Bioinformatics analysis of predicted *Ganoderma boninense* from oil palm (*Elaeis guineensis*)

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**Abstract.** The current report examines the bioinformatics approaches to analyse 13 predicted *Ganoderma boninense* genes from *Elaeis guineensis* Jacq. along with predicted the assembly, pattern, potential transit peptide, and subcellular localisation. The length of the genes was varied with the genes examined, from 209 to 222 bp. It is noteworthy the physicochemical heterogeneity properties consisting comparative molecular weight, theoretical isoelectric point value, the total atomic number, extinction coefficient, instability coefficient, aliphatic index, and general average hydrophaticity along with the analysed genes. Based on stability coefficients, 13 *G. boninense* genes were unstable proteins, mostly stored in the cytoplasm, microbody (peroxisome), and endoplasmic reticulum (membrane). In contrast to this observation, a few genes were existed to the plasma membrane. BLAST search showed that 13 sequences of *G. boninense* isolates show high similarity (89-99%) to the *G. boninense* strain NJ3 in the database of NCBI. These findings pointed the significant knowledge on the diversity and role of physical and chemical characteristics of the distinguishable amino acids in *G. boninense* isolates.

1. Introduction

Indonesia is currently the world's first palm oil producer with 36.5 million metric tons of palm oil production or nearly 70% of world palm oil production [1]. Inappropriately, oil palm plantations (*Elaeis guineensis* Jacq.) in Asia, particularly in Indonesia and Malaysia face the threat of basal stem root (BSR) diseases caused by *Ganoderma boninense* pathogens [2]. *G. boninense* caused loss of yield and finally killed the palm trees. The *G. boninense* control is widely studied and developed nowadays by using tolerant plants; however, so far no particular method available to handle the enduring widespread of the BSR disease [2-3].

Molecular cellular responses of oil palm to these pathogens are not well understood although several lines of genes have been proposed and identified to tolerance or resistance to *G. boninense* [3-5]. These genes are vital to strategise active portions to exterminate BSR. Recently a genome sequence
of the phytopathogenic fungus *G. boninense* from Indonesian strain G3 has been reported [6], showing a severe symptom of BSR disease in North Sumatra, an endemic BSR. However, the bioinformatics analysis of *G. boninense* tolerance from *E. guinensis* has not previously been available. The present report thus purposed to examine 13 sequences of *G. boninenses* isolates from oil palm using the established bioinformatics method [7-8].

2. Materials and method

2.1. Materials

Selected thirteen isolates of *Ganoderma boninense* from oil palm (*Elaeis guineensis*Jacq, Arecaceae) belong to PT Socfindo were collected. The isolates namely 006PTN3, 25, 3J, CS7, 202, Payah Pinang, 192, NJ72, CS1, 191, NJ3, 001MJR, and NJ54 was sequenced as shown in Figure 1. The PCR primers were designed based on a previous study [9], primer 7, F: 5'-TCGGGTAGGCTCGCAGGTGG-3', R: 5'-GGGCCGCACAGGTCGAGAAA-3'. PCR with Primers 7F and 7R was performed by MyTaq Red Mix (Bioline) in line with the manufacturer’s protocol. PCR amplification outline was for 2 min at 94 ºC, followed by 35 cycles of 30 sec at 94 ºC, 30 sec at 60 ºC and 3 min 72 ºC, with the final extension of 10 min at 72 ºC. PCR products were purified with Zymoclean Gel DNA Recovery Kit (Zymo Research) prior sequenced.

2.2. Physicochemical properties of the *G. boninense* gene

The composition, physical and chemical properties of *G. boninense* isolate sequence was performed using ProtParam online (web.expasy.org/protparam/). The calculated factors define the molecular weight, theoretical isoelectric point values, atomic composition, extinction coefficient, half-life estimation, instability index, aliphatic index, and grand typical hydrophaticity as earlier shown [10].

2.3. Potential transit of peptide and subcellular localisation

To measure the transit peptide, the targetP1.1 server online (www.cbs.dtu.dk/services/targetp/) was employed. The site is built on the expected incidence of any of the N-terminal pre-sequences chloroplast transit peptide (cTP), mitochondrial targeting peptide (mTP), and secretory pathway signal peptide (SP). Likewise, PSORT Prediction online (psort.hgc.jp/form.html) was applied to control the subcellular localisation of 13 sequences of *G. boninense* as previously described [10].

![Figure 1](image)

*Figure 1.* 1 µL PCR Products were assessed by electrophoresis with 1% TBE agarose. Sample numbers 1-13 denoted as isolates:006PTN3, 25, 3J, CS7, 202, Payah Pinang, 192, NJ72, CS1, 191, NJ3, 001MJR, and NJ54.

