Tropentag, September 10-12, 2025, hybrid conference

"Reconcile land system changes
with planetary health"

Multiplex PCR to detect *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* and the common bean root pathogens *Fusarium oxysporum* f. sp. *phaseoli* and *Fusarium solani* f. sp. *phaseoli*

Adriane Wendland, Enderson Ferreira, Lobo Jr. Murillo , Maythsulene Inacio de Souza Oliveira

Embrapa - Brazilian Agricultural Research Company, Agricultural Microbiology, Brazil

Abstract

Root diseases that affect common beans (*Phaseolus vulgaris* L.) may share similar symptoms, such as Fusarium wilt (*Fusarium oxysporum* f. sp. *phaseoli*), dry root-rot (*Fusarium solani* f. sp. *phaseoli*) and Curtobacterium wilt (*Curtobacterium flaccumfaciens* pv. *flaccumfaciens*). The difficulties for their accurate diagnosis include multiple infections in the field or a single plant, but molecular tools can overcome such drawbacks and identify causal agents accurately. The objective of this work was to develop a multiplex PCR (m-PCR) method to simultaneously identify three common bean pathogens, *F. oxysporum* f. sp. *phaseoli* (Fop), *F. solani* f. sp. *phaseoli* (Fs) and *C. flaccumfaciens* pv. *flaccumfaciens* (Cff).

The approach included the design and validation of the new primers IGS1FOR and IGS1REV for *F. solani* f. sp. *phaseoli* and adjustments on primer concentration and annealing temperature. Multiplex PCR using IGS1FOR/IGS1REV, CffFOR2/CffREV4 and A280/B310 primers did not change the pre-established procedures for detecting *F. solani* f. sp. *phaseoli*, *C. flaccumfaciens* pv. *flaccumfaciens* and *F. oxysporum* f. sp. *phaseoli*, and reactions with 0.4 to 0.8 μM of primers and annealing at 57 ^{o}C resulted in clear, spaced amplicons in agarose gel, respectively with 143 (Fs), 306 (Cff), and 609 base pairs (Fop).

Multiplex PCR offers significant advantages over the ISTA method for detecting pathogens in bean seeds, mainly due to the drastic reduction in analysis time, from 16 hours to 1 or 4 hours. Despite the shorter preparation time, the multiplex PCR technique demonstrates similar sensitivity to the standard method in identifying the pathogens present. Additionally, multiplex PCR exhibits high specificity, allowing for the precise distinction between different pathogen species, as evidenced by the identification of *Fusarium oxysporum* f. sp. *phaseoli* and the non-detection of *Fusarium solani* f. sp. *phaseoli*. These combined benefits highlight the potential of multiplex PCR to streamline and refine seed phytosanitary analysis processes.

The protocol to differentiate *F. solani* f. sp. *phaseoli*, *C. flaccumfaciens* pv. *flaccumfaciens* and *F. oxysporum* f. sp. *phaseoli* is simple to perform and may be easily implemented in plant disease clinics and seed health laboratories. This is the first report of an m-PCR method to detect common bean phytopathogens.

Keywords: Curtobacterium wilt, dry root rot, Fusarium wilt, plant disease diagnostics, seed health tests, vascular wilt.

Contact Address: Adriane Wendland, Embrapa - Brazilian Agricultural Research Company, Agricultural Microbiology, Rua Sb 20 Qd 11 Lt 03 Portal Do Sol 1, 74884-607 Goiânia, Brazil, e-mail: adriane.wendland@embrapa.br