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Generation of transgenic *Mycosphaerella fijiensis* expressing reporter genes: tools for pathogenicity and mating studies

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Mycosphaerella fijiensis, the causal agent of black leaf streak disease is the most devastating pathogen of banana and plantains. Detailed studies on the plant pathogen interaction as well as mating behavior could be improved by the use of non-destructive reporter gene assays. *M. fijiensis* isolates from different global populations and opposite mating types, CIRAD86 (*Mat1-1*) from Cameroon and CIRAD139A (*Mat1-2*) from Colombia, were selected for our studies. In order to generate transgenic *M. fijiensis* strains, two gene constructs carrying either reporter genes *gfp* (green fluorescent protein) or *dsred* (*Discosoma* sp. red fluorescent protein) driven by the constitutive promoter PtoxA were used. Both constructs also carried the *hph* (hygromycin phosphotransferase) gene for resistance to the antibiotic hygromycin as selective marker.

Transformation procedures mediated by *Agrobacterium tumefaciens* strain LBA1100 were carried out using macerated mycelium from 3-week-old cultures of CIRAD86 and CIRAD139A. After the co-cultivation period, the cultures were transferred to selective medium containing 30 mg/l hygromycin. Transgenic events were identified by fluorescent microscopy observations after 2-3 weeks (Figure 1).

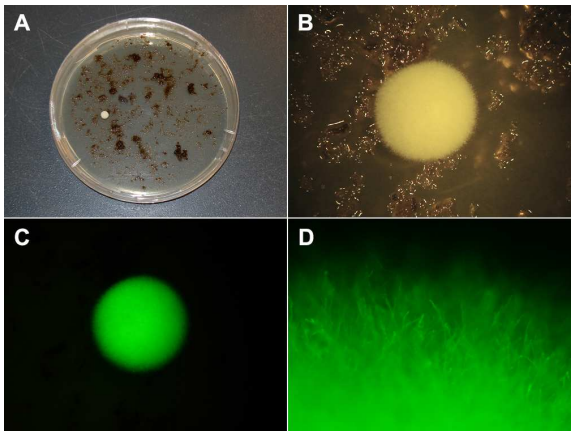

Figure 1. Agrobacterium-mediated genetic transformation of M. fijiensis CIRAD86 with gfp. (A) putative transformed colony growing on selective medium. (B) observation under visible light microscope. (C) observation under fluorescent microscope. (D) detailed view showing fluorescent hyphae.

In this part of the work, we were able to generate transgenic strains from both isolates of *M. fijiensis* expressing either reporter genes, namely CIRAD86::GFP, CIRAD86::DsRed, CIRAD139A::GFP and CIRAD139A::DsRed (Figure 2).

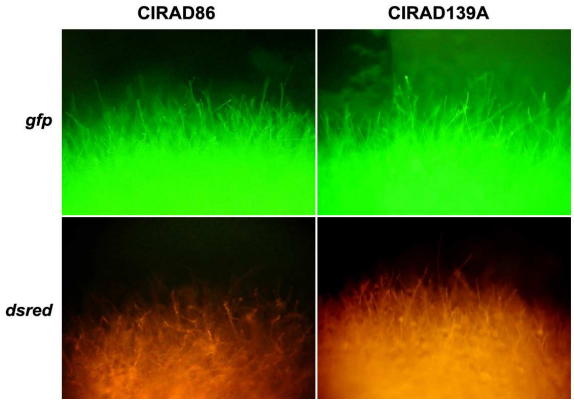

Figure 2. Transgenic strains of CIRAD86 and CIRA139A isolates transformed with gfp and dsred.

Studies on the establishment of mating assays and pathogenicity validation screens using *in vitro* leaf fragments are being conducted in order to characterize the different developmental stages. Preliminary results are shown in the Figures 3 and 4, respectively.

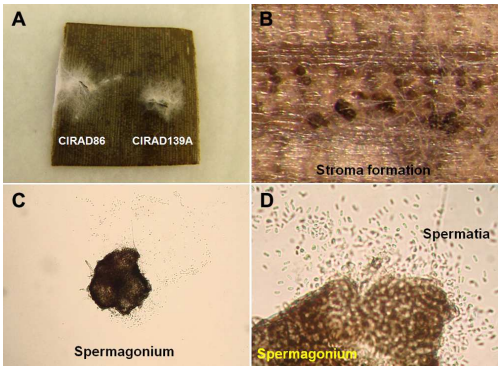

Figure 3. In vitro mating studies. (A) Banana cv. Grand Naine leaf piece inoculated with culture plugs of CIRAD86 and CIRAD139A. (B) Stroma formation. (C) Isolated spermagonium. (D) Closer view of spermagonium and released spermatia.

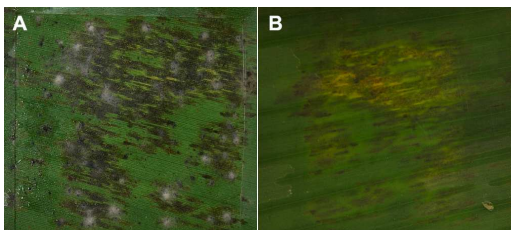

Figure 4. In vitro artificial inoculation of 60 mg mycelium of M. fijiensis on Grand Naine banana cultivar at 91 dpi. (A) Abaxial side where inoculum was applied. (B) Adaxial side of the same leaf piece.

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