3. Results and Discussions

3.1. Physicochemical features

Table 1 depicts some linefactors of physicochemical of *G. boninense* isolates extracted from oil palm plantation. The *G. boninense* sequence consists of thirteen clones. The length of the genes was varied
with the genes examined, from 209 to 222 bp. It is notable the heterogeneity of comparative molecular weight, theoretical isoelectric point value, the total number of atoms, extinction coefficient, instability coefficient, aliphatic index, and general average hydropathicity along with the analysed genes (Table 1). BLAST search shows that the 13 13 sequences of *G. boninenses* isolates from oil palm shows high similarity (89-99%) to the *G. boninense* strain NJ3 in the database of NCBI.

It is important to note that the approximate half-life period was diverse among the genes (1.2-7.2 h). Oxidosqualenecyclase (OSC) genes had the same half-time from Rhizophoraceae tribe (4.4 h) [7], mangrove actin genes have been shown to have a variety half-time (1.3-30 h) [11], *Kandeliaobovata* polyprenolreductase gene and 14 expressed sequence tags from *Rhizophorastylosa* possessed 1.2-30 h of half-time [12, 13]. However, the predictable half-life of polyprenolreductase of plant species was surprisingly to have similar half-time (30 h) [8]. It is noteworthy that these clones had 5.10-5.29 theoretical isoelectric point values (Table 1) and 2188-2347 total numbers of atoms.

### Table 1. Physical and chemical characteristics of *G. boninense* isolates

| *G. boninense* clone | Clone 001MJIR | Clone CS57 | Clone 191 |
|----------------------|---------------|------------|-----------|
| Length of genes/bp   | 214           | 213        | 212       |
| Molecular weight     | 17832.49      | 17829.45   | 17658.27  |
| Theoretical isoelectric point values | 5.10 | 5.11 | 5.28 |
| Total number of atoms| 2214          | 2223       | 2193      |
| Extinction coefficient| 4125          | 4000       | 4000      |
| Half-life period     | 7.2h          | 7.2h       | 1.2h      |
| Instability coefficient| 52.16        | 52.55      | 52.96     |
| Aliphatic index      | 25.23         | 25.82      | 25.00     |
| Grand average of hydropathicity | 0.992 | 0.997 | 0.981 |

| *G. boninense* clone | Clone 192 | Clone J | Clone NJ3 |
|----------------------|-----------|---------|-----------|
| Length of genes/bp   | 212       | 212     | 217       |
| Molecular weight     | 17672.30  | 17612.25| 18370.16  |
| Theoretical isoelectric point values | 5.29 | 5.29 | 5.29 |
| Total number of atoms| 2196      | 2188    | 2293      |
| Extinction coefficient| 4000      | 4000    | 4125      |
| Half-life period     | 4.4h       | 4.4h    | 4.4h      |
| Instability coefficient| 53.07     | 55.43   | 55.41     |
| Aliphatic index      | 25.47      | 26.42   | 26.73     |
| Grand average of hydropathicity | 0.992 | 1.015  | 1.018    |

| *G. boninense* clone | Clone NJ54 | Clone NJ72 | Clone Paya Pinang |
|----------------------|------------|------------|-------------------|
| Length of genes/bp   | 213        | 210        | 212               |
| Molecular weight     | 17867.61   | 17644.27   | 17790.43          |
Theoretical isoelectric point values  5.27  5.11  5.10
Total number of atoms  2215  2199  2214
Extinction coefficient  4250  4000  4000
Half-life period  1.2h  7.2h  7.2h
Instability coefficient  53.53  48.92  52.35
Aliphatic index  25.35  25.71  25.00
Grand average of hydropathicity  1.026  0.987  0.997

Based on stability coefficients, 13 G. boninense genes were non-stable proteins. A few genes have been shown as stable genes from plant polyprenol reductase genes, for example, Glycine max, G. arabretum, and G. raimondii[8]. OSC genes also have been described in Bruguiera gymnorrhiza β-amyrin synthase (BgbAS)and Rhizophora stylosa cycloartenol synthase(RcCAS) also had stable proteins [7], stable proteins were derived from mangrove actin genes: B. gymnorrhiza BgAct1, KcAct1 from K. candel, and RsAct1 from R. stylosa [11]. Very recently, Rs1 from R. stylosa EST had the stable gene (31.52) [14]. These findings demonstrated the significant agreement for the variation and role of physical and chemical characteristics of the distinguishable amino acids in G. boninense isolates [8].

3.2. Prospective transit of peptide and subcellular localisation

Table 2 describes the prospect of the impending transit peptide in 13 clones from G. boninense. There are three options: chloroplast transit peptide, mitochondrial target peptide and signal peptide of secretory pathway together with the estimate possibility. The target chloroplast diversified from 0.090 to 0.274, with the uppermost values of chloroplast be appropriate to clone C5 (0.274), designated that chloroplast transit peptide existing in the nominee genes of Ganoderma tolerance. It is remarkable that the target peptide assessment of mitochondria was a reduced amount of associated with chloroplast transit or mitochondrial peptide. The maximum indicator peptide of the secretory pathway was CS1. Reliability prediction value of 3 (77%) dominated in the G. boninense the genes.

