GENERATION OF TRANSGENIC *MYCOSPHAERELLA FIJIENSIS* EXPRESSING REPORTER GENES: TOOLS FOR PATHOGENICITY AND MATING STUDIES

Kobayashi[1,2], Adilson K.; Diaz Trujillo[1], Caucasella; Lee, Theo van der; Zwiers[3], Lute-Harm; Paiva[5], Luciano V.; Souza Junior[4], Manoel T.; Kema[1], Gert H.J.

[(1)]Plant Research International, P.O. Box 16, 6700 AA Wageningen, The Netherlands
[(2)]Embrapa Mid-North, Av. Duque de Caxias, 5650, CEP64006-220, Teresina/PI, Brazil
[(3)]Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, P.O. Box 85167, 3508 AD Utrecht, The Netherlands
[(4)]Embrapa LABEX Europe, P.O. Box 16, 6700 AA Wageningen, The Netherlands
[(5)]Federal University of Lavras, Caixa Postal 3037, CEP.37200-000, Lavras/MG, Brazil

E-mail: adilson.kobayashi@wur.nl or gert.kema@wur.nl

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 (*Mat*1-1) from Cameroon and CIRAD139A (*Mat*1-2) 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* 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. In this part of the work, we were able to generate transgenic strains from both isolates expressing either reporter genes, namely CIRAD86::GFP, CIRAD86::DsRed, CIRAD139A::GFP and CIRAD139A::DsRed. Simultaneously, pathogenicity validation screens and mating assays using *in vitro* leaf fragments are also being conducted in order to characterize the different stages in the pathogenesis on banana.

Keywords: GFP, DsRed, CIRAD86, CIRAD139A