Development and Validation of a Protocol to Detect *Pseudomonas syringae* pv. *syringae* in Bean (*Phaseolus vulgaris*) Seed

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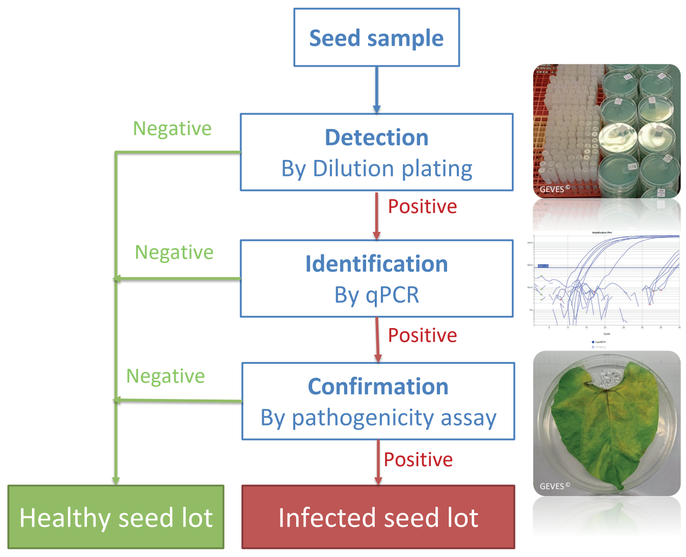
Background and context

The bacterium *Pseudomonas syringae* pv. *syringae* (Pss) is globally distributed and exhibits a high genetic diversity. It has the broadest host range (over 177 hosts described) of *Pseudomonas syringae* pathovars. The observable **brown spot disease** on bean is only caused by certain isolates of Pss, which is a seed transmitted pathogen. Infected seeds contribute to the long-distance dissemination of the pathogen. Therefore, the use of healthy bean seed is a key strategy to manage this disease and to prevent its introduction into new areas.

An International Seed Health Initiative (ISHI) project was launched to internationally develop and validate a protocol to detect Pss in bean seeds. The protocol includes a dilution plating detection test, followed by a real-time PCR identification test of suspect isolates and a pathogenicity confirmation test.

Method Workflow



Controls
- Positive and Negative Process controls: Infected and healthy bean seed lots
- Inhibition Control: Seed extract of each sample spiked with a Pss isolate
- PCR control: Internal Amplification Control, 'Universal' bacterial WU primers

A blurring Target

Collection of 81 isolates characterized by 2 labs using 4 new real-time PCR assays
→ 8 isolates with conflicting PCR results among the 4 PCR assays

Additional tests on 20 isolates:
- 3 pathogenicity tests: Detached Leaf, Spraying Leaf and Rubbing Leaf

