Seed Health Committee update

2023-2024

r.barnhoorn@naktuinbouw.nl

Members

Vice chairs elected:

Applications:

- Eduardo Gálvez Sotelo (Chili)
- Jaiana Malabra (France)
- Shaista Karim (USA)
- Leticia Ruiz (Spain)
- Angela Thüringer (Austria)

	SHCom Members	Country	Active since
1	Ilaria Alberti	Italy	
2	Rouke Bakker	New Zealand	
3	Gary Munkvold	United States	
4	Dorota Szopinska	Poland	
5	Rosa Piña González	Chile	2016
6	Xiulan Xu	China	2017
7	**Vice Chair:** Stephan Brière	Canada	2018
8	**Vice Chair:** Isabelle Serandat	France	2019
9	Marian Mc Ewan	United Kingdom	2019
10	Kohei Osaki	Japan	2019
11	**Chair**: Ruud Barnhoorn	Netherland	2019
12	Dr Mahesh	India	2021
13	Luciana Ferrand	Argentina	2022
14	Dr. Nagamani Sandra	India	2023
15	Shih-Min Su	Taiwan	2023

Rules changes

Taxonomical update:

- Establish rules for taxonomical name changes.

Example

Current Rule	Rule change directly	Rule change in 3 years	Rule change in 6 years
Xanthomonas axonopodis pv. *phaseoli*	*Xanthomonas axonopodis* pv. *phaseoli* (*Xanthomonas phaseoli* pv. *phaseoli*)	*Xanthomonas phaseoli* pv. *phaseoli* (*Xanthomonas axonopodis* pv. *phaseoli*)	*Xanthomonas phaseoli* pv. *phaseoli*

- 2 rules updated with new taxonomical names.

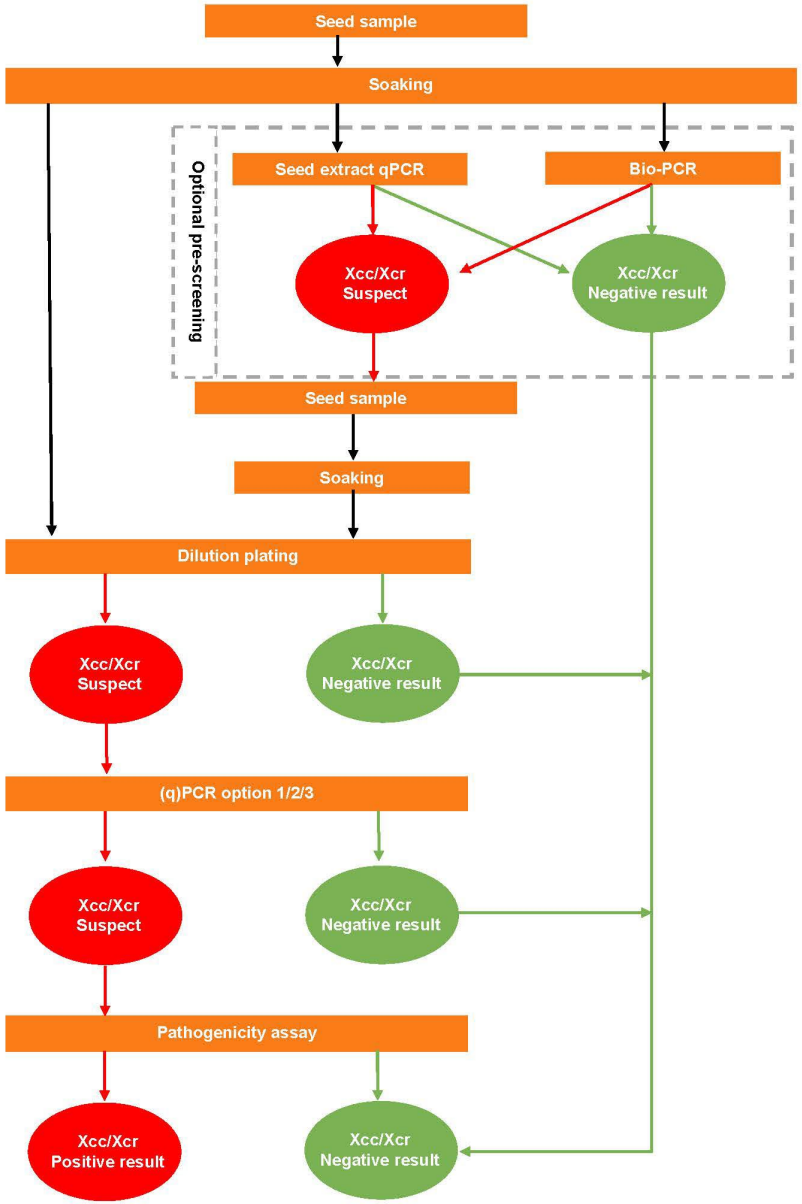
Rules changes

Technical rules changes (via external input)

- ISTA SHCOM own workflow lay-out
  - Introducing in all methods this year

- Adaptation of Rule 7-020 (*Xanthomonas hortorum* pv. *carotea)* due to new scientific knowledge
  - New strain types identified that make the confirmation PCR not specific enough anymore. Rule change submitted

- New: from the Audit Com. Replacing of reference to specific kits
  - The test kit prescribed in Rule 7-07-015 (*Epichloe coenophiala*) not available anymore.
  - All rules will be checked and changed in respect to reference to specific kits.



Method development and validation

Projects under progress:

- ***Fusarium*** **(11 species)/cereals, NIBIO and Kimen Seed Lab:**
  - Method: media grow-out -> suspect analysis via morphological identification
  - Comparative test executed and data analysis currently in progress.
  - New rule suggestion presented before 1 November

- ***Fusarium oxysporum*** **f.sp.** ***lycopersici*** **in tomato, Naktuinbouw:**
  - Method: media grow-out -> suspect qPCR -> pathogenicity assay
  - Comparative test executed and data analysis currently in progress.
  - New rule suggestion presented before 1 November

- **Gray mold on hemp (*Botrytis cinerea*), CREA:**
  - Method: Seed blotter -> suspect analysis via morphological identification
  - Comparative test executed and data analysis currently in progress.
  - New rule suggestion presented before 1 November

Method development and validation

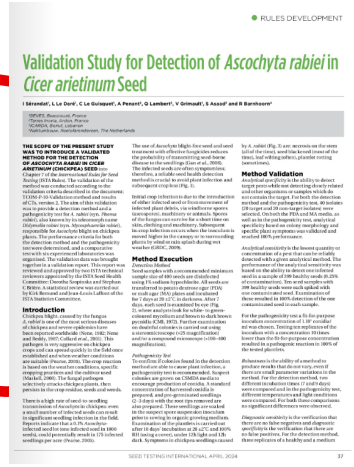
Projects under progress:

- ***Pseudomonas syringae* pv. *glycinea*/soybean, NHSS**
  - Method: Dilution plating, fluorescens screening, pathogenicity assay

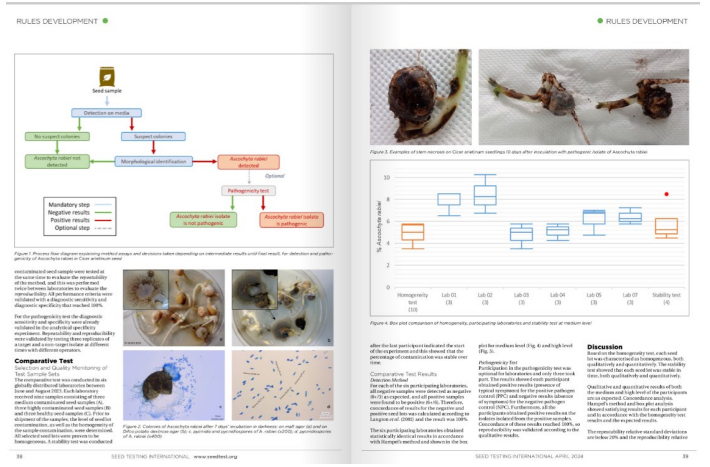
- **ISTA Rule 7-004** *Leptosphaeria maculans* and *Plenodomus biglobosus* in Brassica spp. Seed **updated**
  - Introduction of direct molecular testing via seed extract PCR to the protocol
  - Validation criteria all met.
  - Organisation of CT currently in progress

FINALISED:

Rule 7-033 *Ascochyta rabiei* on chick pea seed


RULES DEVELOPMENT

Validation Study for Detection of *Ascochyta rabiei* in *Cicer arietinum* Seed



Detection method of *Ascochyta rabiei (Phoma rabiei)* on Chickpea seeds

Isabelle.serandat@geves.fr

French project
Ascochyta rabiei on Chickpea seeds

Geves was part of the French research project **AsCoLuP,** led by the Technical Institute Terres Inovia

- 2 pathogens studied: *Ascochyta rabiei* on Chickpea and *Colletotrichum lupini* on Lupin
- Aim: to provide tools to producers for the management of these diseases, both in seed production and consumption.
- Chickpea blight is one of the most serious diseases of chickpea crop. This pathogen is selectively attacking chickpea, then persists in the crop's residues, seeds, and weeds.

