FAMILY OF GENE ENCODING POTENTIAL PATHOGENICITY ASSOCIATED PROTEIN (CYCLOPHILIN) FROM *Ganoderma boninense*

FOOK-HWA LIM; ISKANDAR NOR FAKHRANA; OMAR ABD RASID; ABU SEMAN IDRIS and GHULAM KADIR AHMAD PARVEEZ

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Basal stem rot (BSR) caused by species of *Ganoderma* is a major concern in oil palm plantations especially in Malaysia and Indonesia. The disease could cause significant impact to the economy due to reduction in fresh fruit bunch (FFB) yield and number of productive standing palms (Turner, 1981). Unfortunately, understanding on the infection mechanism of *Ganoderma* especially at molecular and biochemical levels is still obscure (Paterson *et al.*, 2009). Study of pathogenic fungi is difficult because they have diverse lifestyles and modes of interaction with their hosts (Ralf *et al.*, 2011). One of the potential approaches to gain the knowledge is to identify the pathogenicity associated genes from these fungi. Pathogenicity genes refer to genes that are essential for disease development but not necessary for the organism to complete its normal life cycle *in vitro* (Idnurm and Howlett, 2001).

Cyclophilins (CYP) are peptidyl prolyl *cis-trans* isomerases (PPIase) that are highly conserved in bacteria, fungi, plants and animals. The protein implicated in various cellular processes and also as one of the virulence factors in a number of pathogenic fungi. The pathogenicity role of fungal CYP has been well studied in several fungi (Wang *et al.*, 2001; Viaud *et al.*, 2002; Chen *et al.*, 2011). For example, reduced pathogenicity followed by impaired peg formation and appressorium turgor generation were observed in *Magnaporthe grisea* CYP1 null mutant (Viaud *et al.*, 2002). In a recent study by Chen *et al.* (2011), deletion of CYP1 in *Cryphonectria parasitica* resulted in a dramatic reduction in virulence and infection canker size when the fungus strain was inoculated on chestnut stems. The result showed that CYP1 is required for the fungus full virulence although the fungus did not produce similar infection structures as compared to *M. grisea*. The above studies indicated that fungal CYP play different roles during the infection stages. Hence, CYP from *G. boninense* could be involved in the infection process on oil palm.

CHARACTERISATION OF *Ganoderma boninense* CYCLOPHILINS

By using the SMARTer Rapid Amplification of cDNA Ends (RACE) technique, five full-length cDNA clones encoding CYP have been successfully amplified from *G. boninense* (*Figure 1*). The CYP sequence sizes range from 662 bp to 816 bp. They were classified as different family members of CYP because significant differences could be observed in their coding sequence and 5′ or 3′ un-translated regions (UTR). BLASTX results indicated that these sequences shared high similarity compared with CYP from other fungi such as *Coprinopsis cinerea okayama* 7#130, *Laccaria bicolor* S238N-H82 and *Serpula lacrymans* var. lacrymans S7.3. An *in vitro* infection test has been performed by inoculating six-month old oil palm clonal plantlets with clumps of *G. boninense* mycelium in a 250 ml flask and incubating at 28°C for eight weeks (*Figure 2*). Controlled samples were prepared by growing either *G. boninense* or oil palm clonal plantlet in a flask. The mycelium samples were collected every fortnight and total RNA was extracted. Verification of *G. boninense* infection was performed by dissecting and comparing the basal stem of the oil palm plantlets from the inoculation and controlled flasks. Detection of *G. boninense* in the treated palm's basal stem tissue was performed via *Ganoderma* Selective Medium (GSM) (Ariffin and Idris, 1992) (*Figure 3*). The expression of *G. boninense* CYP was studied using real-time quantitative PCR (qPCR). The cDNA samples converted from total RNA samples were used as templates. The qPCR was performed and normalised with three reference genes including α-tubulin, β–tubulin and eEF2 (Lim *et al.*, 2014). Based on the expression results, GbCYP203 and GbCYP205 were found to be upregulated in the mycelium samples collected during early weeks of infection (*Figure 4*). Based on the results, these two CYP transcripts were predicted to be involved in the fungal stress response or pathogenicity. This work provided genetic information and expression profile of the CYP clones encoded by *G. boninense*.

