

Identification of laccase genes in *Ganoderma boninense* draft genome assembly

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Background

Ganoderma boninense, a soil born fungus, is the main agent of basal stem rot, one of the most devastating diseases of oil palm (*Elaeis guineensis*). Observation of oil palm infection by *G. boninense* in natural conditions has shown the fungus capacity to quickly degrade the stem base, leading to important cracks and finally to tree fall (eg. Fig.1). This important degradation of host tissue likely implies ligninolytic enzymes, in particular laccase activity. Laccases are blue copper oxidase and catalyze the one-electron oxidation of phenols, aromatic amines and other electron-rich substrates by reducing molecular oxygen to water through an oxidoreductive multi-copper system (Thurston 1994). Fungal laccase are typically around 500 to 550 amino acids in-length, bears a signal peptide and exhibit a four domain sequence signature encompassing cysteine and histidine residues involved in copper binding (Kumar *et al.*, 2003). Fungal laccases have been well described in several rot disease involving fungus and their role in the pathogenicity of some fungus like the honey mushroom (*Armillaria mellea*) is well established (Yaver *et al.*, 1996, Schwarze & Baum 2000). In this context, the identification and study of *G. boninense* laccase genes seems to be of key interest to a better understanding of basal stem rot disease

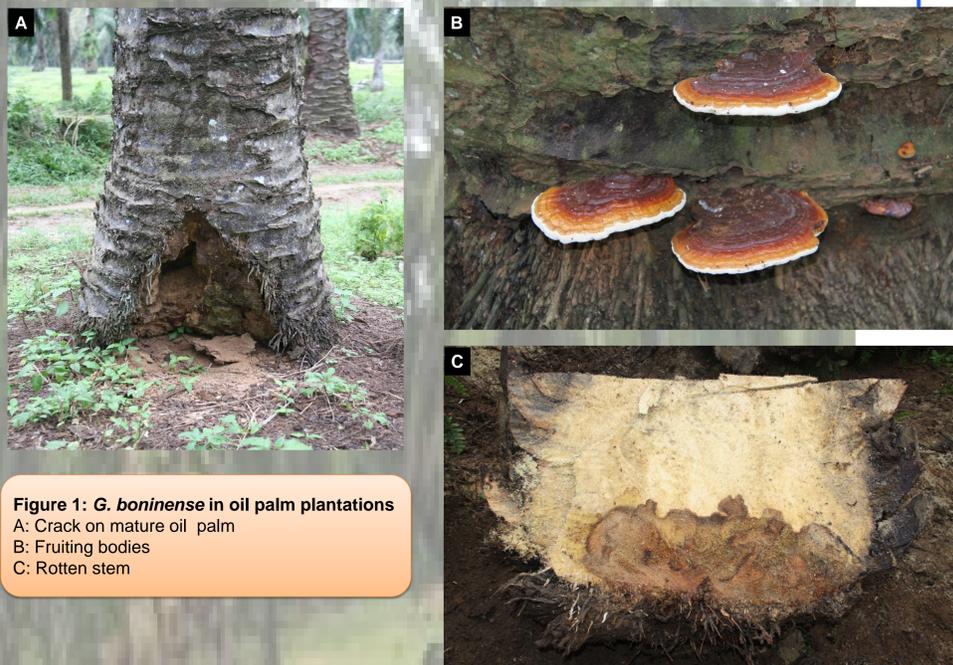


Figure 1: *G. boninense* in oil palm plantations
A: Crack on mature oil palm
B: Fruiting bodies
C: Rotten stem

Preliminary genome assembly characteristics, gene prediction and transcriptome assembly

Our preliminary genome assembly gathers 5,754 scaffolds for a total sequence length of 71×10^6 bp and N50 scaffold length of 11,474 bp. A total of 22,228 gene models were predicted. As transcriptomic data, we obtained 229×10^6 raw reads from Illumina HiSeq 2000 machine, with total sequence length of 23×10^6 bp. 66,807 contigs were obtained with average length of 1,796 bp.

Laccase mining

Requesting "laccase" and "fungi" in the NCBI nr protein database cropped a total of 4,349 entries. After applying the different filters, we retained 841 protein sequences. HMMER software retrieved 33 *G. boninense* gene models showing meaningful similarities to the input of 841 fungus laccase.

G. boninense predicted laccase characteristics

Among these 33 gene models fitting HMMER laccase pattern, 25 bore the 4 domain laccase signature. These 25 gene models display a predicted coding region length between 1,473 bp and 1,926 bp (between 491 and 642 amino acids). These gene models are distributed on seventeen scaffolds. Scaffold 1 bears three gene models with the 4 domain laccase signatures, scaffold 49 bears three gene models with the four domain laccase signatures, scaffold 46, 149 and 197 bears two gene models each with the four domain laccase signatures. Predicted intron number varies between 3 and 12. Twenty four gene models are predicted to be secreted and 23 are displaying a predicted peptide signal. Out of the 25 gene models with 4 domain laccase signatures, 7 showed strong similarity with contigs from assembled transcript (similarity between 93 to 99 % over more than 1,300 bp).

G. boninense predicted laccase among fungal laccases

We performed a phylogenetic analysis on the 25 *G. boninense* predicted laccases displaying the full four domain laccase signature with laccases from four other fungi species (eg fig 2). *G. boninense* predicted laccases are found in two groups. Gene model g9396 and g 4264 gather with a *G. lucidum* and two *L. bicolor* laccases belonging to laccase *sensu lato* (Hoegger *et al.* 2006). These two *L. bicolor* laccases are ferredoxinase. The other 23 *G. boninense* predicted laccase gather in a large group composed of polyporales laccases, including all five *T. versicolor* laccases and 12 *G. lucidum* laccases. The seven laccase predicted gene models with transcript evidence are located in the polyporales gene cluster.

