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

Adriane Wendland, [1] Enderson Ferreira[1], Murillo Lobo Jr.[1]

[1]Brazilian Agricultural Research Corporation (EMBRAPA), National Rice and Beans Research Center (CNPAF), Brazil
email: adriane.wendland@embrapa.br

INTRODUCTION

Brazil is the world's largest producer of common bean (*Phaseolus vulgaris* l.), a basic dietary protein food sourcewith an annual production of approximately 3 million tons. These crops are subjected to yield losses throughout the year, in different environments, primarily by diseases and pests that limit sustainable cropping. The difficulties for their accurate diagnosis include multiple infections in the field or a single plant. This study aimed to develop an m-PCR method to simultaneously detect *F. oxysporum* f. sp. *phaseoli (Fop)*, *F. solani* f. sp. *phaseoli (Fus)* and *C. flaccumfaciens* pv. *flaccumfaciens (Cff)* present in common bean samples.

METHODS

Figure 1 – A. Experiments conducted in a greenhouse and Lab. B.Typical symptoms of diseases in beans.

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 ºC resulted in clear, spaced amplicons in agarose gel, respectively with 143 (Fs), 306 (Cff), and 609 base pairs (Fop).

RESULTS

Isolate	Geographic origin	Species identification
BRM-14991	Recife – PE	F. oxysporum f. sp. phaseoli
BRM-19993	Wenceslau Braz – PR	C. flaccumfaciens pv. flaccumfaciens
BRM-32935	Guarda Mor - MG	F. solani
BRM-31661	Fernandes Pinheiro - PR	F. solani
BRM-31669	Fernandes Pinheiro – PR	F. solani
BRM-33055	Rio Verde – GO	F. solani f. sp. phaseoli
BRM-33085	Buri – SP	F. solani f. sp. phaseoli
*BRM-25302	Ponta Grossa – PR	X. phaseoli pv. phaseoli
*BRM-66463	Brasilia - DF	F. brasiliense
*BRM-66598	Cristalina - GO	F. crassistipitatum
*BRM-66599	Ponta Grossa - PR	F. tucumaniae

Ta
mu

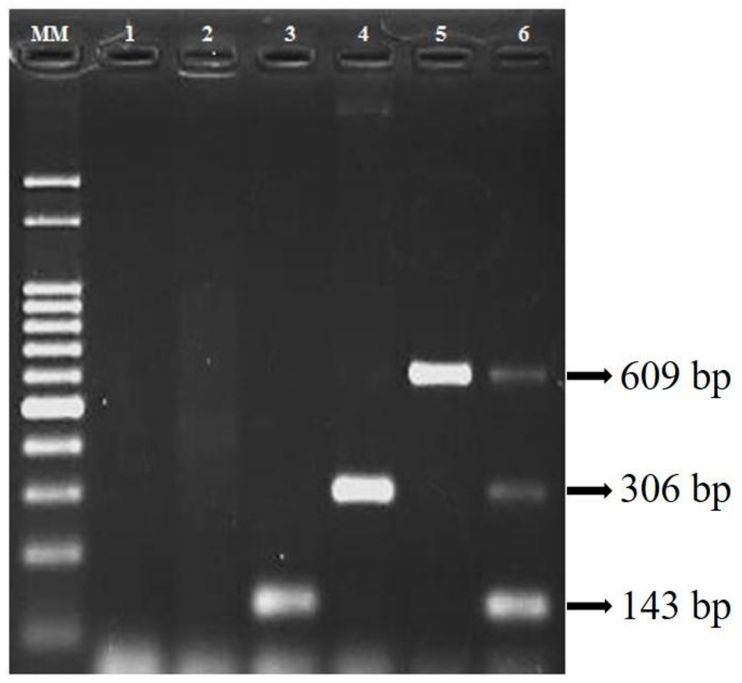

Figure 2. M-PCR products obtained with the FOR2 / REV4 primers for *C. flaccumfaciens* pv. *flaccumfaciens* (306 base pairs), A280 / B310 for *F. oxysporum* pv. *phaseoli* (609 bp) and IG1For / IGS1REV for *Fusarium solani* f. sp. *phaseoli* (143 bp)

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.

CONCLUSIONS

This m-PCR protocol allows the detection of the common bean dry root rot and vascular wilts causal agents and supports their diagnosis in naturally infected seeds. 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.