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Full Length Article

Genetic Diversity, Sequence and Bioinformatic Analysis of *Ganoderma boninense* Isolates

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Abstract

Ganoderma boninense is the most severe pathogen of *Elaeis guineensis* in Sumatra and Sulawesi, Indonesia, owing to the fact that it causes upper or basal stem rot disease. This study aimed to determine the genetic diversity, sequence and bioinformatics of *E. guineensis, Borassus flabellifer, Hevea brasiliensis* and *Cocos nucifera*. As many as 49 individuals of *G. boninense* from six populations were analyzed using the four SSR marker loci. The genetic structure was carried out by the GenAlEx ver 6.502 software. The sequences compared database with the BLASTX program. Physico-chemical, possible transfer peptide, and subcellular localization relationships were analyzed using the bioinformatics methods. The clustering UPGMA and phylogenetic were generated using MVSP ver. 3.22 and CLUSTAL W ver. 1.83 software. Results of the analysis showed that the observed heterozygosity (Ho) was 0.32. The two loci that revealed polymorphism with a polymorphic information content (PIC) value was 0.61 (KT124397 and KT12440). Further, the distribution of the BLASTX analyses indicated that the isolates were more similar to the Transcription factor (*G. sinense* strain ZZ0214-1) homologs in some other fungi. The potential peptides prediction in Ganoderma was similarly localized to the chloroplasts and mitochondria (0.1). The two subcellular principal predicted in the cytoplasm and mitochondria localization were stable (2.0–8.5). They were UPGMA clustered into two distinct geographical distributions. The isolates from the North Sulawesi Province have a genetic correlation close to the isolates from the North Sumatra Province. However, the phylogenetic clearly separated the *G. boninense* and *G. sinense*.

Keywords: Basal stem rot; *Ganoderma sinense*; *Elaeis guineensis; Borassus flabellifer; Hevea brasiliensis; Cocos nucifera*

Introduction

The Ganoderma fungi are responsible for considerable yield losses in oil palm (*Elaeis guineensis*), coconut (*Cocos nucifera*), rubber (*Hevea Brasiliensis*) and palmyra palm (*Borassus flabellifer*) (Chee 2005; Sankaran *et al.* 2005; Kandan *et al.* 2010; Bejo and Vong 2014). Initially, Ganoderma disease had been thought to be caused by the *Ganoderma boninense* alone; however, in recent studies, several species have been reported to be responsible for the basal stem rot disease in oil palm trees, namely, *G. zonatum, G. boninense, G. sinense* and *G. miniatocinctum* (Zhao *et al*. 2007; Rashid *et al.* 2014). *G. lucidum* and *G. applanatum* were the most aggressive pathogen that caused the basal stem rot in coconut trees (Bhaskaran 2000; Rajendran *et al.* 2009). *G. boninense* also occurs on coconut trees, reportedly due to a saprophyte (Pilotti *et al.* 2004). On the other hand, *G. pseudoferreum* and *G. philiplii* are the most common species to infect rubber trees (Zakaria *et al.* 2009; Ogbebor *et al.* 2010).

Pilotti (2005) reported the physiological characteristics of the *G. boninense* causing basal stem rot in the oil palm. The genetic diversity of *G. boninense* has been reported based on species diversity and genetic heterogeneity in oil palm (Miller *et al.* 1999; Pilotti *et al.* 2003). The basidiospores of *G. boninense* implicated in distribution and genetic diversity have been found to cause basal stem rot (BSR) and upper stem rot (USR) (Rees *et al.* 2012). In particular, the availability of new molecular biology tools has made it possible to develop a large number of genetic markers. Microsatellites marker remains the most widely and powerful used tool for studying population genetics and the geographical spread of plant pathogens (Schoebel *et al.* 2013a, b). Purba *et al.* (2019) have reported thirteen species

of *G. boninense* in oil palm in Indonesia, which they discovered through a sequence analysis. Moreover, Hapuarachchi *et al.* (2019) also reported twenty species of *G. sinense* in China found through sequence analysis.

Furthermore, the bioinformatics method provided more information on genetics (Pop and Salzberg 2008; Horner *et al.* 2009). *G. boninense* has a role in the diversity and the physical and chemical characteristics that are distinguishable by amino acids (Basyuni *et al.* 2018a). Moreover, Zhu *et al.* (2015) have reported more than 30 genes of a cluster of *G. sinense*, which they classified using the bioinformatics approach. This study extended our previous work and aimed to determine the genetic diversity within *G. Boninense* populations in some palm trees through sequence analysis and the bioinformatics method.

Materials ad Methods

Sample collection

A total of 49 isolates of the *Ganoderma* sp. belong to Socfindo derived from *C. nucifera* (3), *H. brasiliensis* (6), *E. guineensis* (37), and *B. flabellifer* (3) were collected in some parts of Indonesia (Fig. 1), as follows: Bah-Lias, Simalungun (SL) 3°15' N 99°18' E, Faculty Agricultural University Sumatera Utara (FA) 3°33' N 98˚ 39' E, Gambus Land-Batu Bara (GL) 3°09' N 99°26' E, Sirandorung-Central Tapanuli (CP) 2°02' N 98°21' E, South Sulawesi (SSul) 8°32' S 120°11' E, Dolok-Batu Bara (DB) 3°12' N 99°29' E, Bangun Bandar-Serdang Bedagai (BB) 3°15' N 99°41' E, Sei Dadap-Asahan (SD) 2°57' N 99°41' E, North Labuhan Batu (LB) 2°28' N 99°53' E, and South Sumatera (SSum) 3°19' S 104°00' E.