Detection method of *Ascochyta rabiei* on Chickpea seeds

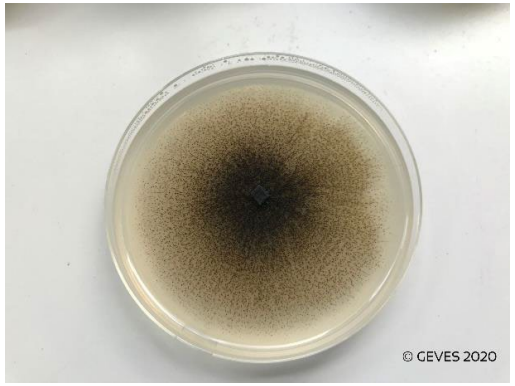
- Geves tasks in AsCoLuP project:

  - ✓ Characterization of the genetic and phenotypic diversity of both pathogens

  - ✓ **Validation of a detection method for *Ascochyta rabiei* on Chickpea**
    → **Propose a new ISTA method on a new crop**

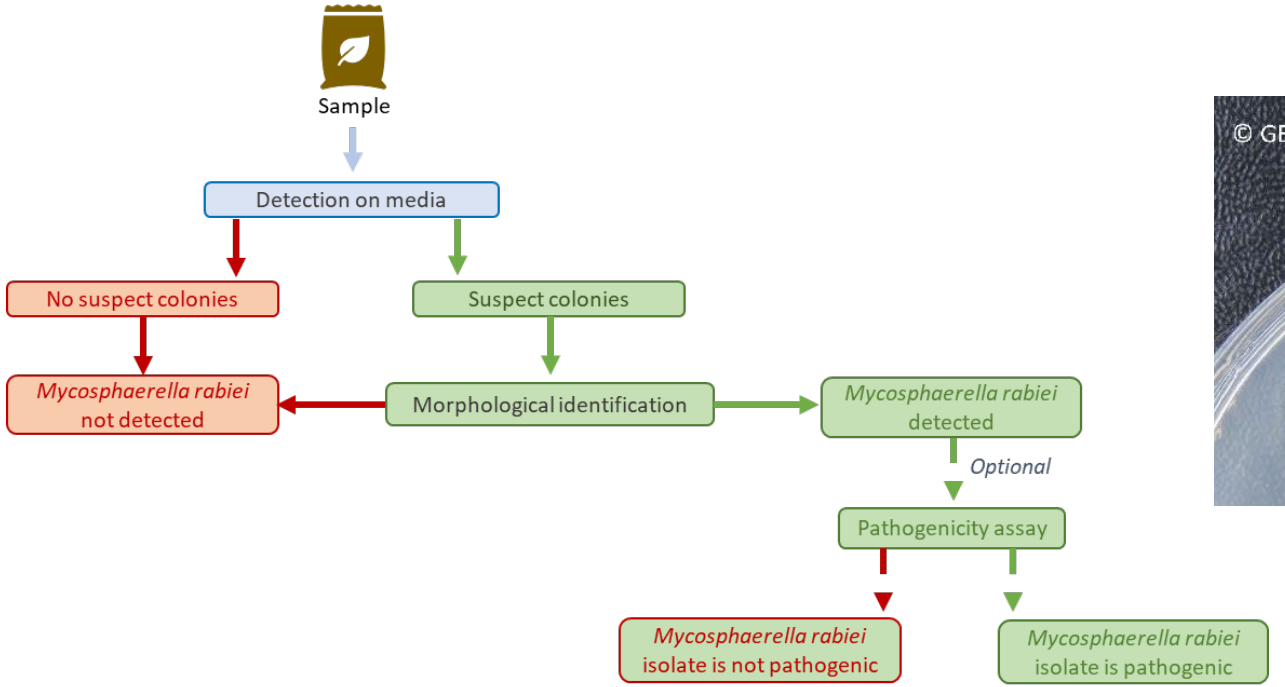
  - ✓ To develop a resistance test in controlled conditions as support of breeding

  - ✓ Alternative seed treatments (Lupin)



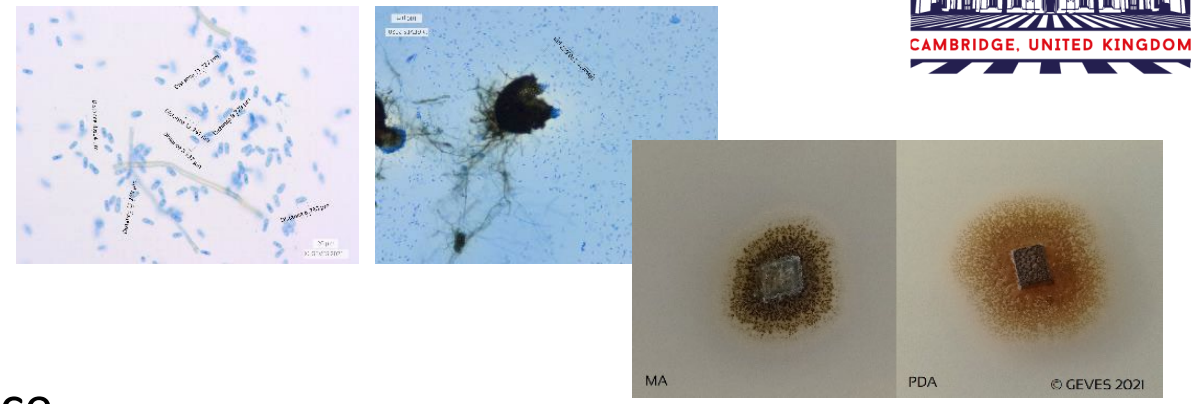
Detection method of *Ascochyta rabiei* on Chickpea seeds

- Detection method:
  - ✓ Based on the test plan prepared by Siham Assad
  - ✓ Quantitative method
  - ✓ Agar plating and morphological identification following by a pathogenicity test (optional)

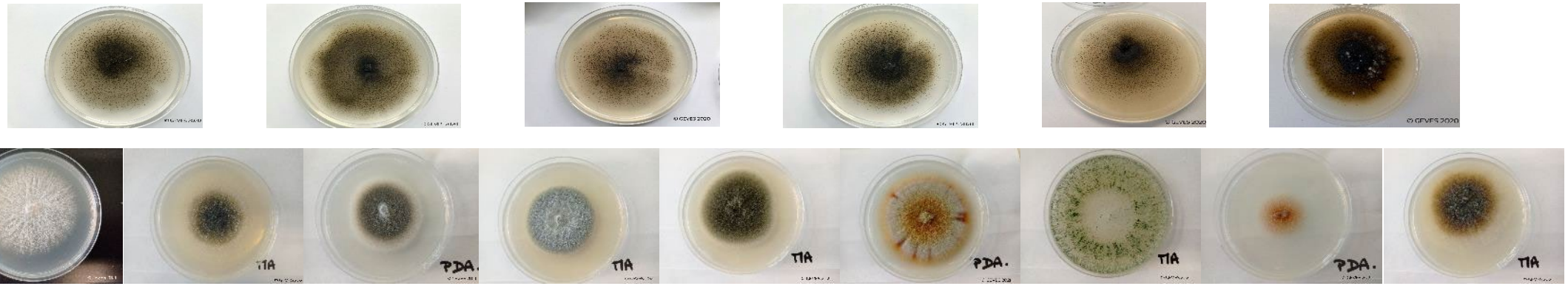


Validation of the detection method

- Test plan accepted, according to ISTA guidelines for method validation
- Seed material obtained
  - ✓ Infected seeds with different levels of infection
  - ✓ Healthy seeds



- Collection constituted and characterized
  - ✓ Targets: 20 isolates from different areas in France
  - ✓ Non targets: 20 other pathogens and saprophytes that could be present on Chickpea seeds
  - ✓ Characterisation: spore size and growth criteria on 2 media (MA and PDA)



Performance criteria validated

- **Analytical specificity**:
  - ✓ Morphological criteria described for the target
  - ✓ Collection compared to the criteria
    - ✓ Targets => meet the criteria
    - ✓ Non targets => do not meet the criteria
  - ✓ **Result of Analytical specificity**:

Target and non target strains	Expected result + (Target)	Expected result - (Non-target)	Specificity
Obtained result +	20	0	100%
Obtained result -	0	20	

→ Analytical specificity validated

Performance criteria validated

- **Analytical sensitivity** :
  - ✓ Validated if 1 infected seed is detected in 400 seeds (10 replicates)
  - ✓ Done by spiking: 1 contaminated seed with 399 healthy seeds (0.25%)
  - ✓ To ensure a 100% contamination of the lot used for the spiking an artificial contamination has been tested and validated.
  - ✓ **Result of Analytical sensitivity** :

Replicate	% Ascochyta rabiei
1	0.25
2	0.25
3	0.25
4	0.25
5	0.25
6	0.25
7	0.25
8	0.25
9	0.25
10	0.25

→ Analytical sensitivity validated

Performance criteria validated

- **Diagnostic sensitivity/specificity (Accuracy)**:
  - ✓ 1 healthy sample
  - ✓ 1 low infected sample (0.25% infection)
  - ✓ 1 medium infected sample (≈ 5% infection)

3 replicates of each level of infection tested at the same time to evaluate the **repeatability**
And performed two times to evaluate the **reproducibility** intra laboratory.

