020

- Rules 7-033: Change of flow diagram to the harmonized ISTA style

Current fllow chart as present in rule 7-033

Figure 1. Process flow diagram explaining method assays and decisions taken, depending on intermediate results until final result, for detection and pathogenicity of *Ascochyta rabiei* in *Cicer arietinum* (chickpea) seed.

Newly proposed flow chart for rule 7-033

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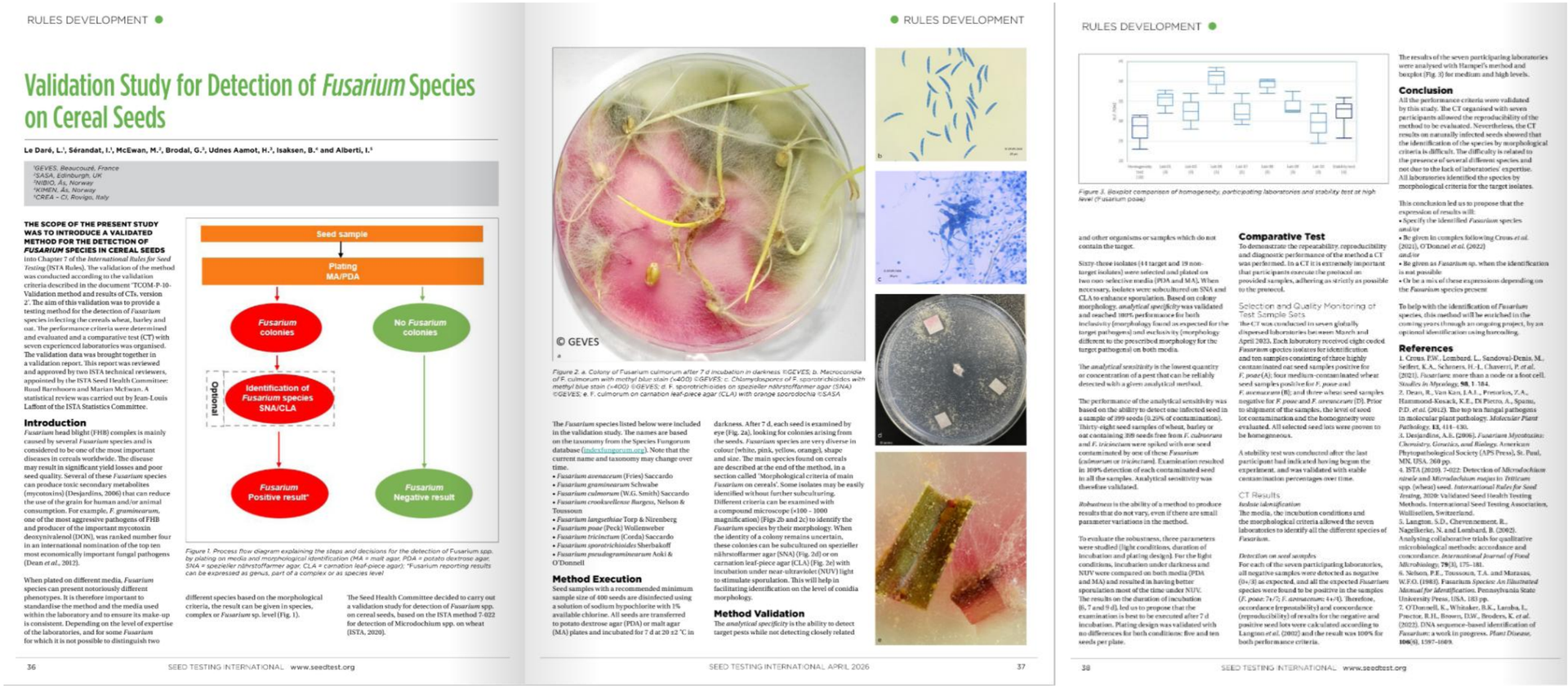
Rules changes (4)

- Publication of Rule 7-034 “Detection of fusarium species in cereal seed” to the chapter 7 rules



RULES DEVELOPMENT

Validation Study for Detection of *Fusarium* Species on Cereal Seeds

Le Daré, L.[1], Sérandat, I.[1], McEwan, M.[2], Brodal, G.[3], Udnes Aamot, H.[3], Isaksen, B.[4] and Alberti, I.[5]

[1]GEVES, Beaucouzé, France
[2]SASA, Edinburgh, UK
[3]NIBIO, Ås, Norway
[4]KUNEN, Ås, Norway
[5]CREA – CI, Rovigo, Italy

THE SCOPE OF THE PRESENT STUDY WAS TO INTRODUCE A VALIDATED METHOD FOR THE DETECTION OF *FUSARIUM* SPECIES IN CEREAL SEEDS

into Chapter 7 of the *International Rules for Seed Testing* (ISTA Rules). The validation of the method was conducted according to the validation criteria described in the document 'TCOM-P-10-Validation method and results of CTs. version 2'. The aim of this validation was to provide a testing method for the detection of *Fusarium* species infecting the cereals wheat, barley and oat. The performance criteria were determined and evaluated and a comparative test (CT) with seven experienced laboratories was organised. The validation data was brought together in a validation report. This report was reviewed and approved by two ISTA technical reviewers, appointed by the ISTA Seed Health Committee: Ruud Barnhoorn and Marian McEwan. A statistical review was carried out by Jean-Louis Laffont of the ISTA Statistics Committee.