Table 3 illustrates the subcellular localisation of partial fragment genes in G. boninense. The subcellular localisation of these genes was generally resided in the cytoplasm, microbody (peroxisome), and endoplasmic reticulum (membrane). In contrast, a few genes kept in the plasma membrane, such as C001MJIR, CS57, CJ, and CNJ54 (Table 3).

Recently, it has been reported that the expression of triterpenoid synthase genes was increased the triterpenoid concentration of entire cell body and plasma membrane portions [7]. Table 3 shows that C001MJIR, CS57, CJ, and CNJ54, which show the uppermost value of cytoplasm were employed on the plasma membrane, supported previous papers on their subcellular localisation of triterpene genes sited in the plasma membrane [7, 13]. The reliability and practically of the plasma membrane are therefore a key element for salt tolerance mechanism in G. boninense[14].

G. boninense become an obstacle of the importance of oil palm as essential commodities in Indonesia to produce crude palm oil from the fruit mesocarp and palm kernel oil from the palm seed or kernel [15-16]. Recently the profile of polyprenols and dolichols in the leaves of oil palm plantations under different land-uses in North Sumatra, Indonesia has been described [17-18]. The finding of pattern polyprenoids in oil palm supported that replanting effort and the resolve of widespread of G. boninense in the oil palm plantation areas both small holders and companies.
Table 2. The possibility of the potential transit peptide in *Ganoderma boninense* polypropenolreductase

| *G. boninense* clone | Chloroplast transit peptide | Mitochondrial target peptide | Signal peptide of secretory pathway | Reliability prediction |
|----------------------|-----------------------------|-----------------------------|-----------------------------------|------------------------|
| C202                 | 0.116                       | 0.184                       | 0.019                             | 3                     |
| CS1                  | 0.110                       | 0.148                       | 0.026                             | 3                     |
| C06PTPN3             | 0.123                       | 0.196                       | 0.027                             | 3                     |
| C5                   | 0.274                       | 0.105                       | 0.021                             | 4                     |
| C001MJIR             | 0.090                       | 0.174                       | 0.015                             | 2                     |
| CS57                 | 0.127                       | 0.155                       | 0.019                             | 3                     |
| C191                 | 0.131                       | 0.187                       | 0.014                             | 2                     |
| C192                 | 0.123                       | 0.191                       | 0.012                             | 3                     |
| CJ                   | 0.152                       | 0.153                       | 0.014                             | 3                     |
| CNJ3                 | 0.196                       | 0.153                       | 0.013                             | 3                     |
| CNJ54                | 0.115                       | 0.155                       | 0.019                             | 3                     |
| CNJ72                | 0.145                       | 0.154                       | 0.016                             | 3                     |
| Cpaya Pinang         | 0.166                       | 0.134                       | 0.016                             | 3                     |

Table 3. Subcellular localisation of predicted *G. boninense*

| *G. boninense* clone | Cytoplasm | microbody (peroxisome) | mitochondrial matrix space | endoplasmic reticulum (membrane) | Plasma membrane |
|----------------------|-----------|------------------------|---------------------------|----------------------------------|-----------------|
| C202                 | 0.450     | 0.252                  | 0.100                     | 0.000                            | nd              |
| CS1                  | 0.650     | nd                     | 0.100                     | 0.000                            | nd              |
| C06PTPN3             | 0.650     | nd                     | 0.100                     | 0.000                            | nd              |
| C5                   | 0.650     | nd                     | 0.100                     | 0.000                            | nd              |
| C001MJIR             | 0.650     | nd                     | 0.100                     | 0.000                            | nd              |
| CS57                 | 0.650     | nd                     | 0.100                     | 0.000                            | 0.100           |
| C191                 | 0.450     | 0.207                  | 0.100                     | 0.000                            | nd              |
| C192                 | 0.450     | 0.305                  | 0.100                     | 0.000                            | nd              |
| CJ                   | 0.650     | nd                     | 0.100                     | 0.000                            | 0.100           |
| CNJ3                 | nd        | nd                     | 0.528                     | nd                               | nd              |
| CNJ54                | 0.650     | nd                     | 0.100                     | 0.000                            | 0.100           |
| CNJ72                | 0.450     | 0.279                  | 0.100                     | 0.000                            | nd              |
| Cpaya Pinang         | 0.450     | 0.491                  | 0.100                     | 0.000                            | nd              |

nd= not detected

4. Conclusions
There are four clones: C001MJIR, CS57, CJ, and CNJ54, showing the utmost value on the plasma membrane, reinforced priorstudies on the subcellular localisation of triterpenoid genes detected in the plasma membrane. The current report indicated the significant role of features of the partial clones of probably *G. boninense* tolerance genes in *E. guinenesis* genomic library.

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