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Malaysian Palm Oil Board, Ministry of Plantation Industries and Commodities, Malaysia
6 Persiaran Institusi, Bandar Baru Bangi, 43000 Kajang, Selangor, Malaysia. *Tel:* 03-8769 4400 *Fax:* 03-8925 9446 *Website:* www.mpob.gov.my

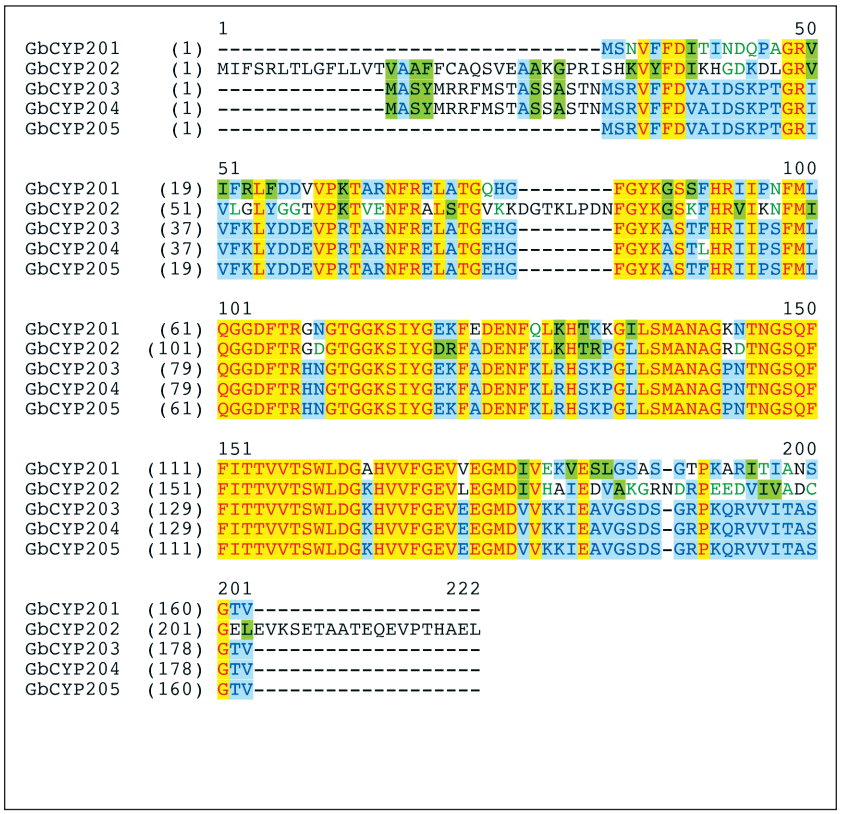

Figure 1. Alignment of deduced amino acid residues encoding CYP clones from G. boninense. *Highly conserved regions highlighted in yellow. Sequences with greater than 50% identities highlighted in blue. Sequences with less than 50% identities highlighted in green.*

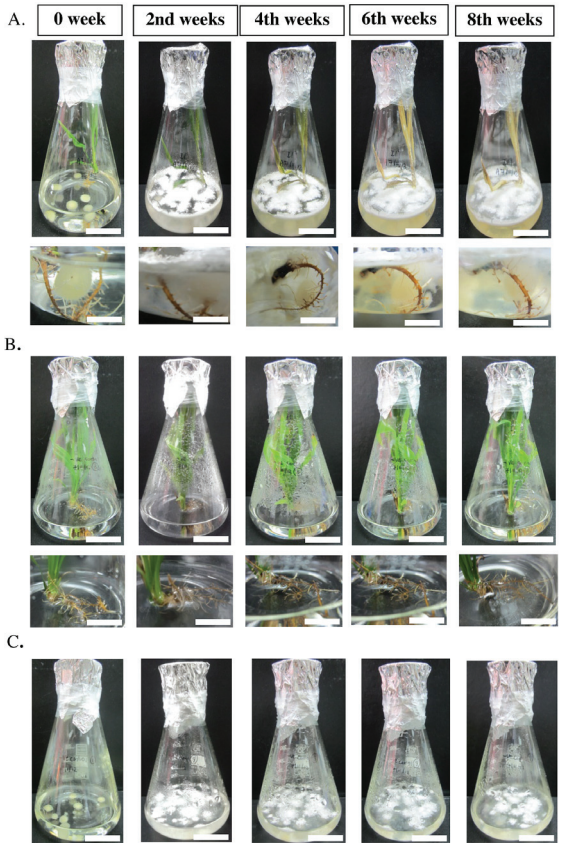

Figure 2. Oil palm clonal plantlet and G. boninense *mycelium clumps up to eight weeks of inoculation. A. Inoculation flask with oil palm clonal plantlet and* G. boninense. *B. Controlled flask with oil palm clonal plantlet only. The upper panel shows the appearance of oil palm clonal plantlets (Bar: 4 cm). The lower panel shows the root condition of the plantlets (Bar: 2 cm). C. Controlled flask with* G. boninense *mycelium only (Bar: 4 cm).*