Introduction

Fusarium head blight (FHB) complex is mainly caused by several *Fusarium* species and is considered to be one of the most important diseases in cereals worldwide. The disease may result in significant yield losses and poor seed quality. Several of these *Fusarium* species can produce toxic secondary metabolites (mycotoxins) (Desjardins, 2006) that can reduce the use of the grain for human and/or animal consumption. For example, *F. graminearum*, one of the most aggressive pathogens of FHB and producer of the important mycotoxin deoxynivalenol (DON), was ranked number four in an international nomination of the top ten most economically important fungal pathogens (Dean *et al.*, 2012).

When plated on different media, *Fusarium* species can present notoriously different phenotypes. It is therefore important to standardise the method and the media used within the laboratory and to ensure its make-up is consistent. Depending on the level of expertise of the laboratories, and for some *Fusarium* for which it is not possible to distinguish two different species based on the morphological criteria, the result can be given in species, complex or *Fusarium* sp. level (Fig. 1).

*Figure 1. Process flow diagram explaining the steps and decisions for the detection of Fusarium spp. by plating on media and morphological identification (MA = malt agar, PDA = potato dextrose agar, SNA = spezieller nährstoffarmer agar, CLA = carnation leaf-piece agar); *Fusarium reporting results can be expressed as genus, part of a complex or as species level*

The Seed Health Committee decided to carry out a validation study for detection of *Fusarium* spp. on cereal seeds, based on the ISTA method 7-022 for detection of Microdochium spp. on wheat (ISTA, 2020).

Figure 2. a. Colony of Fusarium culmorum after 7 d incubation in darkness. ©GEVES; b. Macroconidia of F. culmorum with methyl blue stain (×400) ©GEVES; c. Chlamydospores of F. sporotrichioides with methyl blue stain (×400) ©GEVES; d. F. sporotrichioides on spezieller nährstoffarmer agar (SNA) ©GEVES; e. F. culmorum on carnation leaf-piece agar (CLA) with orange sporodochia ©SASA

The *Fusarium* species listed below were included in the validation study. The names are based on the taxonomy from the Species Fungorum database (indexfungorum.org). Note that the current name and taxonomy may change over time.

- *Fusarium avenaceum* (Fries) Saccardo
- *Fusarium graminearum* Schwabe
- *Fusarium culmorum* (W.G. Smith) Saccardo
- *Fusarium crookwellense* Burgess, Nelson & Toussoun
- *Fusarium langsethiae* Torp & Nirenberg
- *Fusarium poae* (Peck) Wollenweber
- *Fusarium tricinctum* (Corda) Saccardo
- *Fusarium sporotrichioides* Sherbakoff
- *Fusarium pseudograminearum* Aoki & O'Donnell

Method Execution

Seed samples with a recommended minimum sample size of 400 seeds are disinfected using a solution of sodium hypochlorite with 1% available chlorine. All seeds are transferred to potato dextrose agar (PDA) or malt agar (MA) plates and incubated for 7 d at 20 ±2 °C in darkness. After 7 d, each seed is examined by eye (Fig. 2a), looking for colonies arising from the seeds. *Fusarium* species are very diverse in colour (white, pink, yellow, orange), shape and size. The main species found on cereals are described at the end of the method, in a section called 'Morphological criteria of main *Fusarium* on cereals'. Some isolates may be easily identified without further subculturing. Different criteria can be examined with a compound microscope (×100 – 1000 magnification) (Figs 2b and 2c) to identify the *Fusarium* species by their morphology. When the identity of a colony remains uncertain, these colonies can be subcultured on spezieller nährstoffarmer agar (SNA) (Fig. 2d) or on carnation leaf-piece agar (CLA) (Fig. 2e) with incubation under near-ultraviolet (NUV) light to stimulate sporulation. This will help in facilitating identification on the level of conidia morphology.

Method Validation

The *analytical specificity* is the ability to detect target pests while not detecting closely related

*Figure 3. Boxplot comparison of homogeneity, participating laboratories and stability test at high level (*Fusarium poae*)*

and other organisms or samples which do not contain the target.

Sixty-three isolates (44 target and 19 non-target isolates) were selected and plated on two non-selective media (PDA and MA). When necessary, isolates were subcultured on SNA and CLA to enhance sporulation. Based on colony morphology, *analytical specificity* was validated and reached 100% performance for both *inclusivity* (morphology found as expected for the target pathogens) and *exclusivity* (morphology different to the prescribed morphology for the target pathogens) on both media.

The *analytical sensitivity* is the lowest quantity or concentration of a pest that can be reliably detected with a given analytical method.

