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Genetic diversity and structure of *Ganoderma boninense* isolates from oil palm and other plantation crops

AGUSTIAMAN PURBA[1], RAHMAH HAYATI[1], LOLLIE A.P. PUTRI[2], DIANA CHALIL[3], DADANG AFANDI[4], INDRA SYAHPUTRA[4], MOHAMMAD BASYUNI[5♥]

[1]Graduate School of Agrotechnology, Faculty of Agriculture, Universitas Sumatera Utara. Jl. Dr. A Sofyan No. 3, Medan 20155, North Sumatra, Indonesia
[2]Department of Agrotechnology, Faculty of Agriculture, Universitas Sumatera Utara. Jl. Dr. A Sofyan No. 3, Medan 20155, North Sumatra, Indonesia
[3]Department of Agribusiness, Faculty of Agriculture, Universitas Sumatera Utara. Jl. Dr. A Sofyan No. 3, Medan 20155, North Sumatra, Indonesia
[4]Laboratory of Biomolecular, PT Socfin Indonesia, Martebing, Dolok Masihul, Serdang Bedagai 20991, North Sumatra, Indonesia
[5]Department of Forestry, Faculty of Forestry, Universitas Sumatera Utara. Jl. Tri Dharma Ujung No. 1, Medan 20155, North Sumatra, Indonesia.
Tel./fax.: +62-61-8201920, ♥email: m.basyuni@usu.ac.id

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Abstract. *Purba A, Hayati R, Putri LAP, Chalil D, Afandi D, Syahputra I, Basyuni M. 2020. Genetic diversity and structure of* Ganoderma boninense *isolates from oil palm and other plantation crops. Biodiversitas 21: 451-456.* Oil palm is an economically important plant, which one of the most important sources of vegetable oil in the world. However, oil palm plantation and other crops face the treat basal stem rot (BSR) disease by *Ganoderma boninense*. A study on genetic diversity and structure of *G. boninense* is therefore needed in order to formulate improved control strategies for this disease. This work aimed to analyze the genetic diversity and structure of the *G. boninense* isolates derived from different hosts, 131 oil palm (*Elaeis guineensis*), six rubber (*Hevea brasiliensis*), three coconuts (*Cocos nucifera*), and three lontar palm (*Borassus flabellifer*). Genetic diversity and population structure of *G. boninense* isolates were investigated using six SSR markers with GenAlex 6.502 software. Results showed that several microsatellite loci indicated specific primary success rates, such as KT124402, KT124399, and KT124394, depicting high polymorphism content (>75%). This result suggested that these markers were equally effective in determining the polymorphisms of *G. boninense* isolates. A hierarchical analysis of molecular variance (AMOVA) revealed that genetic diversity mostly found among individuals within a population (88%), then among populations (8%), and within individuals (4%).. Phylogeny analysis showed two clusters of *Ganoderma* isolates. which was considered variation as dissimilar across with origin. The present study indicated that *G. boninense* from oil palm was predominantly comprised of a genetically distinct individual.

Keywords: AMOVA, *Ganoderma disease*, microsatellite, polymorphism

Abbreviations: FBSR-CN: fruiting body basal stem rot-*Cocos nucifera*, FBSR-BF: fruiting body basal stem rot-*Borassus flabellifer*, FBSR-EG: fruiting body basal stem rot-*Elaeis guineensis*, FUSR-EG: fruiting body upper stem rot-*E. guineensis*, FBSR-HB: fruiting basal stem rot-*Hevea Brasiliensis*, FRWB-HB: fruiting body rubberwood block-*H. brasiliensis*, TUSR-EG: tissue upper stem rot-*E. guineensis*, TBSR-EG: tissue basal stem rot-*E. guineensis*, SBSR-EG: spore basal stem rot-*E. guineensis*, FB-EG: fruiting body-*E. guineensis*, T-EG: tissue-*E. guineensis*, FBD-EG: fruiting body died-*E. guineensis*, RFBS-EG: re-isolation fruit body seedling-*E. guineensis*, PDA: potato dextrose agar, WA: water agar.