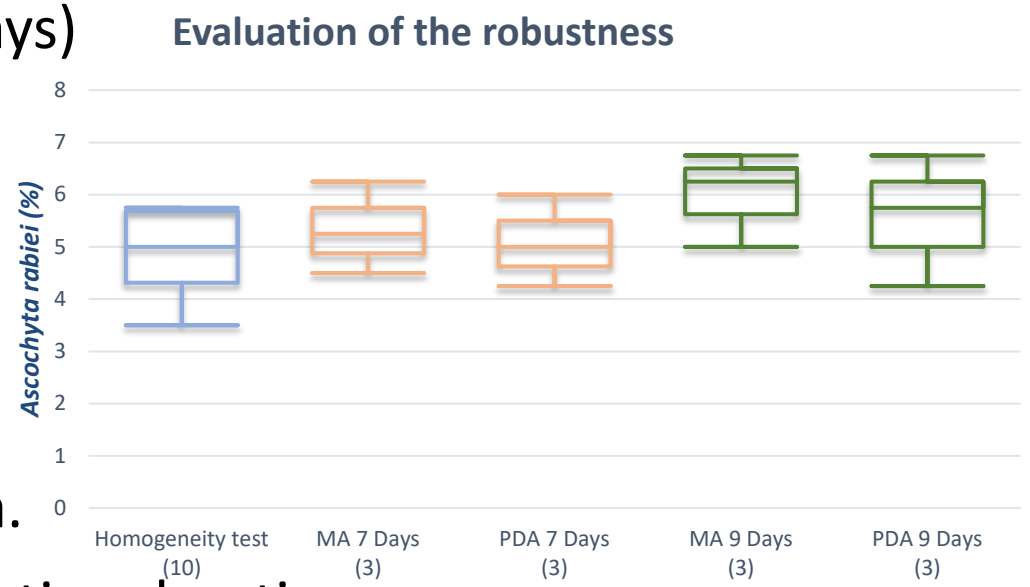
- ✓ **Result of Accuracy**:

	Expected result +	Expected result -	Diagnostic sensitivity	Diagnostic specificity
Obtained result +	6	0	100.00%	100.00%
Obtained result -	0	6		

→ Accuracy validated

Performance criteria validated

- **Robustness**:
  - ✓ A medium level infection lot (3.2%) tested
    - ✓ two different media (MA and PDA)
    - ✓ two different incubation durations (7 and 9 days)



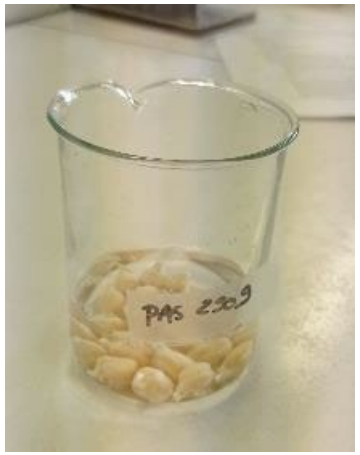
- ✓ **Result of robustness**
  - ✓ No significant differences between the two media.
  - ✓ No significant differences between the two incubation durations

→ **Robustness validated**

Pathogenicity pretests

- **Comparison of 3 pathogenicity test methods:**
  1. **Inoculation by soaking germinated seeds in a conidial suspension (1.10^5 conidia/mL)**
  2. Inoculation by deposit of conidial suspension on germinated seeds on media
  3. Inoculation by deposit of conidial suspension on germinated seeds in potting soil

- **Inoculation by soaking germinated seeds chosen**



Performance criteria validated

- **Analytical specificity (*Sensitivity/specificity diagnostic*)**:
  - ✓ Performed by testing 20 target and 20 non-target isolates from the collection (1 plant per strain)
  - ✓ Comparison of symptoms: presence of necrosis at the base of the stem, wilting of the leaves.

- ✓ **Result of Analytical specificity** :
  - ✓ All targets show expected symptoms
  - ✓ All non-targets show different symptoms.

➡ **Analytical specificity validated**

Target and non-target strains	Expected result + (Target)	Expected result - (Non-target)	Specificity
Obtained result +	20	0	100%
Obtained result -	0	20	

Negative control

Target
M. rabiei

Non target

Performance criteria validated

- **Analytical sensitivity** :
  - ✓ 2 concentrations were tested to choose a fit for purpose concentration so that a maximum of seedlings show symptoms: 1.10^5 and 1.10^4conidia/mL
  - ✓ For each concentration 10 seedlings were tested compared to 10 seedlings of the negative control

  - ✓ **Result of analytical sensitivity** : Symptoms are more severe and typical on the highest concentration (1.10^5)

Concentration	10^5	10^4	NC
Rep 1	+	+	-
Rep 2	+	+	-
Rep 3	+	+	-
Rep 4	+	+	-
Rep 5	+	+	-
Rep 6	+	+	-
Rep 7	+	+	-
Rep 8	+	+	-
Rep 9	+	+	-
Rep 10	+	+	-

→ **Analytical sensitivity validated: 1.10^5 concentration chosen**

Performance criteria validated

- ✓ **Repeatability/Reproducibility:**
  - ✓ Performed with one target (*Ascochyta rabiei*) and one non-target (*Botrytis cinerea*)
  - ✓ 3 replicates have been tested at the same time and performed two times intra laboratory
- ✓ **Result of Repeatability/Reproducibility:**
  - ✓ All seedlings inoculated with the target strain showed symptoms
  - ✓ All seedlings inoculated with the non-target strain did not show typical symptoms

Strain	Replicate 1	Replicate 2
Ascochyta rabiei	3+/3	3+/3
Botrytis cinerea	0+/3	0+/3
Negative control	0+/3	0+/3

Negative control

Target
Ascochyta rabiei

Non-Target
Botrytis cinerea

Repeatability/Reproducibility validated

Performance criteria validated

- **Robustness**:
  - ✓ Different parameters have been evaluated :
    - ✓ Temperatures: 20°C - 25°C
    - ✓ Light conditions: 8h light/16h darkness – 12h light/12h darkness
  - ✓ Each condition was tested on 5 seeds
  - ✓ A negative control for each condition (3 seeds)
- ✓ **Result of Robustness:**
  - ✓ No significant differences between the different conditions
  - ✓ All negative controls conform

Temperature / Light	20°C	25°C
8h light/ 16h light	5+/5	5+/5
12h light / 12h light	5+/5	5+/5

→ **Robustness validated**

Performance criteria validated

- **Repeatability/Reproducibility interlaboratory**:
  - ✓ Validated through a comparative test, 6 Laboratories participating
    - ✓ 9 coded samples (400 seeds)
    - ✓ 3 levels of naturally contamination (medium, high, healthy)
    - ✓ Checked by homogeneity and stability test

➡ **All performance criteria validated**

- **New ISTA method to be voted on for publication in january 2025**

Thank you for your attention

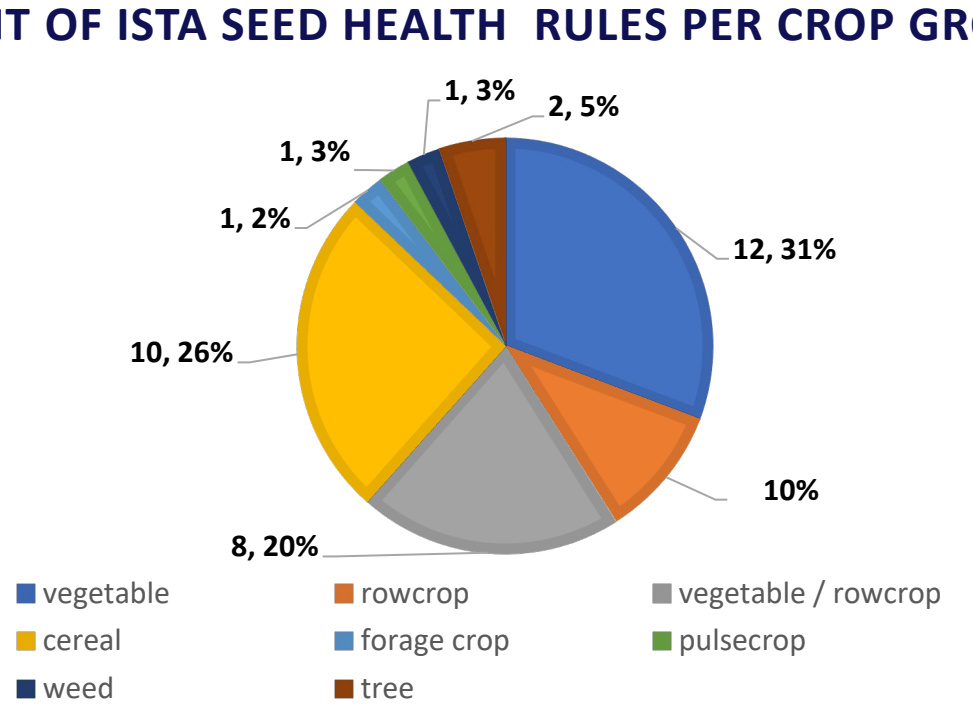
GEVES
Expertise & Performance
Groupe d'Étude et de contrôle des Variétés Et des Semences

Thank you to the myco team especially Lorine Le Dare

ISTA 100 YEARS
A CENTURY OF PROGRESS IN SEED QUALITY ASSURANCE

Isabelle.serandat@geves.fr

Gap analysis SH rules / crop group



Actions taken:

- Contacted the Chairs and Vice Chairs of the flower seed testing committee and the forest tree and shrub committee:
  1. To address if there is need for SH Rules for their crops
  2. To identify Seed Health specialists for the crops that fall under their umbrella