The performance of the analytical sensitivity was based on the ability to detect one infected seed in a sample of 999 seeds (0.25% of contamination). Thirty-eight seed samples of wheat, barley or oat containing 399 seeds free from *F. culmorum* and *F. tricinctum* were spiked with one seed contaminated by one of these *Fusarium* (*culmorum* or *tricinctum*). Examination resulted in 100% detection of each contaminated seed in all the samples. Analytical sensitivity was therefore validated.

Robustness is the ability of a method to produce results that do not vary, even if there are small parameter variations in the method.

To evaluate the robustness, three parameters were studied (light conditions, duration of incubation and plating design). For the light conditions, incubation under darkness and NUV were compared on both media (PDA and MA) and resulted in having better sporulation most of the time under NUV. The results on the duration of incubation (6, 7 and 8 d), led us to propose that the examination is best to be executed after 7 d incubation. Plating design was validated with no differences for both conditions: five and ten seeds per plate.

Comparative Test

To demonstrate the repeatability, reproducibility and diagnostic performance of the method a CT was performed. In a CT it is extremely important that participants execute the protocol on provided samples, adhering as strictly as possible to the protocol.

Selection and Quality Monitoring of Test Sample Sets

The CT was conducted to seven globally dispersed laboratories between March and April 2023. Each laboratory received eight coded *Fusarium* species isolates for identification and ten samples consisting of three highly contaminated oat seed samples positive for *F. poae* (A); four medium-contaminated wheat seed samples positive for *F. poae* and *F. avenaceum* (B); and three wheat seed samples negative for *F. poae* and *F. avenaceum* (D). Prior to shipment of the samples, the level of seed lot contamination and the homogeneity were evaluated. All selected seed lots were proven to be homogeneous.

A stability test was conducted after the last participant had indicated having begun the experiment, and was validated with stable contamination percentages over time.

CT Results

Isolate identification

The media, the incubation conditions and the morphological criteria allowed the seven laboratories to identify all the different species of *Fusarium*.

Detection on seed samples

For each of the seven participating laboratories, all negative samples were detected as negative (0+/3) as expected, and all the expected *Fusarium* species were found to be positive in the samples (*F. poae*: 7+/7; *F. avenaceum*: 4+/4). Therefore, accordance (reputability) and concordance (reproducibility) of results for the negative and positive seed lots were calculated according to Langton *et al.* (2002) and the result was 100% for both performance criteria.

The results of the seven participating laboratories were analysed with Hampel's method and boxplot (Fig. 3) for medium and high levels.

Conclusion

All the performance criteria were validated by this study. The CT organised with seven participants allowed the reproducibility of the method to be evaluated. Nevertheless, the CT results on naturally infected seeds showed that the identification of the species by morphological criteria is difficult. The difficulty is related to the presence of several different species and not due to the lack of laboratories' expertise. All laboratories identified the species by morphological criteria for the target isolates.

This conclusion led us to propose that the expression of results is:

- Specify the identified *Fusarium* species *and/or*
- Be given in complex following Crous *et al.* (2021), O'Donnel *et al.* (2022) *and/or*
- Be given as *Fusarium* sp. when the identification is not possible
- Or be a mix of these expressions depending on the *Fusarium* species present

To help with the identification of *Fusarium* species, this method will be enriched in the coming years through an ongoing project, by an optional identification using barcoding.

References

1. Crous, P.W., Lombard, L., Sandoval-Denis, M., Seifert, K.A., Schroers, H.-J., Chaverri, P. *et al.* (2021). *Fusarium*: more than a node or a foot cell. *Studies in Mycology*, **98**, 1-184.
2. Dean, R., Van Kan, J.A.L., Pretorius, Z.A., Hammond-Kosack, K.E., Di Pietro, A., Spanu, P.D. *et al.* (2012). The top ten fungal pathogens in molecular plant pathology. *Molecular Plant Pathology*, **13**, 414–430.
3. Desjardins, A.E. (2006). *Fusarium Mycotoxins: Chemistry, Genetics, and Biology*. American Phytopathological Society (APS Press), St. Paul, MN, USA. 260 pp.
4. ISTA (2020). 7-022: Detection of *Microdochium nivale* and *Microdochium majus* in *Triticum* spp. (wheat) seed. *International Rules for Seed Testing*, 2020: Validated Seed Health Testing Methods. International Seed Testing Association, Wallisellen, Switzerland.
5. Langton, S.D., Chevennement, R., Nagelkerke, N. and Lombard, B. (2002). Analysing collaborative trials for qualitative microbiological methods: accordance and concordance. *International Journal of Food Microbiology*, **79**(3), 175–181.
6. Nelson, P.E., Toussoun, T.A. and Marasas, W.F.O. (1983). Fusarium *Species: An Illustrated Manual for Identification*. Pennsylvania State University Press, USA. 183 pp.
7. O'Donnell, K., Whitaker, B.K., Laraba, I., Proctor, R.H., Brown, D.W., Broders, K. *et al.* (2022). DNA sequence-based identification of *Fusarium*: a work in progress. *Plant Disease*, **106**(6), 1597–1609.

