Trichoderma TRACKING IN PLANTS USING GREEN FLUORESCENT LABELLING

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Trichoderma spp. has been commercially introduced as biocontrol agent in the agricultural industry to enhance plant growth and defence mechanism (Chacón *et al*., 2007; Angel *et al*., 2016; Węgrzyn and Górzyńska 2019), to provide a root shield against pathogen infestation (Lu *et al*., 2004; Sundram 2013a, b), to control the population of pathogenic microorganisms in the rhizosphere (Benítez *et al*., 2004); and reduce disease severity (Michereff *et al*., 1995). *Trichoderma* spp. are known as excellent candidates in agriculture, include *T. virens*, *T. harzianum* and *T. hamatum*. In oil palm, *T. virens* and *T. harzianum* were proven to be the most effective agents to combat against *Ganoderma boninense*, a pathogen causing basal stem rot (BSR) disease (Sundram *et al*., 2010; Sundram 2013a, b). The *Trichoderma* transformation system utilises a synthetic green fluorescent protein (SGFP) gene sequence that contain a serine-to-threonine substitution at amino acid 65 (S65T), mediated by *Agrobacterium tumefaciens*, serves as a useful tool in monitoring antagonistic activity of biocontrol *Trichoderma* against various plant pathogens and its interactions with other organisms. SGFP variant has been used in the transformation of filamentous fungi, providing a high level of fluorescence in fungi (Lorang *et al*., 2001). SGFP-tagging methods have been widely used to track and monitor the distribution of fungi *in planta*, and to estimate their biomass.

PROCEDURES

The SGFP-tagged *Trichoderma* transformation system uses a SGFP variant which is soluble, emits highly fluorescence, and can reduce photobleaching (Cubitt *et al*., 1995). SGFP has been frequently used for the transformation of filamentous fungi due to its high GFP yield and increased fluorescence level in transformants (Chiu *et al*., 1996; Lorang *et al*., 2001). The insertion of SGFP fragment into *T. virens* was mediated by *A. tumefaciens* AGL1 strain through T-DNA transfer.

The experiment involved the transformation of plasmid containing SGFP (pCAMBIAgfp) into chemically competent *A. tumefaciens* AGL1, via heat-shock method. Successful transformation of *Agrobacterium* was confirmed by PCR amplification of SGFP at approximately 711 bp, and *Sal*1 restriction analysis which produces three fragments at 9000 bp, 2800 bp and 1400 bp. The minimal inhibitory concentration (MIC) for the selection of putative *T. virens* transformants using hygromycin (HygB) was identified by subjecting 1 x 10^6 spores ml^{-1} from the wild type *T. virens* on potato dextrose agar (PDA) containing HygB prior to fungal transformation.

The transfer of T-DNA harbouring SGFP into *T. virens* was mediated by *A. tumefaciens* AGL1 strain. Following the transformation procedures, the mixture of fungal spores and AGLI suspension was spread on co-cultivation medium and selected on PDA media supplemented with 175 µg ml^{-1} HygB and 200 µM cefotaxime to eliminate excess bacteria. The HygB resistant isolates were selected and visualised using Olympus BX41 microscope equipped with a fluorescence filter to detect green fluorescence.

NOVELTY OF TECHNOLOGY

- SGFP-tagged *Trichoderma* is a simple and effective tool to monitor the fungal growth, activity and population of biocontrol agents introduced into the rhizosphere and plant systems.
- Optimised method for transformation of *Trichoderma* using a SGFP variant, mediated by *A. tumefaciens* AGL1 strain has been developed to facilitate research according to the need of researchers.

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SERVICE OFFERED AND COST

Trichoderma transformation procedures are outline in *Figure 1*. The cost of fungal transformation service per sample is estimated at RM 2200. The time required to complete the procedures is approximately 10 weeks. Prices are subjected to change depending on customer's enquiry and cost of consumables.

BENEFITS OF SGFP-TAGGED *Trichoderma*

1. **Why *Trichoderma?***

   Trichoderma is a special workhorse fungus with high productivity. It can increase plant nutrient availability, efficiently promote growth, enhance plant defence, aggressively respond towards pathogen infestation, able to withstand difficult conditions, and modify the rhizosphere.

2. **Why SGFP-tagged *Trichoderma* is important?**

   Economic and new tool to efficiently monitor the biocontrol *Trichoderma* development and *Trichoderma*-pathogen-plant interaction *in situ* using SGFP marker. It provides fundamental information on the colonisation potential of the planta system, and facilitates the elucidation of its mechanism as a mycoparasites.

3. **How it benefits the industry?**

   The tracking of *Trichoderma* through SGFP-tagging could increase trust and confidence of customers on *Trichoderma*-containing biofertilisers and biocontrol agents.

CONCLUSION

Inoculation of *T. virens* inoculum into oil palm tissue culture ramets through dipping technique has shown positive colonisation by the presence of regenerating hyphae penetrating and colonising the root tissues (*Figure 2*). The colonisation of endophytic fungi in oil palm root system was shown by SGFP-tracking (*Figure 3*) in successfully transformed *T. virens,* demonstrating that *Trichoderma* could be an endophyte in oil palm roots.

THE CLIENTS

The service is available to all stakeholders in the agricultural industries, the scientific community from universities and research institutions.