- Supported Nicolas Denancé (Pestlist) with finances to promote ISTA SHCOM at the IUFRO Congress

Webinars / Workshop

Webinars

- 28 May 2024 **ISSS-ISTA Webinar**

- 11 July 2024 **APSA Seed Technology Webinar Session 2**

Seed Health Testing

- To be announced 2024/2025 **The use of statistics in validating seed health test methods**
  - Collaborative action between SHCom and StatCom
  - Multiple webinars on different topics

Workshops

- Nov 2024 in collaboration with ATC, Angers, France, **Insect detection in seeds**
- Sep 2024, Bangalore, India, **Validation, Quality assurance and Accreditation of Seed Health** methods.
- 2025/ 2026 Canada, ***Leptosphaeria maculans* and *Plenodomus biglobosus* in *Brassica* spp. seed**

Special projects

ISTA Reference pest list

- Version 12 launched 24 May 2024, contains 34 crops species – 411 pests reviewed
- Last year :

  Dec 2023: vers. 10 – Chickpea

  Feb 2024: vers. 11 – Lupin, Lentil and Potato (true seed)

  May 2024: vers 12 – Cedar, Chestnut, False cypress, Poplar, Oak, Red-cedar and Walnut

Thank you **Caroline Bellenot** and reviewers for all your help

Project 23-1 Seed Health image collection

- Project is led by Nicole Calliou (Canada)
- Web designer Terry Harker
- ISTA assistance Sejal

ISTA Congress 2024 – Cambridge, UK

Seed Health Testing – Image Collection Project

Nicole Calliou | July 2024

Guest speaker

Nicole Calliou
Disease Diagnostics Lead
SGS
Nicole.calliou@sgs.com

Graduated from University of Alberta with Immunology and Infection Bachelor of Science degree, with Honors in 2010. 14 years of seed health testing, and molecular biology diagnostics, for SGS BioVision. Member of Canadian Phytopathology Society and Plant Pathology Society of Alberta. Accredited by Canadian Food Inspection Agency for *Ustilago nuda* detection in Barley.

ISTA lead for special project – Seed Health Image Database construction (expected to be live mid-2024).

Lead on green lab initiatives at Sherwood park, achieved LEAF Gold certification in 2023.

The Team

Terry Harker

Website Designer and Developer, Co-Founder of byteKultur

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Sejal Patel

IT Business Solutions Manager at ISTA

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Ruud Barnhorn

ISTA Seed Health Committee Chair

r.barnhoorn@naktuinbouw.nl

Nicolas Denancé

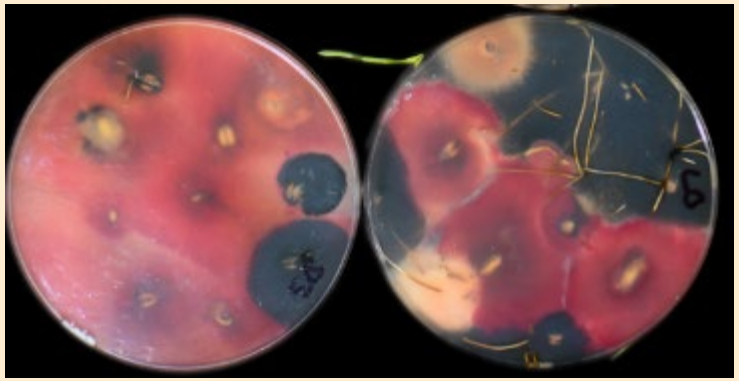
GEVES Seed Health Deputy Manager and Nematology Activity Manager

nicolas.denance@geves.fr

Contents – Initial Proposal (2022)

- Purpose
- Goal - ISTA Reference Pest List
- Factors to consider
  - Agar
  - Chamber Lighting
  - Chamber Temperature
  - Growth Time
  - Spore Images

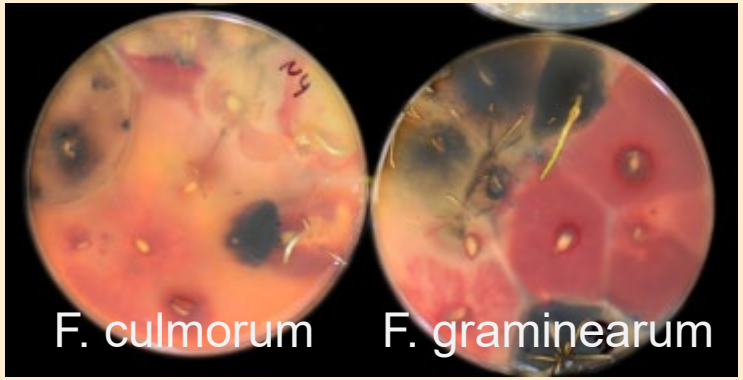
Why should we share ‘extraneous’ data on images?



PDA - Difco

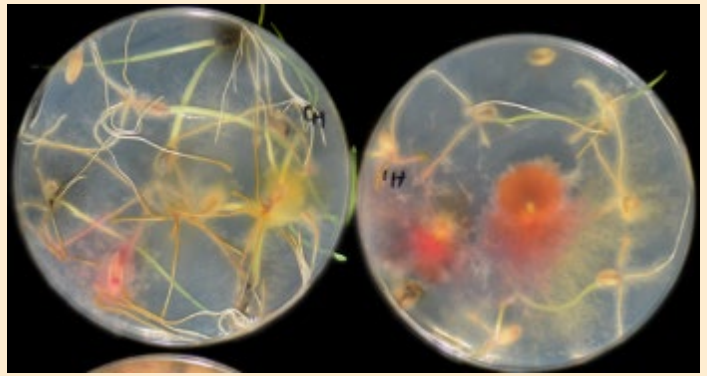
- Good fungal growth. Strong pigment production. Good spore production.

Agar 15 g/L
Dextrose 20 g/L
Potato Extract/Starch 4 g/L



PDA - Neogen

- Good fungal growth. Range of colours produced by Fusarium species - Fusarium culmorum obviously ‘orange’. Good spore production.

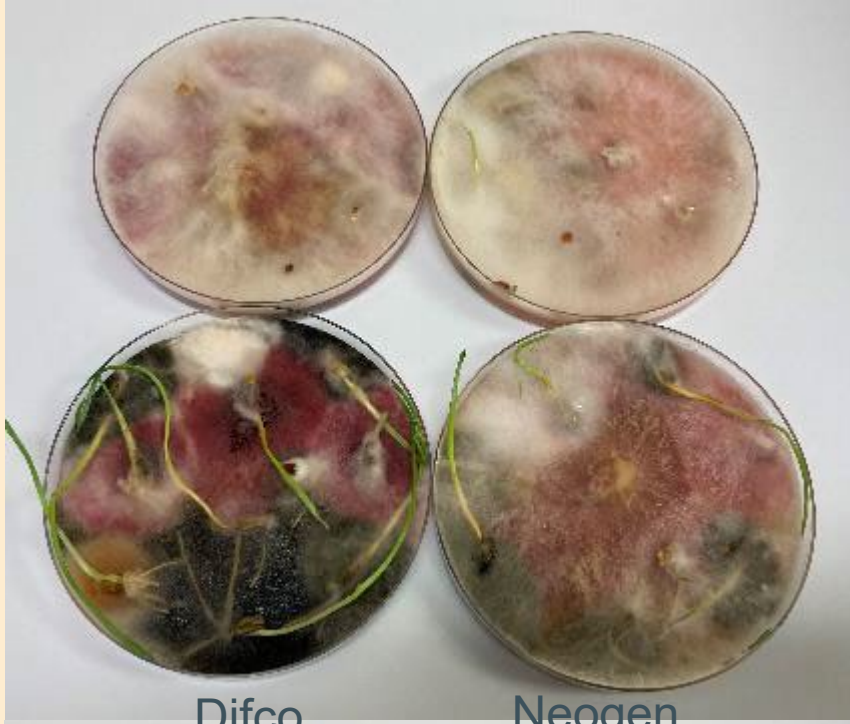


PDA - Homemade

- Weak growth of fungal colonies. Poor colour production. Good spore production.