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Method development and validation

Gray mold on hemp (*Botrytis cinerea*), CREA:

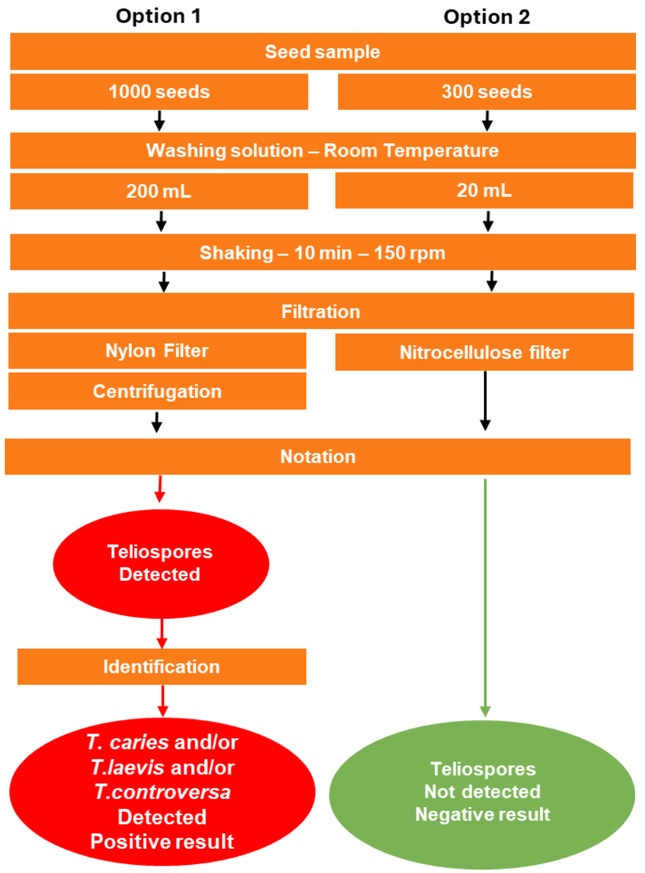
- Method: Seed blotter -> suspect analysis via morphological identification
- Comparative test executed and data analysis for healthy seed rejected.
- Comparative test repeated
- New rule suggestion presented before 1 November

Pseudomonas syringae pv. *glycinea on* soybean, NSHS:

- Method: Dilution plating, fluorescens screening, pathogenicity assay
- Analytical specificity and Analytical sensitivity executed
- CT to be finalized mid 2026

Tilletia spp on wheat seed, GEVES – AGES:

- Two way approach protocol established
- Validation plan approved



Method development and validation

ISTA Rule 7-004: *Leptosphaeria maculans* and *Plenodomus biglobosus* in Brassica spp. seed (CFIA)

- Splitting current rule 7-004 into two separate rules
  a. Being the current 7-004 rule
  b. Being a more sensitive approach using direct Seed Extract-PCR
- Discussion ongoing on value of two different methods with a different sensitivity
- Organization of a CT is limiting factor for introduction of this method, investigation of method-based approach by using data from Canadian labs under execution.

ISTA Rule 7-034: Detection of *Fusarium* species on cereal seeds (*Triticum aestivum L.; Hordeum vulgare L.; Avena sativa L.*)

- Adding a Barcode sequence approach to the method to facilitate more in-depth discrimination between *Fusarium* species found in seed of cereals.
- Validation plan approved

Detection of Seed-born Pathogen *Acidovorax citrulli* by Multipectral Imaging

Xiulan Xu

Vegetable Research Center
Beijing Academy of Agriculture and Forestry Sciences

Application of High-Throughput Sequencing in the Detection of Viruses In Seed

Yanhong Qiu

Vegetable Research Center

Beijing Academy of Agriculture and Forestry Sciences

Seed Health Accreditation 2.0

- Mar 2024 the Seed Health committee shared a “letter of concern” with ECOM
- Oct 2025 the Seed Health committee shared an accreditation 2.0 proposal
- Nov 2025 – Feb 2026 proposal shared and cleaned by SH-WG
- Feb 2026 ECOM votes positive to proposal

- Seed Health Accreditation 2.0
- Method based accreditation instead of protocol-based accreditation
- Installation of regional centers of expertise for organizing CT/PT’s
- Regional inventory of pathogens of interest
- Acceptance and referral to other well validated test methods
- Acknowledgement of PT & CT results of other organizing bodies
- ISTA SHCOM more a review than a method producing group

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ISTA Strategic Initiative Funded project: Seed Health Web-based platform

Project proposal for ISTA special funding shared with ECOM

Seed Health Test Image Database

ShareSeeds

ISTA Reference Pest List

Unknown Pathogens Image identification tool

Seed health community Forum

ISTA Seed Health Statistics Toolbox

PHASE I

PHASE II

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ISTA Seed Health Test Image Database