INTRODUCTION

Oil palm (*Elaeis guineensis*) is an economically important plant, which one of the most important sources of vegetable oil in the world and a good fuel of biodiesel (Pleanjai and Shabbir 2009). A number of studies have been reported that *Ganoderma boninense* was a major pathogen causing diseases to attack oil palm (Paterson 2007), even to a lethal level (Hushiarian et al. 2013), coconut tree (Kandan et al. 2008), rubber tree (Sariah et al. 1994), lontar palm (Sankaran et al. 2005). Stem basal rot (SBR) has been reported to appear in various regions in Africa, includes Angola, Cameroon, Ghana, Nigeria, Zambia, Sao Tome, Tanzania, Zimbabwe and the Republic of the Congo; in America occurred in Honduras, and in Oceania found in Papua New Guinea, and South East Asia countries (Wang et al. 2009).

BSR can cause significant damage (Ishaq et al. 2014). Areas that constitute the BSR zone were North Sumatra and along the west coast of Peninsular Malaysia. Many infections occur with the appearance of the fruit body at the base of the plant. The spear leaves do not open, and canopy turns yellow (Breton et al. 2008). Oil palm, rubber, and tropical plantations crop suffer losses due to rootstock rot, caused by *G. boninense* (Caro et al. 2014), which is a fungus that causes death to plants. Many oil palm plantations experience considerable losses caused by the impact of disease of *Ganoderma*, used of planting materials that are resistant is needed (Purba et al. 2019). Total resistance to fungi has rarely been reported, but many examples have been observed, including in oil palm (Durand-Gasselin et al. 2014), rubber (Lim 1997), coconut (Zakaria 2005) and lontar palm (Rajendran et al. 2014).

Furthermore, this disease was difficult to diagnose, and pathogens can be presented without the appearance of symptoms but have naturally infected plants (Corley and Tinker 2003). On the other hand, *G. boninense* has a slow growth development (Ho and Nawawi 1986) but has a long-lasting ability because of the resting spore and *pseudosclerotium* (Blanchette 1984). Darmono (1998) has reported that *G. boninense* found in Indonesia to have a molecular difference. In this circumstance, the phenotypic variation of *G. boninense* from several regions in Indonesia does not have very close relations (Purba et al. 2019). However, genetic diversity on *G. boninense* is not fully understood. Recently molecular performances showing the grouping of plant resistance to *G. boninense* occurred in healthy leaves of mature oil palm (Afandi et al. 2018). Furthermore, polyisoprenoid carbon chain-length has been shown to be a chemotaxonomic marker for the screening of oil palm resistance to *G. boninense* (Afandi et al. 2019. Therefore, a study on genetic diversity and structure of *G. boninense* is needed in order to formulate improved control strategies for this disease. The present work aimed to investigate the genetic diversity and structure of the *G. boninense* isolates from mostly *E. guineensis* and other plantation crops.

MATERIALS AND METHODS

Ganoderma boninense isolates

The materials used in this work were 147 *Ganoderma boninense* isolates, which were from different hosts, 131 oil palm (*Elaeis guineensis*), six rubber (*Hevea brasiliensis*), three coconuts (*Cocos nucifera*), and three lontar palm (*Borassus flabellifer*). These isolates were derived from the Pathology Laboratory of PT Socfin Indonesia collections. The isolates were initially from North Sumatra (Serdang Bedagai, Asahan, Simalungun, Tebing Tinggi, Central Tapanuli, Batubara, Labuhan Batu Utara, and Medan), West Sumatra, South Sumatra, and Sulawesi, Indonesia. Source of isolates were grouped as 13 populations that previously described (Purba et al. 2019) as follows. FBSR-CN (fruiting body basal stem rot-*Cocos nucifera*), FBSR-BF (fruiting body basal stem rot-*Borassus flabellifer*), FBSR-EG (fruiting body basal stem rot-*Elaeis guineensis*), FUSR-EG (fruiting body upper stem rot-*E. guineensis*), FBSR-HB (fruiting basal stem rot-*Hevea Brasiliensis*), FRWB-HB (fruiting body rubberwood block-*H. brasiliensis*), TUSR-EG (tissue upper stem rot-*E. guineensis*), TBSR-EG (tissue basal stem rot-*E. guineensis*), SBSR-EG (spore basal stem rot-*E. guineensis*), FB-EG (fruiting body-*E. guineensis*), T-EG (tissue-*E. guineensis*), FBD-EG (fruiting body died-*E. guineensis*), RFBS-EG (re-isolation fruit body seedling-*E. guineensis*). The majority of isolates were sourced from oil palm (*Elaeis guineensis* Jacq., Arecaceae).

