

Standardized bioassays: an improved method for studying *Fusarium oxysporum* f. sp. cubense race 4 (FocR4) pathogen stress response in *Musa acuminata* cv. ‘Berangan’

ABSTRACT

To date, there is no standardized *Fusarium* bioassay protocol established owing partly to the wide variety of *Fusarium oxysporum* f. sp. cubense (Foc) isolates and banana cultivars present. Thus, validation of the infection parameters is deemed essential prior to each bioassay experiment. In the current study, a simple standardized workflow was developed based on available assays for testing *Fusarium* wilt disease response in *Musa acuminata* using *M. acuminata* cv. ‘Berangan’ of tissue-culture origin as a model. The phenotypic assays were able to detect external disease symptoms less than one week post-inoculation, while the molecular approach using RT-qPCR identified differential expression of catalase (CAT), pathogenesis-related 10 (PR10), phenylalanine ammonia-lyase (PAL) and xylanase (XYL) genes as early as day 0. The transcript levels of PR10 and XYL fluctuated over 4 days of Foc Race 4 (FocR4 C1 HIR isolate) infection while the expression of CAT steadily increased over time. In contrast, PAL was highly upregulated at 2 days post-inoculation. These signature changes suggest that all genes tested might be involved in the early defense response of ‘Berangan’ plants against FocR4 infection. ‘Berangan’ cultivar was found to be highly susceptible to Foc Race 4 (C1 HIR isolate) with leaf symptoms index (LSI) and rhizome discoloration index (RDI) scores of 4.257 and 5.971, respectively. The procedure elaborated in this study can be used as a reference Foc bioassay for reproducible and comparable results possibly across cultivars and test isolates due to its simple steps aided by integration of phenotypic and molecular approach.

Keyword: Banana; Foc bioassay; *Fusarium oxysporum* f. sp. cubense; *Fusarium* wilt; *Musa acuminata* cv. ‘Berangan’; Plant microbe interaction