Seed Health Test
Image Database

Search | About | Submit an image | Knowledge base | Links | Contact

Search the database

Search

Search

Clear all

Crop

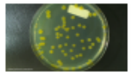
- Select Host - ×

Search by genus

- select genus - ×

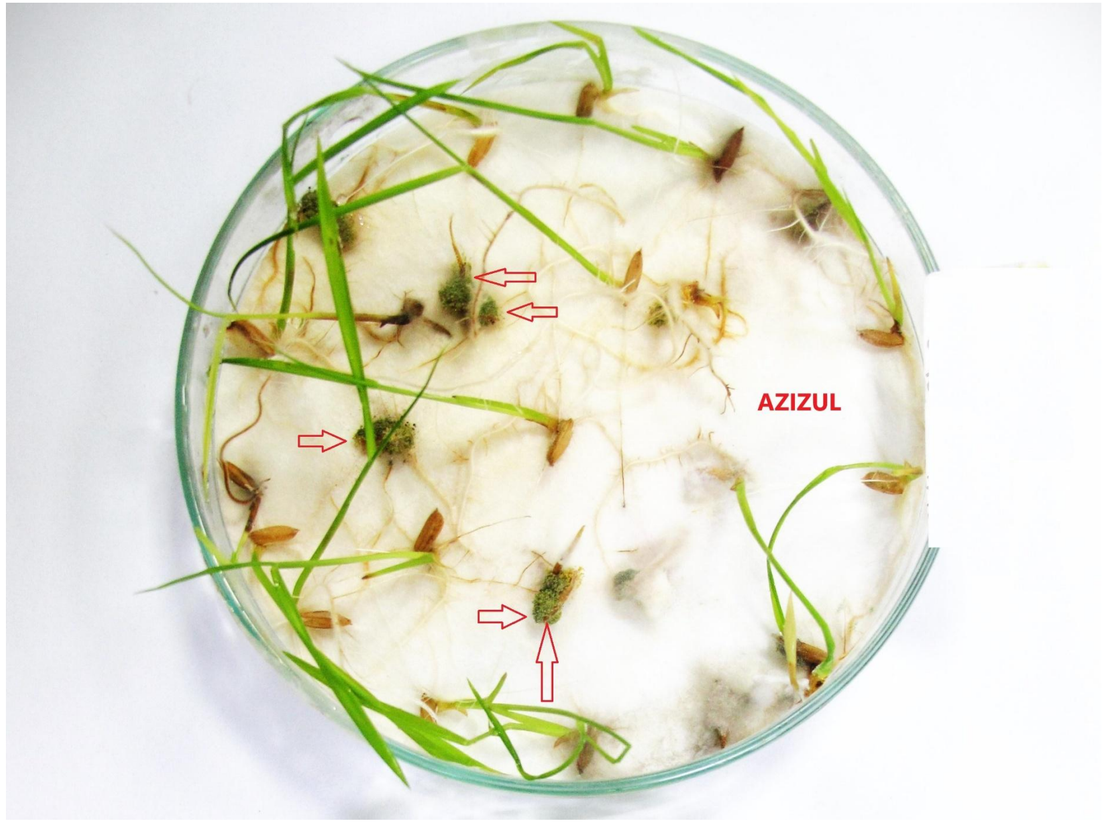
Pathogen

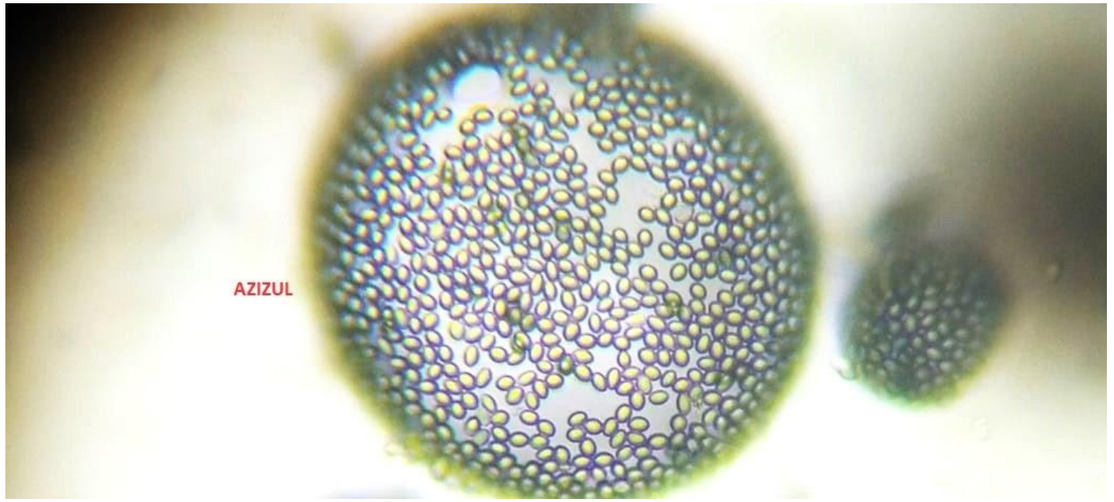
- Select Pathogen - ×

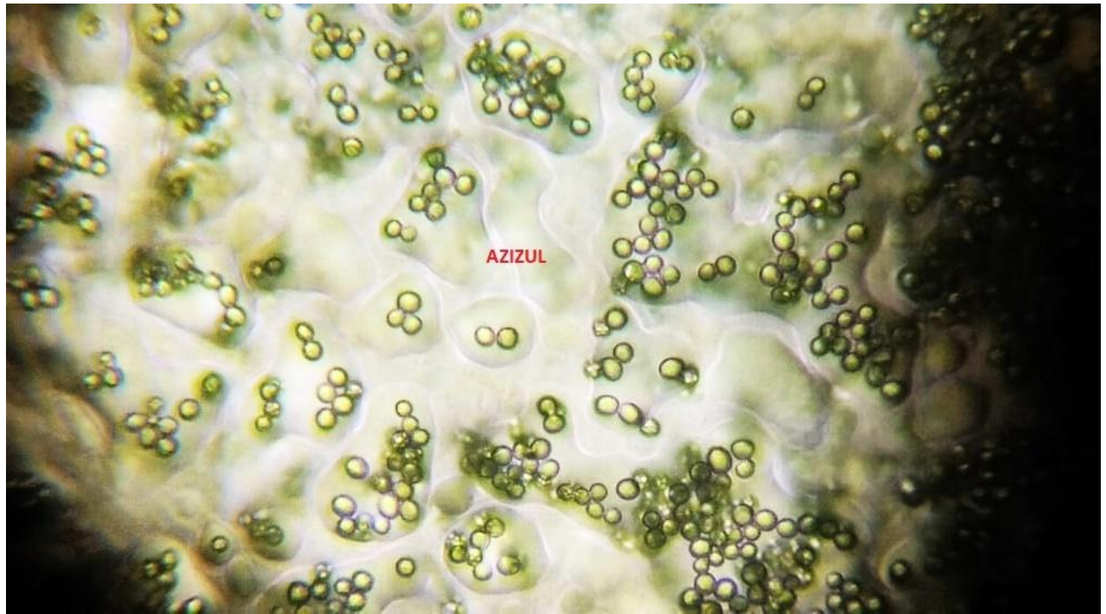
IMAGE	SPECIES	CROP	GENUS	PATHOGEN	EPPO	DISEASE NAME	AGAR
	Allium porrum	Leek	Pseudomonas	Pseudomonas syringae pv. porri	PSDMPR	Bacterial blight	KBBC
	Allium porrum	Leek	Pseudomonas	Pseudomonas syringae pv. porri	PSDMPR	Bacterial blight	MSP

Unknown Pathogens Image identification

Crop: Rise
Pathogen type: Fungi
Medium: PDA
Etc.







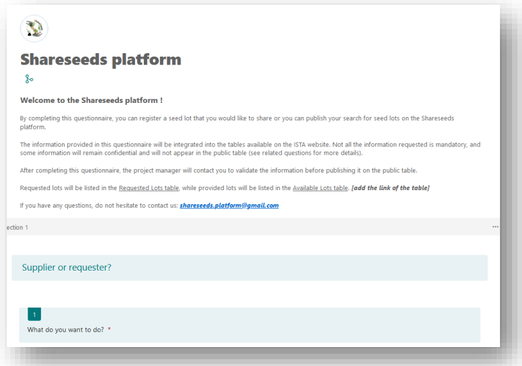
ShareSeeds: Request and Provide

Publish a searched seed lot

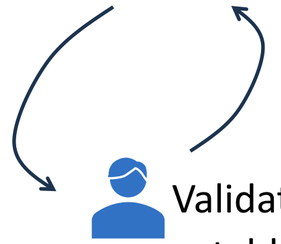
Requester
→ Want to post a request for a seed lot