Relevant Information - Discuss

- Images need to include:
  - Pathogen name, and host
  - 2 pictures, top and bottom
  - Agar used (& Manufacturer)
  - Growth conditions (temp/lighting)
  - Growth time (# of days)
- Images should include:
  - Spore image (with scale bar)
- Additional information:
  - Genetic information (primers/probe sequence, PCR conditions)
  - Verbal description



F. culmorum

F. graminearum

Process

Our Inputs

Collect quality images, with extraneous data

Grow Database

Integrate these images into the ISTA Reference Pest List

Our Outputs

Analysts can compare what they see, to what others see, and find what works best for them

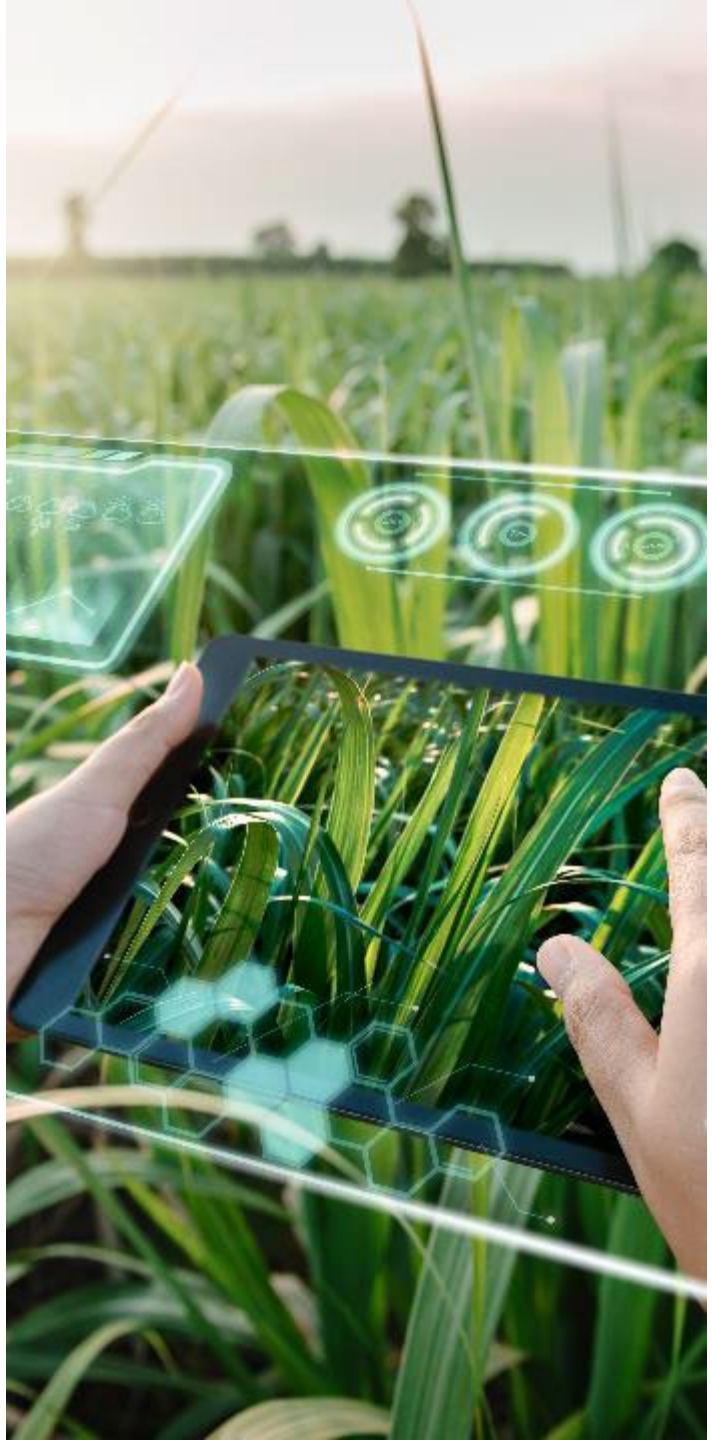
Our Value

Global harmonization of testing, minimizing differences across labs

Contents – Seed Health database

- Why an image database?
- Introduction to the website:
  - Search features
  - Sample Image Submission
  - Nematode Image Submission
  - Plant/Seedling Health Image Submission
  - ‘Extraneous’ info collected
- Milestones to meet
  - ISTA Chapter 7
  - ISTA Reference Pest List

Why an Image Database?



- **Training challenges**

- Artificially inoculated seed
- Seed storage
- Poor published images
- Reference isolates
- Multi infections
- Climate change
- International trade
- Disease pressure – monoculture, irrigation, rotation, new crops

Website Introduction

- www.seedhealthtestimagedb.info

- Home: Search
- Submit
  - Sample Images
  - Isolate or Nematode Images
  - Seedling/Plant Images

You should find one of these in your welcome bag

THE PLATFORM

A searchable, online image database, with detailed information on pathogen growth influences.

Researchers can submit images to share their knowledge and data with the world. Our team of experts will review them for accuracy before publishing on the website.

WHY CHOOSE US?

This website efficiently bridges the knowledge gap between experts and young scientists. It's accessible to all, accepting image and data submissions from any researcher for free.

www.seedtest.org

www.seedhealthtestimagedb.info

contact@seedhealthtestimagedb.info

STORY

It began with PDA (Potato Dextrose Agar). A manufacturer altered their formulation to cut costs, resulting in significant changes in culture color and morphology despite ostensibly keeping the ingredients and concentrations the same. This prompted an investigation into whether other labs were aware of the potential impacts of manufacturing changes.

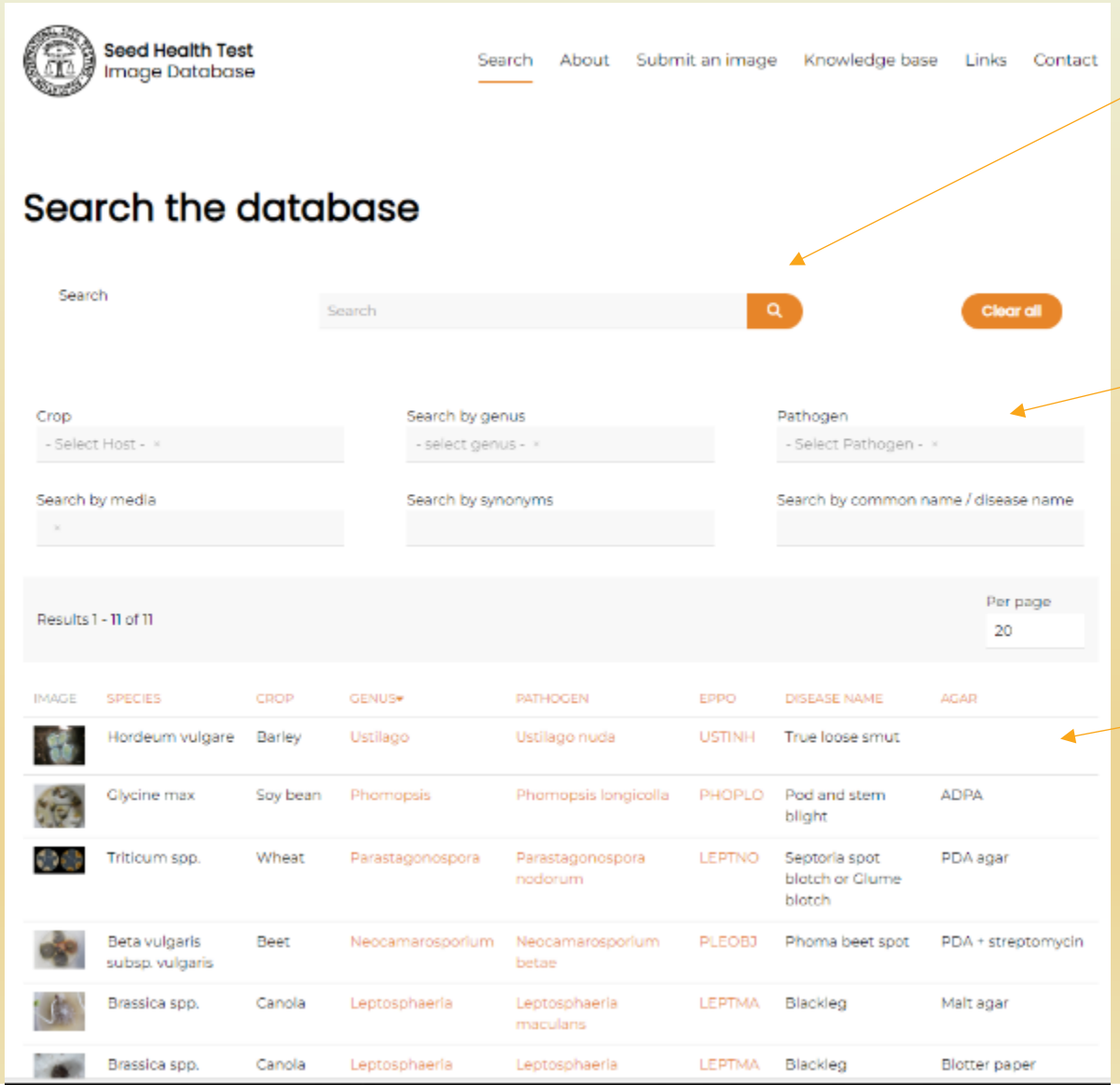
VISION

ISTA promotes information sharing, disseminates knowledge, and trains young seed pathologists. However, the current reference images lack crucial details. Prioritising the education of future plant pathologists is essential amid concerns about food security and seed health.

MISSION

We prioritise seed quality through standardised testing in any accredited lab to ensure consistent results. Comprehensive training encompassing all factors influencing pathogen growth in the lab is essential. Our experts will review the images you submit, fostering shared knowledge and mutual benefits.