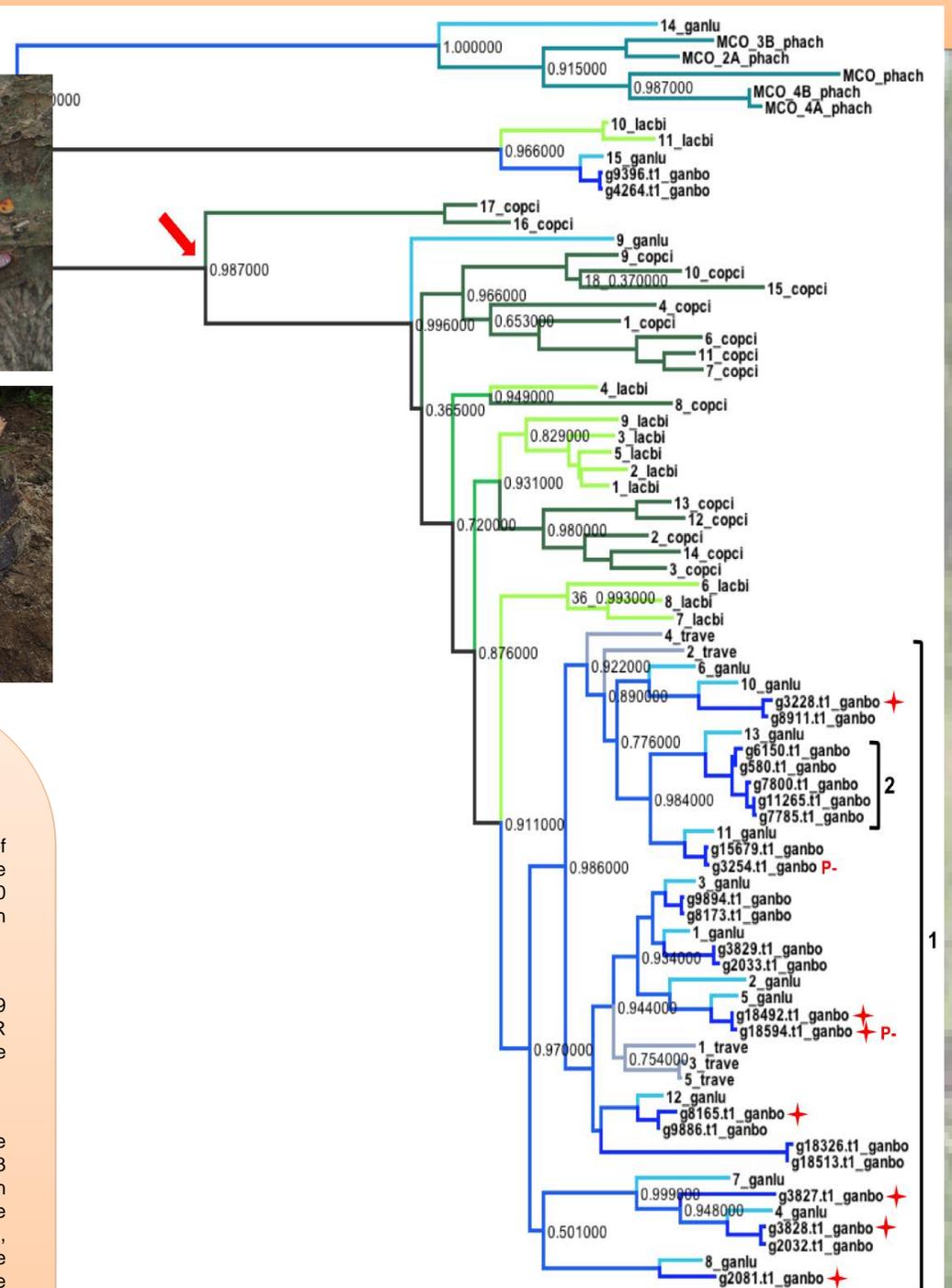


Figure 2: Phylogenetic tree for *G. boninense* predicted laccases Phylogenetic tree was obtained with PhyML (Guindon *et al.* 2010) using default values for amino acid analysis. It is rooted on the median point. ganbo: *G. boninense* predicted laccase gene models (electric blue), ganlu: *G. lucidum* (cyan), trave: *T. versicolor* (grey), phach: *P. chrysosporium* (Turkish green), copci: *C. cinerea* (dark green), lacbi: *L. bicolor* (light green). Plain blue indicates branches specific to polyporales and plain green indicates branches specific MCO: Multi Copper Oxidase. Branch support values were obtained with LRT SH-like test. A red arrow indicates *sensu stricto* laccases. Bracket one underline a laccase clade specific to polyporales and bracket two pinpoint a possible copy expansion in *G. lucidum*. Red star indicates *G. boninense* gene model with transcript match and red "P-" indicates *G. boninense* gene models with no peptide signal

Conclusions

We identified 25 gene models exhibiting the 4 domain fungal laccase signature and the main characteristic of fungal laccases in term of length, cellular addressing and signaling and intron number. Transcript evidence supports seven of these gene prediction. Our results suggest the existence of a specific group of polyporales *sensu stricto* laccases (Hoegger *et al.* 2006) for which ancient duplications occurred (e.g. fig 2). This group includes laccases from the white rot *G. lucidum*, *G. boninense* and *T. versicolor* but not *P. chrysosporium*. Within the polyporales *sensu stricto* laccase group, the phytopathogenic *G. boninense* exhibit a noticeable behavior, with the specific expansion of some gene sub-family.

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