Go on the ISTA website →

ShareSeeds page
Fill the questionnaire →



Information supplied will be formatted in a table (excel file) →



Validation of the table by the requester

Publication of the request on the public table (excel file)

Available on the ShareSeeds page

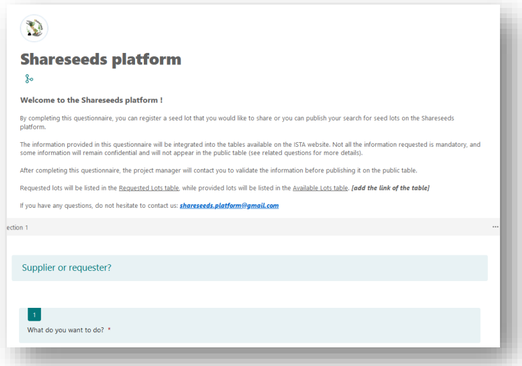
Requester information							
Request ID	Requester code or name	Importer country	Contact mail address	Plant species	Variety	Quantities (kg)	
eg : 002	*eg : GEVES*	*eg : France*	*eg : justine.foucher@*	*eg : Sunflower*	*eg : Var340*	*eg : 5 kg*	*eg :*

Publish an available seed lot

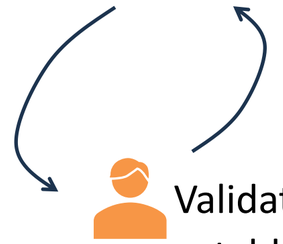
Provider
→ Want to share a seed lot

Go on the ISTA website →

ShareSeeds page
Fill the questionnaire →



Information supplied will be formatted in a table (excel file) →



Validation of the table by the provider

Publication of the seed lot on the public table (excel file)