Home: Search

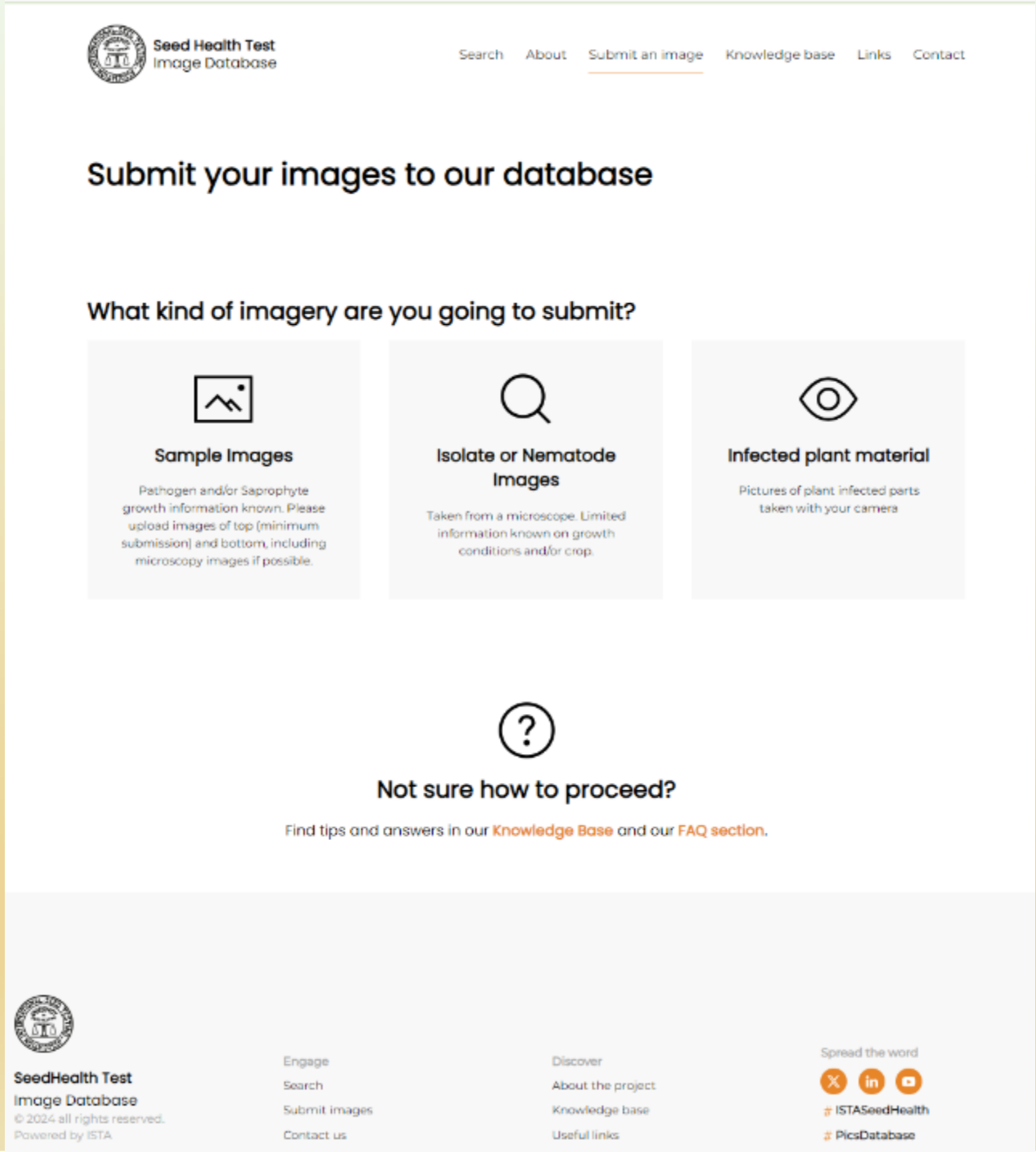


User Type 1: Knows (or thinks they know) exactly what they have and just wants to spend an extremely short amount of time to find comparable images

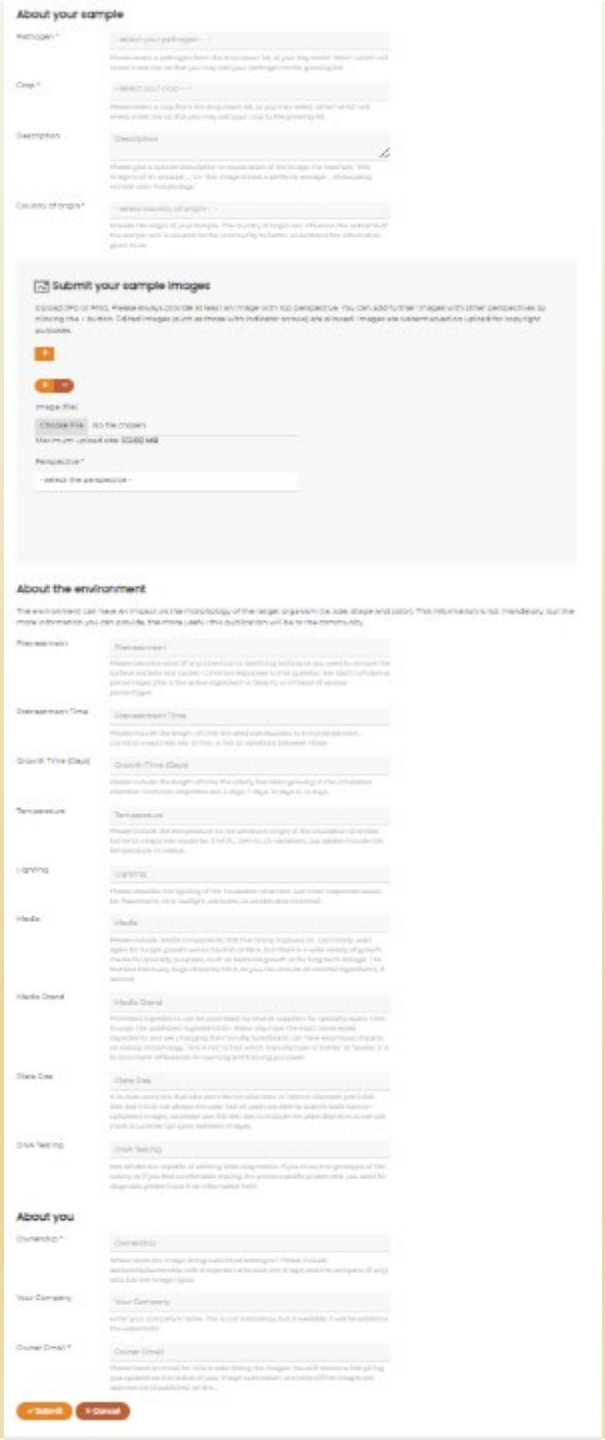
User Type 2: Has limited knowledge of what they have (may only know Crop, or Genus, etc.). Able to layer drop downs to reduce image results

User Type 3: Has no idea what is going on, wants to see every image ever uploaded. Doesn't want to miss a thing