Growth of *Ganoderma boninense* isolates on potato dextrose agar (PDA)

The growth of *G. boninense* isolates was carried out as previously reported (Purba et al. 2019). Briefly, the isolates were conserved, stored in an incubator at 20°C. Preparation of isolates was carried out in laminar airflow under sterile conditions. Mycelium was taken from pieces of PDA (potato dextrose agar) from a Wheaton tube using a needle, then subcultured into water agar (WA) media (+chloramphenicol, streptomycin) in three petri dishes, which was prepared for five days before the subculture performed. Mycelium, which grows on WA media, was subcultured into the PDA (+chloramphenicol) medium of five petri dishes, which have been prepared five days before subculture was carried out (Naher et al. 2012). Then, the *G. boninense* isolates incubated in dark conditions at 28°C for ten days, as shown in Figure 1.

The DNA extraction

Ganoderma boninense mycelium, which has been grown on the PDA media, were used for DNA extraction. Total DNA was extracted from *B. boninense* using the CTAB method, as previously described with minor modification (Basyuni et al. 2017). The DNA quality was tested based on UV-illuminator and documented using the Gel doc. DNA was quantified using the nanophotometer method, using wavelengths (λ) 260 and 280 nm.

PCR Amplification

In this study, the SSR (Simple Sequence Repeat) primers for the Polymerase Chain Reaction (PCR) used 6 primers pairs (Table 1) as previously reported (Merciere et al. 2015). Amplification reaction for PCR was done in 10 µl of the total volume containing 3 µL of DNA templates mixed with 2.5 µL Gotaq master, 0.5 µL forward primer and 0.5 µL reverse, primer and 3.5 µL ddH_2O.

PCR amplification was performed on Eppendorf Mastercycler ep 384 (Eppendorf, Westbury, New York, USA). The amplification program consisted 35 cycles at 95°C for 4 min, followed by 10 sec at 94°C, annealing at 52°C for 75 sec, and elongation were processed at 72 °C for 90 sec and a final extension at 72°C for 8 min. PCR product was performed by electrophoresis and documented with UV-transilluminator (UV-Doc) and Gel-Doc (U Doc) as previously described (Afandi et al. 2018).

Figure 1. The mycelium *Ganoderma boninense* have been grown on PDA

Table 1. Description of primer sequences used to this study

Primer	Primer sequences (5'-3')	Amplicon (bp)
KT124397	F: CGCCATGCCCACCACCAGAG R: GACCCGGCTGCCCGAATGAG	283-325
KT124402	F:ACAAGGCTCAAGGCAGCGCA R: GCACACCCCAGCAACAGGAGG	212-224
KT124403	F: GGCGACGAGGGCACGAGAGA R:CCGCACTTTCGCCAACCACC	273-297
KT124400	F:AGCTCCCCTCCCAGCTCCAAC R:GAATGCGGCGGGGAAACGGA	171-186
KT 124399	F:GCACAGGCACAAGCGCAAGG R:CGACGACCGCCCCAAAGGAT	204-267
KT 124394	F:CGGGAAGTGGTGAACGGTGGT R:GGGTGGCTTGACAGCGGCAT	234-243

Tabel 2. F-Statistics, total migrant and polymorphic information overall population