Available on the ShareSeeds page

Requester information							
Request ID	Requester code or name	Importer country	Contact mail address	Plant species	Variety	Quantities (kg)	
eg : 002	*eg : GEVES*	*eg : France*	*eg : justine.foucher@*	*eg : Sunflower*	*eg : Var340*	*eg : 5 kg*	*eg :*

ShareSeeds: Aquire and Share

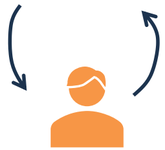
Request an available seed lot

Requester
→ Want to have access to an available seed lot

Go on the ISTA website →

ShareSeeds page
Look at the public table and contact the generic email address
Shareseeds.platform@gmail.com →

Received the mail and contact the seed lot provider to ask if they could respond to this request →



If the provider is OK :

Exchange between provider and requester MTA available

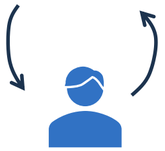
Respond to a seed lot search

Provider
→ Want to respond to a search and provide a seed lot

Go on the ISTA website →

ShareSeeds page
Look at the public table and contact the generic email address
Shareseeds.platform@gmail.com
Or directly the email address of the requester →

Received the mail and contact the requester to ask if they are interested in the lot →

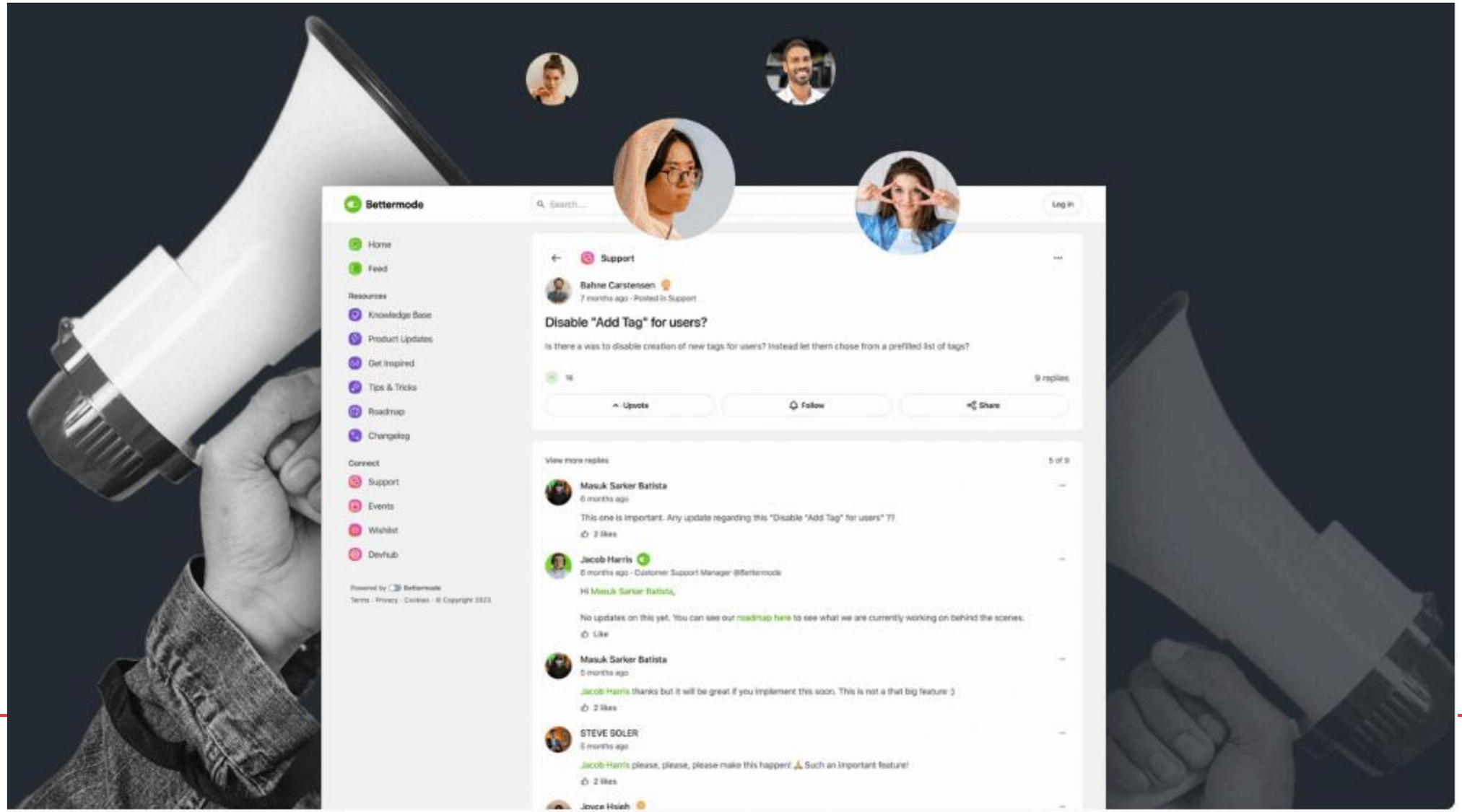


Requester

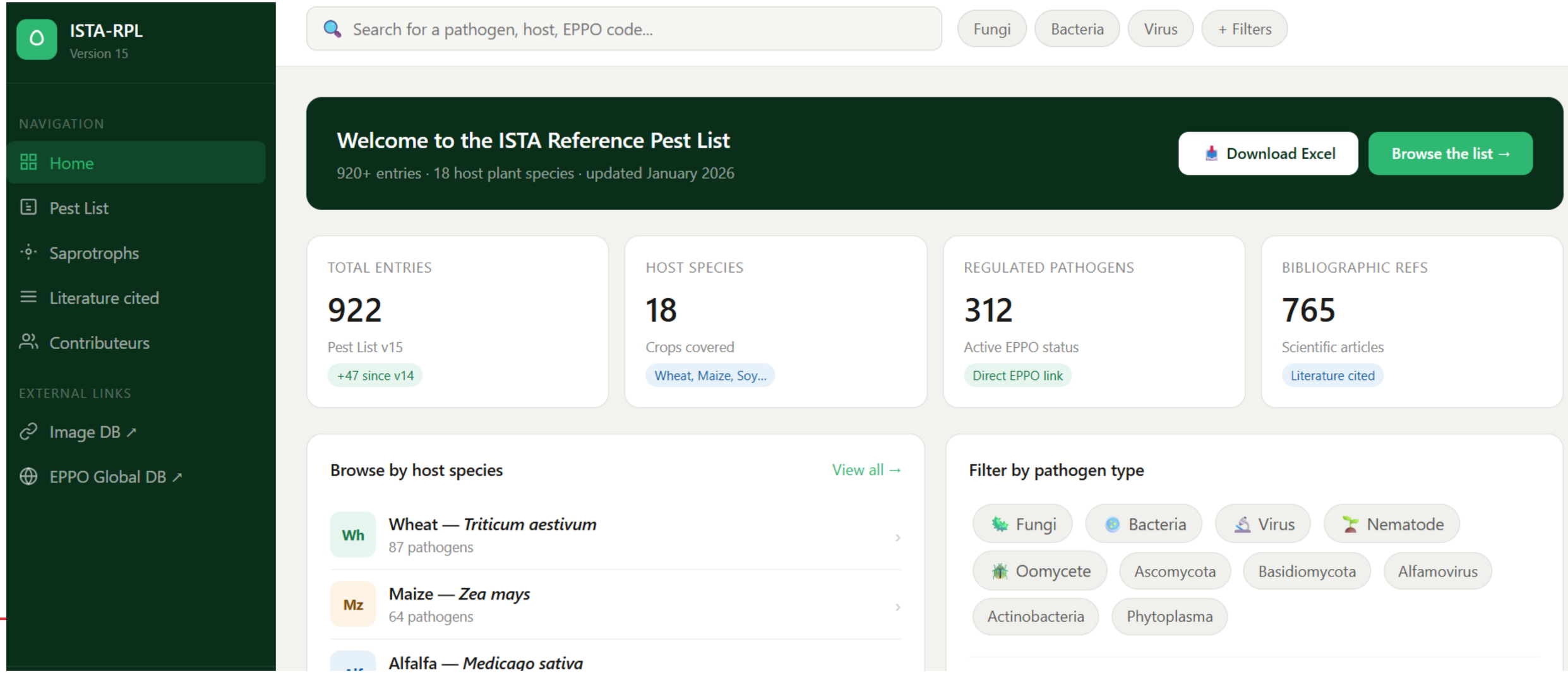
If the requester is OK :

Exchange between provider and requester MTA available

Seed Health community Forum



ISTA reference pest list