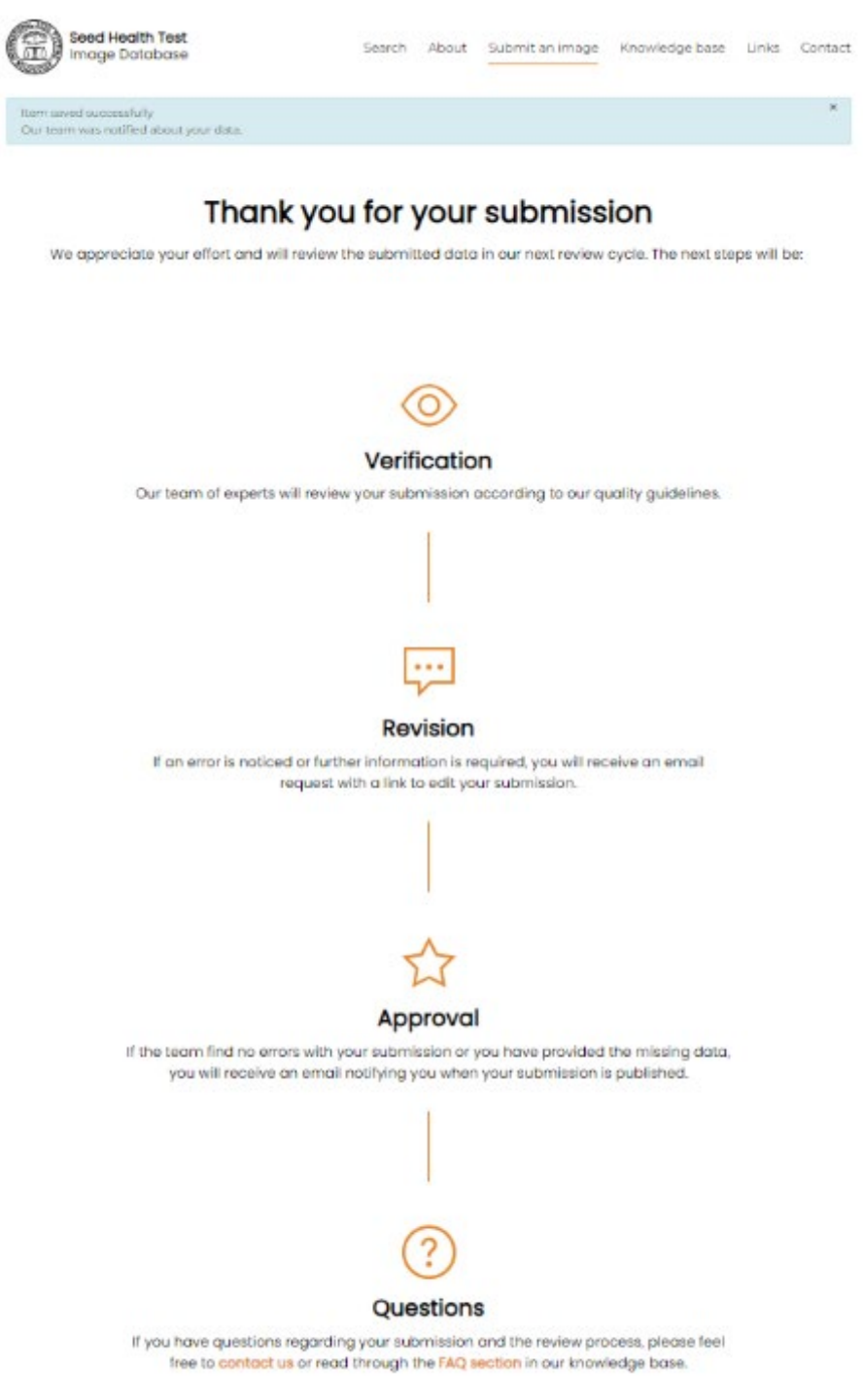
Submission


Seed Health Test
Image Database
Search
About
Submit an image
Knowledge base
Links
Contact
Submit your images to our database
What kind of imagery are you going to submit?
Sample Images
Pathogen and/or Saprophyte growth information known. Please upload images of top (minimum submission) and bottom, including microscopy images if possible.
Isolate or Nematode Images
Taken from a microscope. Limited information known on growth conditions and/or crop.
Infected plant material
Pictures of plant infected parts taken with your camera
Not sure how to proceed?
Find tips and answers in our Knowledge Base and our FAQ section.
SeedHealth Test
Image Database
© 2024 all rights reserved.
Powered by ISTA
Engage
Search
Submit images
Contact us
Discover
About the project
Knowledge base
Useful links
Spread the word
ISTASeedHealth
PicsDatabase

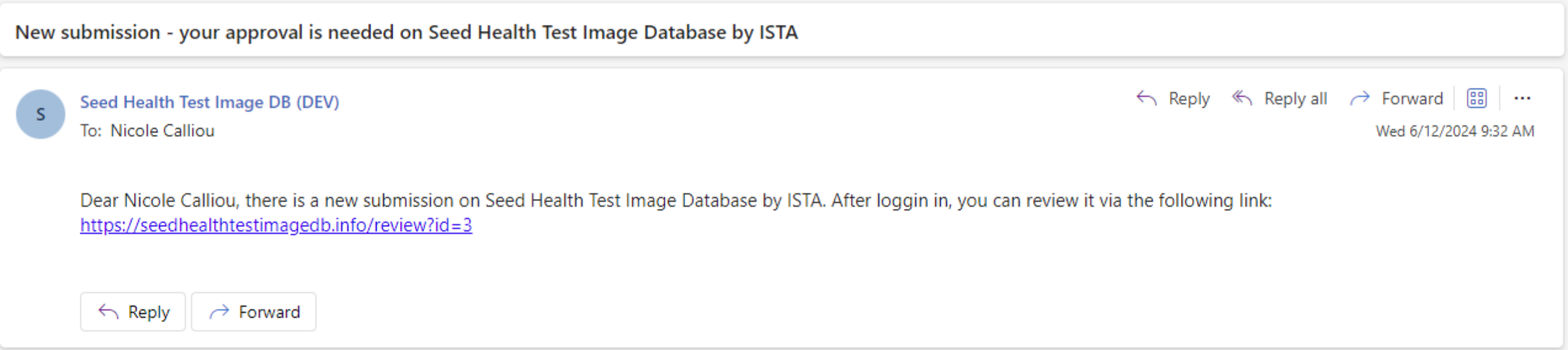
Submission


About your sample
Submit your sample images
About the environment
About you

Submission


Seed Health Test
Image Database
Search
About
Submit an image
Knowledge base
Links
Contact
Item saved successfully
Our team was notified about your data.
Thank you for your submission
We appreciate your effort and will review the submitted data in our next review cycle. The next steps will be:
Verification
Our team of experts will review your submission according to our quality guidelines.
Revision
If an error is noticed or further information is required, you will receive an email request with a link to edit your submission.
Approval
If the team find no errors with your submission or you have provided the missing data, you will receive an email notifying you when your submission is published.
Questions
If you have questions regarding your submission and the review process, please feel free to contact us or read through the FAQ section in our knowledge base.

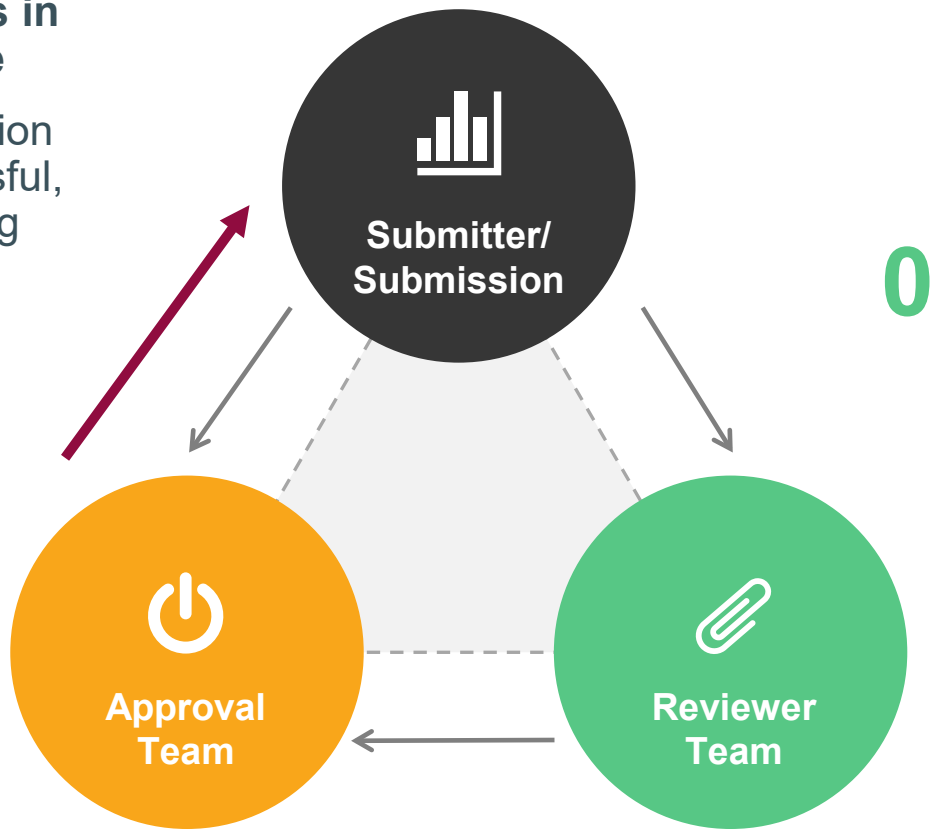
Submission Email – to Approvers/Reviewers



Work Flow

01 **A Submitter uploads images in a submission to the website**

Submitter will receive notification if the submission was successful, as well as instructions notifying them their submission will be reviewed.



02 **Reviewer Team AND Approval Team receive email notification**

2 weeks are allowed before a decision must be made to reject or accept submission.

Ideally, each submission has 3 different people approve it, prior to publishing

03 **Approval Team**

Submission is accepted and published on website, or notification is sent to submitter asking for editing. Admins are able to make edits directly in backend of website, if necessary.

The Review Team

Dorota Szopinska

Seed pathology specialist, Poznań University of Life Sciences and ISTA Seed Health Committee Member

Shaista Karim, Ph.D.

QC Seed Pathology Tech Lead at Bayer Crop Sciences

Rosa Piña

Pinto Piga Seeds S.A. and ISTA Seed Health Committee Member

Xiulan Xu

Associate Researcher, Beijing Vegetable Research Center and ISTA Seed Health Committee Member

Luciana Ferrand

Plant Pathologist at INASE and ISTA Seed Health Committee Member

Laixin Luo, Ph.D.

Professor of Plant Pathology Dept in China Agricultural University (CAU). Vice Director of Seed Health Center, CAU

Dipl.-Ing. Angela Thueringer

Seed Health Analyst at AGES, Vienna

Annu Albert, M.Sc.

Disease Diagnostics at SGS

Ernestine Lippert

Miriam Lechner

Submission Editing

Seed Health Test
Image Database

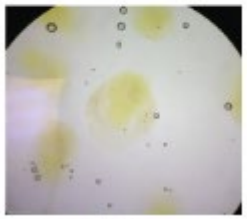
Search About Submit an image Knowledge base Links Contact

An email was just sent to your inbox with a temporary editlink. Please edit your submission in the next 30 minutes because the link will expire after that. ×

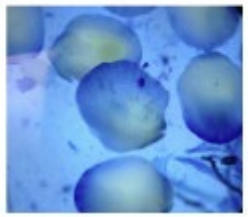
Submission Review

Ustilago nuda (USTINH)

Images



Perspective: top
Magnification: x25



Perspective: top
Magnification: x25

About the Sample

Pathogen	Ustilago nuda (USTINH)
Crop	Barley (Hordeum vulgare)
Description	Barley embryos isolated according to ISTA 7-013. 1)Infected, unstained embryo showing golden brown hyphae in scutellum 2) Infected embryo with Methyl Blue stain
Country of origin	Canada

About the owner

Ownership	Nicole Calliou, SGS
Owner Email	Nicole.Calliou@sgs.com

Manage your submission

ID: 2

Created: Monday, 10 June 2024 19:25

Reviewer: Annu Albert

Comment by the review team

Review decision
rejected (to improve)

Reviewer Comment
Although it would look similar to the methyl blue image, another image with trypan blue staining would be good since that is a common method used in many labs. AA

What to do next?