- Sequencing of *cts* gene
- MALDI-TOF Mass Spectrometry

Species	Crop	Variety	Matrix	Country	Year	qPCR assays						Seq. assay	MALDI	Pathogenicity assays		
						Avr Pto	*hop AB1*	*Wu*	*SBP*	*Pepti dase*	*Wu*	*cts* gene		Detached leaf	Spraying leaf	Rubbing leaf
Pss	Bean	Duplex	leaf	FR (63)	2019	15.8	17.1	12.2	19.3	16.3	14.5	gp1	I	Pos	Pos	Pos
Pss	Bean	Mogex	leaf	FR (41)	2009	16.4	17.7	12.8	18.5	15.1	13.7	gp1	I	Mixed	Pos	Pos
Pss	Bean	Duplex	leaf	FR	2020	16.7	17.7	13.1	19.5	16.1	14.7	gp1	I	Pos	Pos	Pos
Pss	Bean	Duplex	pod	FR (41)	2021	16.4	17.9	13.1	20.4	17.3	15.7	gp1	I	Pos	Pos	Pos
Pss	Bean	MB 3343	leaf	FR (49)	2021	15.5	17.2	11.8	20.3	17.1	15.2	gp1	I	Pos	Pos	Pos
Pss	NA	NA	NA	NA	NA	17	18.4	13.4	21.9	18.3	16.4	gp1	I	Pos	Pos	Neg
Pss	Bean	NA	seed	NL	2020	N/A	17.5	13.1	20.7	17.1	15.3	gp1	I	Pos	weak	Pos
Pss	Bean	NA	NA	NL	2012	N/A	17.7	13.2	20	16.7	14.8	gp1	I	Pos	Pos	Pos
Pss	Bean	NA	seed	NL	2021	N/A	16.5	12.4	19.5	16.5	14.5	gp1	I	Pos	Pos	Pos
Pss	Bean	NA	seed	NL	2021	N/A	N/A	13.7	20.8	17.5	15.7	gp1	I	Mixed	Pos	weak
Pss	*Prunus*	NA	NA	NA	2017	N/A	N/A	13.6	21.4	17.5	15.6	gp1	I	Neg	Pos	weak
Pss	Bean	NA	seed	NL	2022	N/A	N/A	13	20	16.7	15	gp1	I	Mixed	weak	Neg
Pss	NA	NA	NA	NA	2005	N/A	N/A	12.7	20.5	17.3	15.5	gp1	II	Mixed	Pos	Neg
Pss	NA	NA	NA	NA	NA	N/A	N/A	13.5	23	15.8	13.9	gp1	II	Neg	weak	weak
Pss	NA	NA	NA	NA	NA	N/A	N/A	12.4	N/A	38.6	13.6	*P. congelans*	II	Neg	Neg	Neg
Pss	NA	NA	NA	NA	NA	N/A	N/A	13.6	N/A	N/A	15.1	*P. congelans*	II	Neg	Neg	Neg
Pss	NA	NA	NA	NA	NA	N/A	37.9	13.3	38	N/A	15.5	gp2	III	Neg	Neg	Neg
Pss	NA	NA	NA	NA	NA	N/A	N/A	13.5	N/A	N/A	14.9	gp2	III	Neg	Neg	Neg
Pss	NA	NA	NA	NA	NA	N/A	N/A	13	N/A	N/A	14.4	*P. coronafaciens*	*Ps porri*	Neg	Neg	Neg
Ps peponis	NA	NA	NA	NA	NA	N/A	N/A	13.8	N/A	N/A	15.5	NA	NA	Mixed	Pos	Neg

→ Definition of targets and non targets but presence of a grey area
→ Choice of the protocol: hopAB1/Peptidase primers and Detached Leaf assay

Validation study – First results

Analytical Specificity

23 target isolates + 23 non-target isolates
Analyses in duplicates

Dilution Plating assay (on MSP and KBC media)
+ Biochemical tests (Oxydase, Hydrolysis on MT and Fluorescence)
→ 100% inclusivity
→ 47% exclusivity

PCR assay (Triplex: hopAB1/Peptidase/WU)
→ 100% inclusivity
→ 91% exclusivity

Selectivity of the Dilution Plating Test

Spike of 6 bean seed lots in triplicate with a Pss isolate at a concentration near the limit of detection

	Variety	Year of production	TSW	Saprophytic level
Lot 1	Var A	2025	534	low
Lot 2	Var B	2025	399	medium
Lot 3	Var C	2024	157	low
Lot 4	Var D	2025	527	low
Lot 5	Var E	2023	157	Low
Lot 6	Var F	2023	102	High

Detection of Pss in all seed lots
→ 100% of selectivity

Conclusion and perspectives

Development of a real-time PCR identification test was challenging due to the broad host range of Pss and the need to discriminate it from closely related species and pathovars on bean (pathogenic and epiphytic). Four real-time PCR assays were designed within this project and two were selected (*hopAB1/Peptidase*). The Detached leaf test was selected among the three pathogenicity tests.

The exclusivity of the dilution plating assay is too low (47%) and this assay must be followed by a PCR confirmation assay. The selectivity was 100% for the dilution plating test.

Next steps: Evaluation of the analytical specificity for the pathogenicity assay, the diagnostic performances, the repeatability, and the reproducibility (Organisation of an ISHI Comparative Test).