Workshops / Seminars

- 23-Feb 2026: ISTA Seed Health seminar, New Delhi, India.

“Quality Assurance of Seed Health: Classical and Modern methodologies supportive to one another”

- 24-Apr 2026: EPPO Conference, Vienna, Austria

“Towards Safer Seeds: The Changing Landscape of Seed Health Testing”

- 15-17 Jun 2026: Edminton, Canada.

"ISTA Workshop on Seed Health Testing, a deep dive into mycology method execution and validation."

- 1-2 Jul 2027: Angers France. ISTA Seed Health Method Validation Workshop

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Intercross TCOM collaborations

- T&S Com – Survey prospecting the field of important pathogens and the needof SH tests in T&S.
- ATC, PUR, StatCom – Discussion session on the impact of IA use for ISTA
- StatCom – Workshop for ISF-ISHI on Stat tool use in executing a CT

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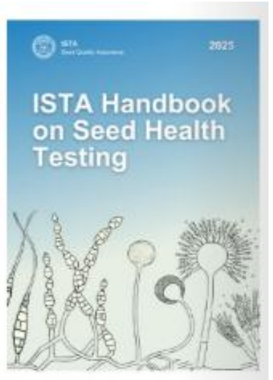
Other research and running SHCOM topics

Insect detection on seed – help in the establishment of an ISTA policy on this topic.

Near ultraviolet fluorescent light will no longer be available after 2027, this has an impact on some of the ISTA SH Fungal methods.

Is there an alternative for NUV?

Update the SHCom document: “Validation of seed health methods and organization and analysis of interlaboratory comparative tests (CT)” to vers. 3.0



Available since June 2025

ISTA ANNUAL MEETING 2026