Loci	Fis	Fit	Fst	Nm	PIC
KT124397	0.80	0.86	0.31	0.57	0.56
KT124402	1.00	1.00	0.44	0.32	0.92
KT124403	0.78	0.85	0.31	0.56	0.53
KT124400	1.00	1.00	0.42	0.34	0.15
KT124399	0.76	0.85	0.38	0.41	1.00
KT124394	0.71	0.83	0.40	0.38	0.76
Mean	0.84	0.90	0.37	0.43	0.65
SE	0.05	0.03	0.02	0.04	0.12

Note: Allele frequency correlations between individuals in the subpopulation (Fis), allele frequency in the population caused by Fis and Fst (Fit), allele frequency correlations between subpopulation (Fst), the total of migrants (Nm), polymorphic information content (PIC).

Microsatellite data analysis

Genetic differentiation for each population and locus were assessed by calculating using GenAlEx ver 6.502 (Peakal and Smouse 2012) as frequency by alleles correlations between individuals in subpopulations (Fis), correlation of frequency between subpopulations (Fst), frequency by alleles in population caused by both factors (Fit), total migrants (Nm), number of different alleles (N), number of different allele frequencies >0.5% (Na), Number of active alleles (Ne) and Shannon of Information index (I). The polymorphisms for each population and locus were assessed by calculating the observed average heterozygosity (Ho), expected heterozygosity (He), and fixation index (F) (Nei 1978). Polymorphic Information Content (PIC) was determined by Avval (2017). The genetic structure analyzed was calculated using the GenAlEx analysis of molecular variance (AMOVA) ver 6.502 package (Peakal and Smouse 2012).

Phylogenetic analysis

Phylogenetic analysis was done based on the accession and grouping analysis of the phylogenetic tree of *Ganoderma boninense* isolates. Forward, they were analyzed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) by MVSP ver. 3.22 software (Basyuni et al. 2018).

RESULTS AND DISCUSSION

Selection of polymorphism marker

We identified of *G. boninense* isolates in six loci (KT124397, KT124402, KT124403, KT124400, KT124399 and KT124394) and 13 populations. Interpretation of genetic variation and interaction of locus frequencies between alleles was a collaboration between individual alleles and indicated Fis (0.84), as displayed in Table 2. The average frequency of the different isolate populations in the alleles identified was 0.90 (Fit). Wherein the second factor was obtained by Fst (0.37), while the maximum Fis (1.00) and Fit (1.00), the minimum Fis (0.71), and Fit (0.83). Total migration (Nm) by the mean number of alleles was 0.43 within populations; PIC 0.65 mean observed (Table 2). The PIC value of each SSR primers was determined by the number of alleles and the frequency of distribution within a population, wherein high PIC was > 0.5, moderate 0.5 > PIC > 0.25, and low PIC was < 0.25 (Bhattacharya et al. 2010). The polymorphic of this study ranged from 0.15 to 1.0 within thirteen populations. This genetic variation indicated that the genetic diversity within the population was very low in KT124400 loci (0.15) but higher in KT124399 loci (1.00). Among three loci, as reported by Merciere et al. (2015) showed the same mean PIC was KT124397 (0.56), KT124403 (0.53), and KT124394 (0.76) respectively.

This study suggested that some microsatellite loci data indicated specific primary success rates, this result showed only for locus KT124402, KT124399, and KT124394. There are not effective polymorphism locus within KT124400. The high level for a polymorphic recommendation (PIC > 0.5) of SSR markers and suggested that these markers were effective in determining the polymorphisms of *Ganoderma* isolates. The present study supported the previous reports (Afandi et al. 2018) that molecular markers to be considered as promising markers for *G. boninense* resistance screening oil palm. In addition to the polyisoprenoid pattern in *E. guineensis* as a potential biochemical marker in response to *G. boninense* infection (Afandi et al. 2019).