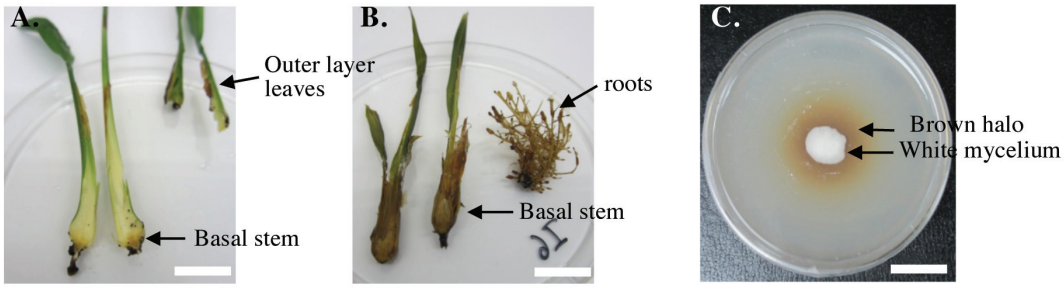

Figure 3. Dissection of controlled and infected oil palm clonal plantlets and detection of G. boninense *on GSM. A: Controlled oil palm (Bar: 1.0 cm). B:* Ganoderma *infected oil palm, 4 weeks after inoculation (Bar: 1.2 cm). C. Detection of* G. boninense *in oil palm tissue using GSM (Bar: 2.0 cm).*

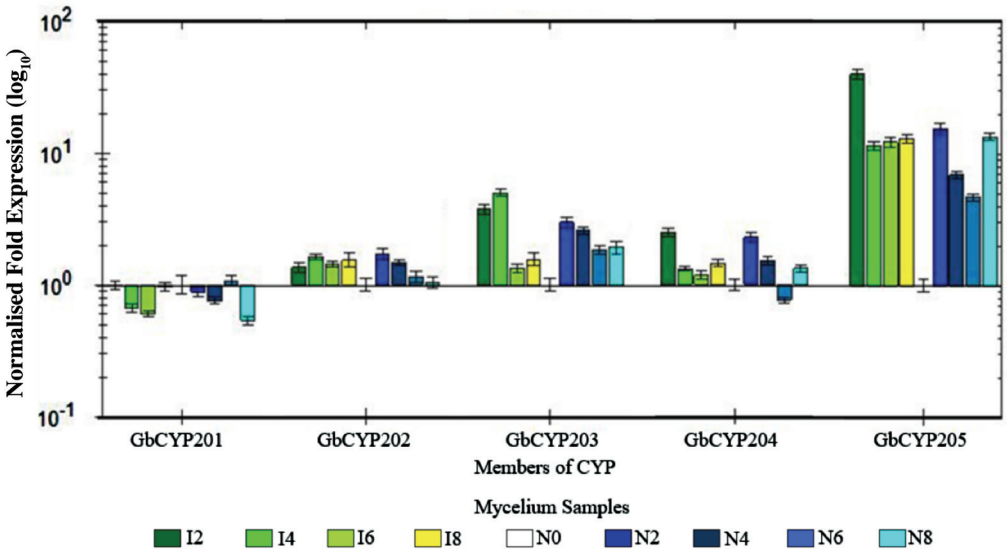

Figure 4. Expression of five CYP clones in G. boninense *mycelium samples during* in vitro *infection test. The relative normalised expression is presented in fold change (log_{10}). Standard deviations are shown by vertical bars. "N" indicates the controlled samples. "I" indicates the treated samples. Figures after the alphabet indicate weeks after inoculation.*

BENEFITS OF THE STUDY

Understanding the functions of CYP transcripts especially those involved in fungal pathogenicity could lead to understanding of the infection mechanism of *G. boninense*. The CYP clones, GbCYP203 and GbCYP205 could be used to enhance tolerance of oil palm against BSR. In addition, qPCR was developed to study the gene expression of *G. boninense* in different type of tissues. This method could be used to profile the expression of other putative pathogenicity genes or other genes such as xylanase and cellulase in *G. boninense*. The *in vitro* infection technique is less labour intensive and less time consuming than rubber wood block (RWB) sitting (Idris *et al.*, 2006) or root inoculation (Idris *et al.*, 2000) technique. This technique offers an option for preliminary study prior to nursery / field trial.

WHO WOULD BENEFIT?

Plant pathologists, molecular biologists, academicians and researchers could benefit from using the cDNA encoding *G. boninense* CYP to enhance tolerance of oil palm against BSR.

CONCLUSION

Five full-length cDNA encoding CYP have been successfully amplified from *G. boninense*. Based on qPCR expression analysis, two CYP clones, GbCYP203 and GbCYP205 were found to be upregulated during early weeks of infection and could be involved in *Ganoderma* stress response or pathogenicity. The genetic information of these two CYP clones could be used to enhance the tolerance of oil palm against BSR.

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For more information, kindly contact:

Director-General
MPOB
6, Persiaran Institusi,
Bandar Baru Bangi,
43000 Kajang, Selangor,
Malaysia
Tel: 03-8769 4400
Fax: 03-8925 9446
www.mpob.gov.my