Detection of oil palm root penetration by Agrobacterium-mediated transformed Ganoderma boninense, expressing green fluorescent protein

ABSTRACT

A highly efficient and reproducible Agrobacterium-mediated transformation protocol for Ganoderma boninense was developed to facilitate observation of the early stage infection of basal stem rot (BSR). The method was proven amenable to different explants (basidiospore, protoplast, and mycelium) of G. boninense. The transformation efficiency was highest (62%) under a treatment combination of protoplast explant and Agrobacterium strain LBA4404, with successful expression of an hyg marker gene and gus-gfp fusion gene under the control of heterologous p416 glyceraldehyde 3-phosphate dehydrogenase promoter. Optimal transformation conditions included a 1:100 Agrobacterium/explant ratio, induction of Agrobacterium virulence genes in the presence of 250 μm acetosyringone, co-cultivation at 22°C for 2 days on nitrocellulose membrane overlaid on an induction medium, and regeneration of transformants on potato glucose agar prepared with 0.6 M sucrose and 20 mM phosphate buffer. Evaluated transformants were able to infect root tissues of oil palm plantlets with needle-like microhyphae during the penetration event. The availability of this model pathogen system for BSR may lead to a better understanding of the pathogenicity factors associated with G. boninense penetration into oil palm roots.

Keyword: Basidiomycetes; Oil palm root penetration; Agrobacterium-mediated transformed; Ganoderma boninense; Green fluorescent protein