Microsatellite analysis for each population

Table 3 shows the microsatellite for the population of *G. boninense* isolates. The average number of alleles (N) for each population from 2.50 to 21.00, was observed. The highest value found in RFBS-EG was 21.00. The mean number of different alleles frequency >0.5% found (8.10); this value was similar to those previously reported for *Ganoderma boninense* (8.00) (Merciere et al. 2015). Estimated Ne values averaged 6.64 varying from 1.00 to 21.57 (Table 3), indicating that most individuals were necessary for one population or another one. However, I value ranging from 0.00 to 3.24, with an average was 1.45.

Table 3. The profile of microsatellite loci for all population of *Ganoderma boninense* isolates

Population	N	Na	Ne	I	Ho	He	uHe	F
FBSR-CN	2.50	3.17	2.93	1.11	0.31	0.66	0.83	0.52
FBSR-BF	2.83	3.00	2.84	1.05	0.14	0.63	0.77	0.79
FBSR-EG	51.33	30.83	21.57	3.24	0.03	0.95	0.96	0.97
FUSR-EG	20.00	16.33	12.82	2.66	0.03	0.92	0.94	0.97
FBSR-HB	3.00	2.33	2.23	0.69	0.28	0.42	0.50	0.39
FRWB-HB	3.00	3.50	3.07	1.06	0.50	0.57	0.68	0.19
TUSR-EG	17.00	12.33	10.18	2.37	0.00	0.89	0.91	1.00
TBSR-EG	11.00	9.00	8.20	2.13	0.00	0.87	0.91	1.00
SBSR-EG	3.00	1.67	1.53	0.42	0.00	0.30	0.36	1.00
FB-EG	5.00	4.00	3.62	1.29	0.00	0.69	0.77	1.00
T-EG	3.00	1.00	1.00	0.00	0.00	0.00	0.00	1.03
FBD-EG	3.00	1.00	1.00	0.00	0.00	0.00	0.00	0.50
RFBS-EG	21.00	17.17	15.26	2.78	0.00	0.93	0.96	0.89
Mean	11.21	8.10	6.64	1.45	0.10	0.60	0.66	0.82
SE	1.53	0.99	0.76	0.12	0.03	0.04	0.04	0.04

Note: A number of different alleles (N), number of different alleles frequency> 0.5% (Na), number of effective alleles (Ne), and Shannon of information index (I), observed heterozygosity (Ho), expected heterozygosity (He), unbiased expected heterozygosity (uHe), fixation index (F). FBSR-CN (fruiting body basal stem rot-*Cocos nucifera*), FBSR-BF (fruiting body basal stem rot-*Borassus flabellifer*), FBSR-EG (fruiting body basal stem rot-*Elaeis guineensis*), FUSR-EG (fruiting body upper stem rot-*E. guineensis*), FBSR-HB (fruiting basal stem rot-*Hevea Brasiliensis*), FRWB-HB (fruiting body rubberwood block-*H. brasiliensis*), TUSR-EG (tissue upper stem rot-*E. guineensis*), TBSR-EG (tissue basal stem rot-*E. guineensis*), SBSR-EG (spore basal stem rot-*E. guineensis*), FB-EG (fruiting body-*E. guineensis*), T-EG (tissue-*E. guineensis*), FBD-EG (fruiting body died-*E. guineensis*), RFBS-EG (re-isolation fruit body seedling-*E. guineensis*)

Table 4. Summary analysis of molecular variance (AMOVA) of *Ganoderma boninense* isolates population

Source	df	SS	MS	Est. Var.	% var
Among populations	12	122.51	10.21	0.24	8
Among individuals	134	724.88	5.41	2.65	88
Within individuals	147	16.50	0.12	0.11	4
Total	293	863.89		3.00	100

Note: df: Degree of Freedom, SS: Source of Variation, MS: Mean Squares, Est.Var: Estimation of Variant, Var: Variant

