Validation of a *Ascochyta rabiei* detection method on Chickpea seeds.

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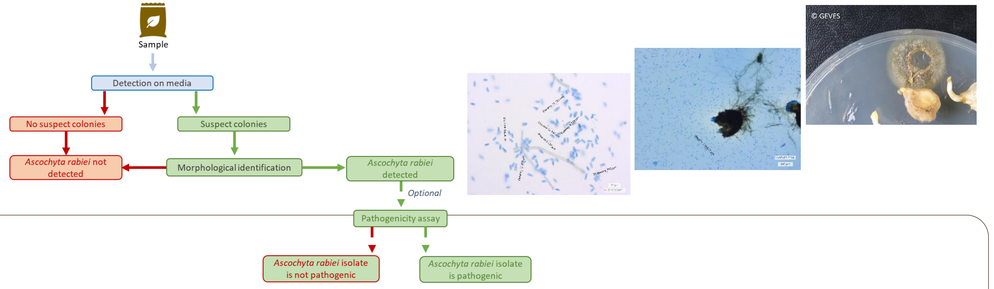
The French project AsCoLuP (CASDAR-IP) aims to understand the development and contamination dynamics of *Ascochyta rabiei* (chickpea) in a French context, through the development of standardized methods for common and uniform management of these diseases from seed to field, the identification of agronomic or technological levers to control diseases in seed or consumer production and the impetus of a territorial dynamic between the actors of these sectors. Chickpea is of real interest for cereal rotations. Although this crop is tending to develop today in both organic and conventional farming, one of the factors is strongly hindering the increase in surface areas is the management of diseases, particularly blight of chickpea caused by Ascochyta rabiei. In addition to agronomic levers, the use of healthy seeds remains the most important lever in the fight against this disease.
The validation of the detection method including a pathogenicity test, therefore addresses this issue.

Seed Detection method

The method has been adapted from the ISTA method 7-005 for the detection of *Ascochyta pisi* on Pea seeds. The method will include:

- Detection by plating 400 seeds on media (PDA or MA)
- Morphological identification under stereomicroscope/microscope
- Optional pathogenicity assay

The method has been validated according to ISTA guidelines for method validation and including study of performance criteria of the method



Performance Criteria validation

Seed material : Healthy and naturally infected seeds with different levels of infection.
Isolates collection : characterized by conidia size and growth criteria on 2 media (MA and PDA) Target: 20 isolates from different areas in France Non targets: 20 other pathogens and saprophytes that could be present on Chickpea seeds.

Pathogenicity assay performance criteria : Inoculation by soaking germinated seeds chosen after comparison of 3 different pathogenicity tests.
Symptoms : Necrosis at the base of the stem, wilting of the leaves
Analytical specificity: 20 target and 20 non-target (1 plant per strain), all targets showed expected symptoms, all non-targets showed different symptoms.
Fit for purpose concentration :2 concentrations were tested 10 seedlings tested for each concentration compared to 10 seedlings of the negative control.
Sensitivity/specificity diagnostic (Accuracy) : tested at the same time as the analytical specificity.
Repeatability/Reproducibility intra laboratory : 3 replicates tested at the same time and performed 2 times.
Robustness : Temperatures: 20°C - 25°C, light conditions: 8h light/16h darkness – 12h light/12h darkness, compared.

Targets (*Ascochyta rabiei*)

Non targets

Media detection performance criteria : Agar plating and morphological identification
Analytical specificity: collection compared to the criteria, all targets met the criteria, all non target did not meet the criteria
Analytical sensitivity: detection threshold defined : 0.25%, 1 infected seed was detected in 400 seeds in 10 replicates
Diagnostic sensitivity/specificity (Accuracy): 1 healthy, 1 low infected (0.25% infection), 1 medium infected (≈ 5% infection) samples
Repeatability/Reproducibility intra laboratory: 3 replicates of each level of infection tested at the same time and performed 2 times.
Robustness : a medium level infection lot (3.2%) tested on 2 different media (MA and PDA), at 2 different incubation durations (7 - 9 days).

Performance criteria	Media detection	Pathogenicity assay	Result
Analytical specificity	✓	✓	100%
Analytical sensitivity Cc fit for purpose	✓	✓	100% 1.10^5 conidia/mL
Diagnostic specificity	✓	✓	100%
Diagnostic sensitivity	✓	✓	100%
Repeatability	✓	✓	100%
Reproducibility intra laboratory	✓	✓	100%
Robustness	To be validated	✓	
Reproducibility inter laboratory	Performed in a comparative test planned end of 2022 (currently 8 participants)		

Negative control

Ascochyta rabiei

Non target

Perspectives

Validation report will be submitted to ISTA - ISTA rule proposal to publish a new ISTA method

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