

## **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  $\mu$ m 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