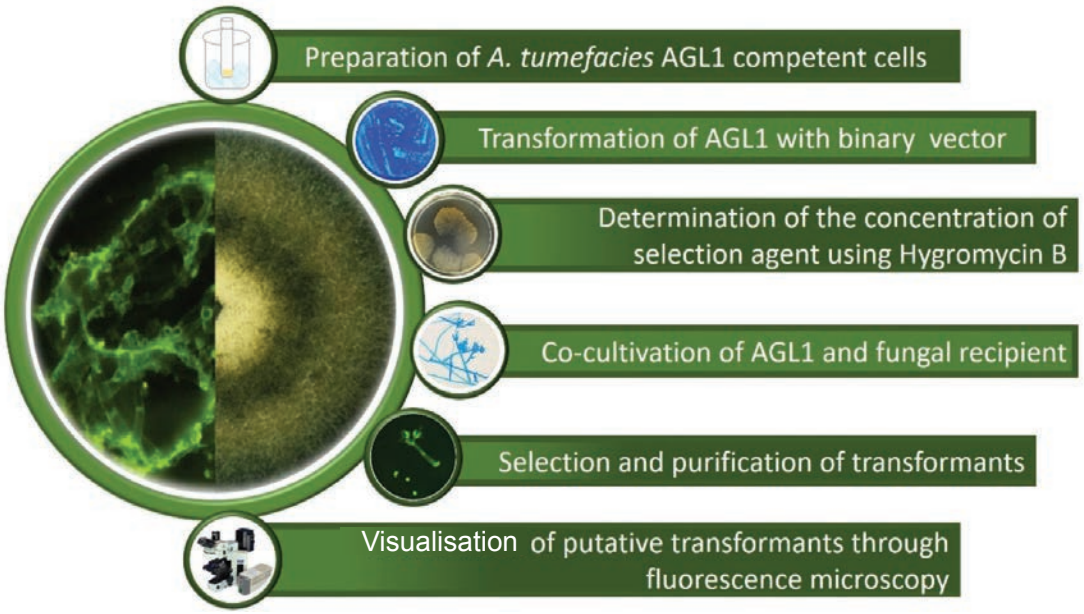

Figure 1. General workflow / flowchart for fungal transformation with Green Fluorescent Protein (GFP) mediated by Agrobacterium tumefaciens.

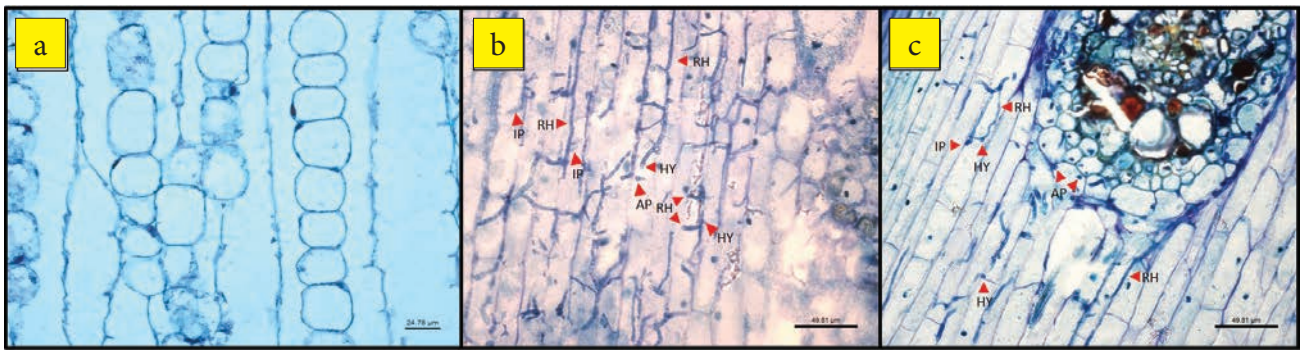

Figure 2. a) Microscopic visualisation of the roots of oil palm tissue culture ramets without Trichoderma virens *7b showed absence of fungal hyphae, b) and c) Presence of appressoria (AP), simple hyphopodia (HY), infection pegs (IP) and runner hyphae (RH) penetrating and colonising the root of inoculated oil palm roots with* T. virens *7b.*

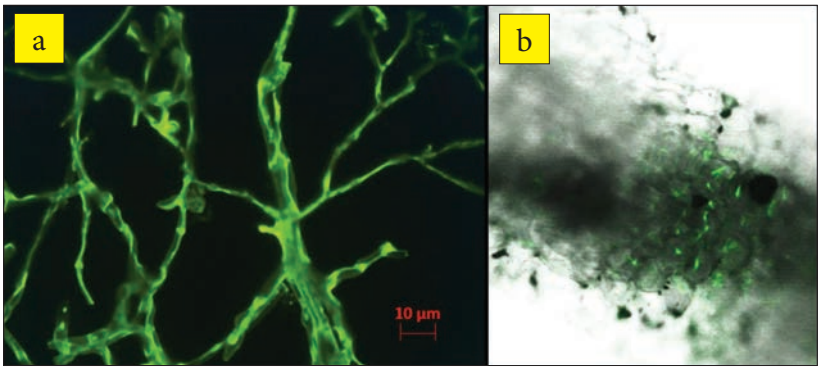

Figure 3. a) Fluorescence micrographs of putative transformants of T. virens *7bis23::gfp. b) Colonisation of* T. virens *7bis23::gfp in oil palm tissue culture roots was confirmed through confocal laser scanning microscopy (CLSM).*

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