Allelic diversity by samples was estimated using heterozygosity (Ho and He). It is interesting to note that seven populations had Ho (0.00); these values could not be detected in samples TUSR-EG, TBSR-EG, SBSR-EG, FB-EG, T-EG, FBD-EG, and RFBS-EG. Furthermore, the others were successfully detected, the range value Ho from 0.03 to 0.31. A more appropriate measure of genetic variation in population was genes diversity (mean expected heterozygosity). He, in this population, was average (0.60), which was in the range (0.30 to 0.95) to be useful for measuring genetic variation with population T-EG and FBD-EG were not detected (He= 0.00). One of the factors that affects the dendrogram is the level of heterozygosity, in some organisms with average heterozygosity higher than 0.1 to construct a reliable dendrogram (Nei 1978). Genetic diversity was assessed by calculating unbiased expected heterozygosity (Raymond and Rousset 1995). uHe among population T-EG and FBD-EG are not detected (0.00), and using an average, the fixation index (F) of the population was 0.82. On the other hand, the *Ganoderma* isolates from Belitung Island have been reported with He of 0.77 (Jiat et al. 2019). Moreover, Merciere et al. (2015) have reported that the average He was 0.59 for *G. boninense* isolates, was similar to this study.

Genetic structure of *Ganoderma boninense*

The genetic structure of *G. boninense* was carried out by hierarchical analysis of molecular variance (AMOVA) using on the infinite alleles model (F-statistics). Table 4 shows locus by AMOVA analysis, which was done to undertake the populations as sources of variation. In this case, results derived from GeneAlex programs provide strong support of a *G. boninense* isolates origin *C. nucifera*, *B. flabellifer*, *E. guineensis*, and *H. brasiliensis*. This SSR marker seems to be worthy, mainly genetic diversity. The high average percentage of the source of variation (724.88) among individual, pointed out the genetic differences among individuals within populations.

In addition, AMOVA indicated the degree of freedom that 134 of the total genetic variation. While the mean square was 5.41 corresponded to the distinction among individuals. Percentages among individuals within the population were 88% variant, among populations was low (8%), and within an individual (4%) were estimated. This

genetic structure of *G. boninense* varied among the individuals was supported by the previous report that population structure could be the result of an arrangement of long-surviving isolates, possibly from adjacent areas planted with coconut and oil palm (Pilotti et al. 2003). Recently, Merciere et al. (2017) have reported on the AMOVA results that *G. boninense* had a very low variation between regions (1.59 %) and between plantations (0.97), but a very high variation within samples (97.44 %,), totally agreed with the present study.

Phylogenetic analysis of *G. boninense*

We identified the genetic variation from thirteen populations of FBSR-CN (fruiting body basal stem rot-*Cocos nucifera*), FBSR-BF (fruiting body basal stem rot-*Borassus flabellifer*), FBSR-EG (fruiting body basal stem rot-*Elaeis guineensis*), FUSR-EG (fruiting body upper stem rot-*E. guineensis*), FBSR-HB (fruiting basal stem rot-*Hevea Brasiliensis*), FRWB-HB (fruiting body rubberwood block-*H. brasiliensis*), TUSR-EG (tissue upper stem rot-*E. guineensis*), TBSR-EG (tissue basal stem rot-*E. guineensis*), SBSR-EG (spore basal stem rot-*E. guineensis*), FB-EG (fruiting body-*E. guineensis*), T-EG (tissue-*E. guineensis*), FBD-EG (fruiting body died-*E. guineensis*), RFBS-EG (re-isolation fruit body seedling-*E. guineensis*). It showed in Figure. 2. The dendrogram (UPGMA) showed two large groups in the population. The first group consisting of population was RFBS-EG, FB-EG, FBD-EG, T-EG, SBSR-EG, TBSR-EG, TUSR-EG, FUSR-EG and FBSR-EG. The second group consisted mainly of only four populations from FBSR-HB, FRWB-HB, FBSR-BF, FBSR-CN. Dendrogram was concluded that the clades represented separated populations of origin. *G. boninense* distinct most closely related to isolates from *E. guineensis* (cluster I) dissimilar with isolates from *H.Brasiliensis* (cluster II), *B. flabellifer* (cluster III) and *C.nucifera* (cluster IV). This study supported likewise *Ganoderma boninense* is the major disease factor to *E. guineensis* plantations in the world (Gorea et al. 2019). On the other hand, *Ganoderma sp.* have been reported from the tea and rubber plantation were more closely related compared to oil palm and the forest trees, furthermore, the species were identified especially to *G. boninense* from *E. guineensis* and *C.nucifera*, whereas *G. philippii* from *H. brasiliensis* (Nusaibah et al. 2011). *Ganoderma* disease caused by *G. psuedoferreum* has been reported caused fruiting bodies were seen in the *H. brasiliensis* stumps in the plantation (Ogbebor et al. 2010).

