

Production of tomato seeds artificially infected with a GFP – tagged strain of *Clavibacter michiganensis* subsp. *michiganensis* for studies on seed transmission. Ribeiro, DH^{1,2}; Souza, RM²; Van der Wolf, JM¹. ¹Plant Research International, P.O. Box 16, 6700AA, Wageningen, The Netherlands. ²Laboratory of Plant Bacteriology/DFP/UFLA, CP 3037, CEP 37200-000, Lavras, MG, Brazil. E-mail: dh_ribeiro@hotmail.com. Produção de sementes de tomate artificialmente infectadas com um isolado de *Clavibacter michiganensis* subsp. *michiganensis* marcado com GFP para estudos na transmissão por sementes.

Clavibacter michiganensis subsp. *michiganensis* (Cmm) is the causal agent of canker and bacterial wilt of tomato, and responsible for high losses worldwide. To study transmission of Cmm from seed to seedling, a method was developed to generate tomato seeds internally-infected with a GFP-tagged strain of Cmm. Seeds were first wounded by scarification and incubated on a wetted blotter for 16 h to soften the seeds. Seeds were inoculated with a Cmm suspension by vacuum infiltration. To enhance (internal) growth of Cmm in seeds, they were inoculated at 25°C on blotters or on a Cmm-selective medium. Seeds were incubated with and without prior disinfection to eliminate Cmm on the seed coat. Seed infection and translocation of Cmm from seed to seedling were studied with plating, epifluorescence stereomicroscopy (ESM) and confocal laser scanning microscopy (CLSM). The Cmm population directly after inoculation were 10⁴ cfu/seed and 10³ cfu/seed in the not disinfected and disinfected seeds, respectively. Incubation of both non-disinfected and disinfected seeds resulted in a strong increase of Cmm populations to densities higher than 10¹⁰ cfu/seed after 3 days of incubation. The GFP signal could be observed in the seedlings, on the seed coat, cotyledons, stem and roots by ESM and CLSM. The GFP-tagged Cmm strain were most frequently found in roots from non-disinfected seeds, suggesting that during radicle protrusion, contact infections occur from the heavily infested seed coat. Incidentally, colonization of xylem vessels was observed, indicating systemic infections.

Pathogen: bacteria

Pathogen specie: *Clavibacter michiganensis* subsp. *michiganensis*

Host specie: *Lycopersicon esculentum*

Host common name: Tomato

Area: Seed Pathology

The study involves a new pathogen description or a new pathogen-host association for Brazil?
() yes (X) No

Trabalho envolve novo relato de patógeno ou associação patógeno-hospedeiro para o Brasil? () sim, (X) não