Request editing

What does that mean? +

Not sure how to proceed? +

Edit Link

[EXTERNAL] Edit your submission on Seed Health Test Image DB (DEV)

SH

Seed Health Test Image DB (DEV) <contact@seedhealthtestimagedb.info>

To Calliou, Nicole (Sherwood Park)

Reply | Reply All | Forward

Thu 6/13/2024 12:31 PM

This sender contact@seedhealthtestimagedb.info is from outside your organization.

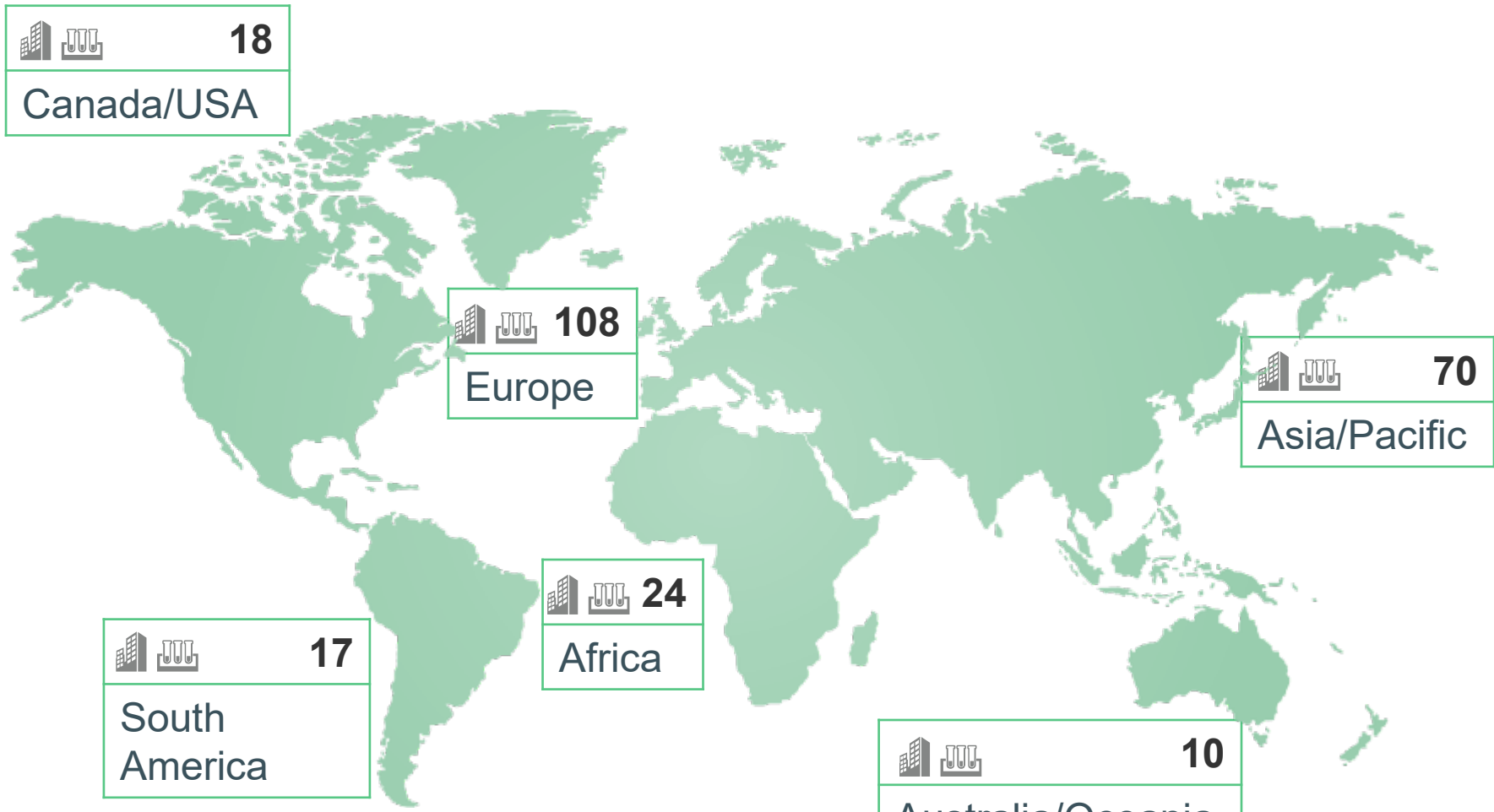
*** WARNING: this message is from an EXTERNAL SENDER. Please be cautious, particularly with links and attachments. ***

Someone has requested a link on Seed Health Test Image DB (DEV) to edit a submission. If this was you, you can edit your submission with the followint link: https://seedhealthtestimagedb.info/edit-submission?id=2&et=6c61a2af1e5385addebfce4bc32cd49f If this was not you, please ignore this email.

ISTA
100 YEARS
A CENTURY OF PROGRESS IN SEED QUALITY ASSURANCE

COUNTRIES

68 Designated Authorities
37 Personal Members
59 Associate Members
247 Laboratory Members



SGS

Milestones – Solicit Image Submissions

- ISTA Chapter 7 Pathogens
- ISTA Member labs have been requested to assist

- ISTA Reference Pest List
- Researchers from around the world are able to submit images to achieve this goal

- Pathogens of significance (Location dependent)
- Saprophytes (as per request at 2022)

Path to Success

ISTA Approval

And funding!

Website Creation

Clear goals and expectations

Review Team

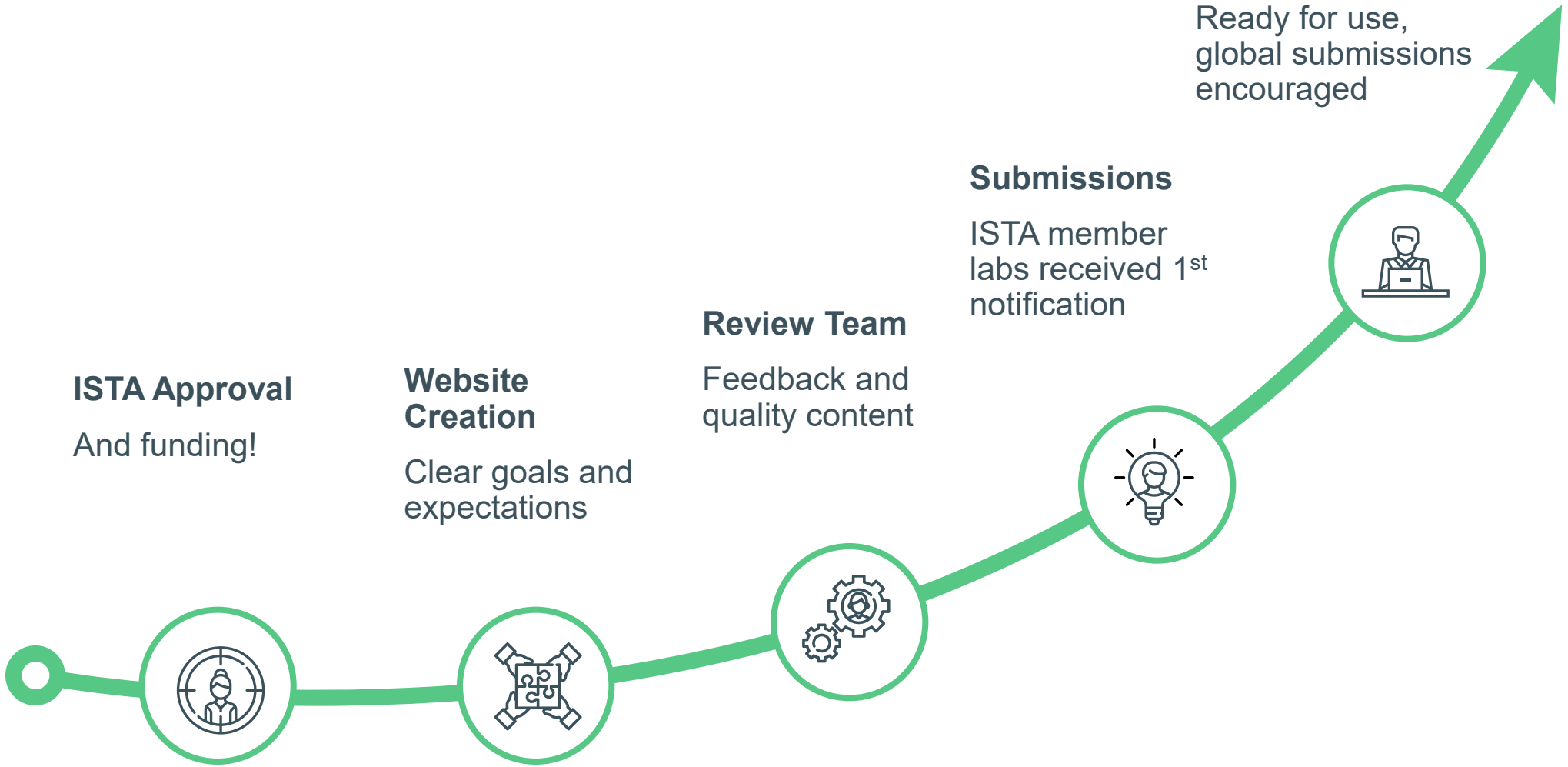
Feedback and quality content

Submissions

ISTA member labs received 1st notification

Marketing

Ready for use, global submissions encouraged



Questions?