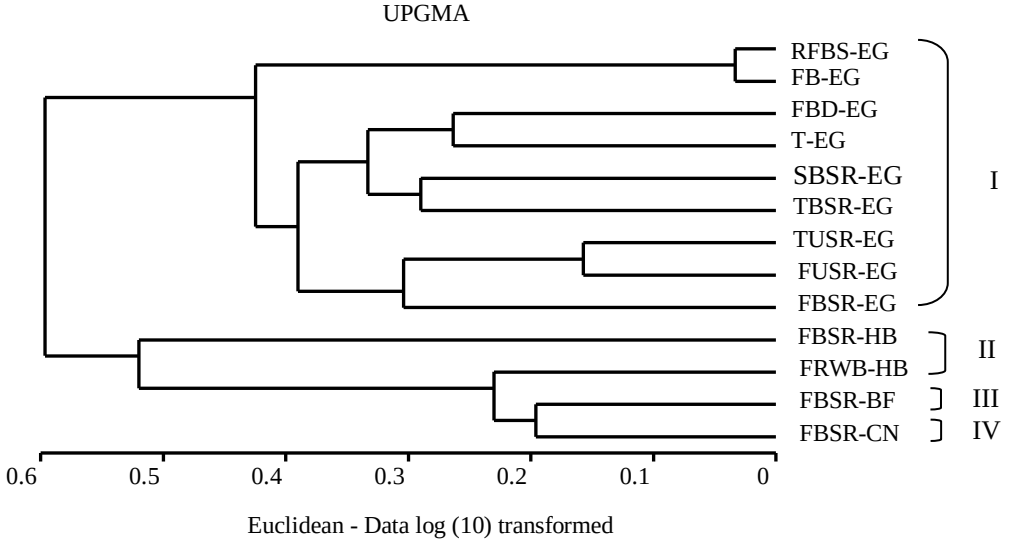

Figure 2. Cluster analysis of *Ganoderma boninense* isolates from 13 populations. FBSR-CN (fruiting body basal stem rot-*Cocos nucifera*), FBSR-BF (fruiting body basal stem rot-*Borassus flabellifer*), FBSR-EG (fruiting body basal stem rot-*Elaeis guineensis*), FUSR-EG (fruiting body upper stem rot-*E. guineensis*), FBSR-HB (fruiting basal stem rot-*Hevea Brasiliensis*), FRWB-HB (fruiting body rubberwood block-*H. brasiliensis*), TUSR-EG (tissue upper stem rot-*E. guineensis*), TBSR-EG (tissue basal stem rot-*E. guineensis*), SBSR-EG (spore basal stem rot-*E. guineensis*), FB-EG (fruiting body-*E. guineensis*), T-EG (tissue-*E. guineensis*), FBD-EG (fruiting body died-*E. guineensis*), RFBS-EG (re-isolation fruit body seedling-*E. guineensis*).

In conclusion, this work confirmed that *G. boninense* had high genetic diversity among the individual within populations. Management factors such as the origin between isolates could be the significant cause *G. boninense* population isolates. Several SSR markers were promisingly effective in determining the polymorphisms of *G. boninense* isolates. This study clarified that *G. boninense* from oil palm was predominantly comprised of a genetically distinct individual.

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