Growth of *G. boninense* in the PDA

The fungi were maintained on *Potato Dextrose Agar* (PDA) and the mycelial growth for a minimum of ten days' subculture (Nasreen *et al.* 2005).

DNA extraction

Total DNA was extracted using cetyl trimethyl ammonium bromide (CTAB) method (Brandfass and Karlovsky 2008). The quantity DNA test was carried out following the nanophotometer method (Gallagher and Desjardins 2006) and the quality test with agarose gel (1–2%) and quantified using the UV-Tex method (Lee *et al.* 2012).

Polymerase chain reaction

A set of specific primers SSR selected is shown in Table 1 (Mercière *et al.* 2015). The amplification reaction for the PCR product was performed in 10 μL of a total volume containing 3 μL of DNA templates mixed with 2.5 μL Gotaq master, 0.5 μL primer forward and 0.5 μL primer reverse, and 3.5 μL ddH_2O. The amplification was operated for 35 cycles (30 sec at 95°C 30 sec at 60°C, 40 sec at 78°C) and after the final to extensions of 8 min at 72°C. The PCR product was analyzed with the electrophoresis agarose gel stained with GelRed® and visualized with Ultraviolet Translumination (Voytas 2000).

Microsatellite analysis

The polymorphism data for each population and locus was assessed by calculating the mean for allele frequency correlations between individuals in the subpopulation (Fis), allele frequency correlations between subpopulations (Fst), allele frequencies in the population caused by both factors (Fit), the total of migrant (Nm), the number of different alleles (N), the number of different alleles frequency > 0.5% (Na), the number of active alleles (Ne) and the Shannon of information Index (I).

The genetic polymorphism for each population and locus was assessed by calculating the mean observed heterozygosity (Ho), expected heterozygosity (He), and the fixation index (F) (Nei 1978). The polymorphic information content (PIC) was determined by Avval (2017). Genetic structure analyzed was calculated using the analysis of molecular variance (AMOVA) package GenAlEx ver. 6.502 (Peakal and Smouse 2012).

DNA sequencing analysis

The polymerase chain reaction (PCR) products were purified and sequenced. The nucleotides to confirm our microsatellite data were selected, and other sequences, which are available in the NCBI database (*https://blast.ncbi.nlm.nih.gov/*) at BLASTX program (Altschul *et al.* 1997), were searched for sequence similarity. The sequence of the comparable database with BLASTX score were an E-value $<10^{-4}$, considered to have significant similarities (Bohnert *et al.* 2001).

Physical and chemical characteristics

The structure and physico-chemical characteristics of selected data on *G. sinense* were analyzed analysis with Protparam after an online search (*web.expasy.org/protparam/*). The calculated factors designated of the length of genes, molecular weight, theoretical isoelectric point values, total number of atoms, extinction coefficients, half-life period, instability coefficient, aliphatic index, and grand average of hydropathicity (Basyuni *et al.* 2018b).

Possible peptide transfer and subcellular localization

The transit peptide of selected data on *G. sinense* was analyzed by accessing the online P1.1 target server (*www.cbs.dtu.dk/services/targetp/*). This position

Table 1: The primer specific for *G. boninense*

Primer	Primer Sequences (‘5-‘3)	Amplification (bp)
KT124397	F: CGCCATGCCCACCACCAGAG	283-325
	R: GACCCGGCTGCCCGAATGAG	
KT124403	F: GGCGACGAGGGCACGAGAGA	293-297
	R: CCGCACTTTCGCCAACCACC	
KT124399	F: GCACAGGCACAAGCGCAAGG	204-267
	R: CGACGACCGCCCCAAAGGAT	
KT124394	F: CGGGAAGTGGTGAACGGT	234-243
	R: GGGTGGCTTGACAGCGGCAT	

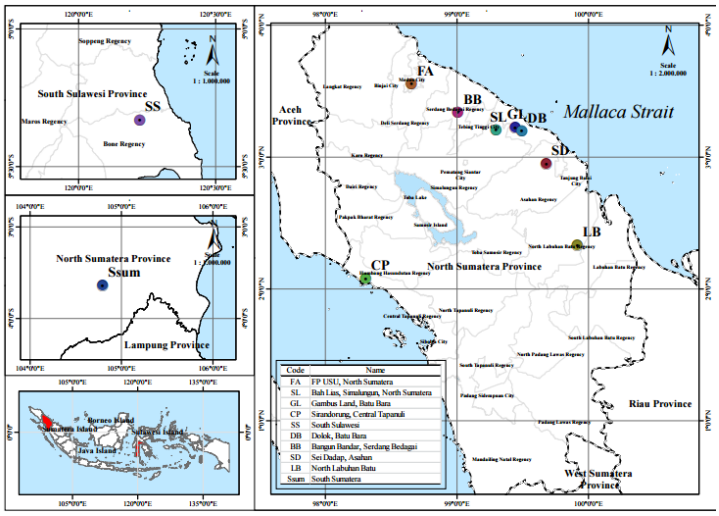

Fig. 1: Map of location of *Ganoderma* sampling

corresponds to the estimated presence of one of the chloroplast pre-sequential N-terminal terminals of transit peptide (cTP), mitochondrial targeting peptide (mTP), and also the peptide signal (SP) peptide pathway. The prediction tool for subcellular localization proteins (PSORT) was used to access online predictions with *psort.hgc.jp/form.html* to control the protein-induced of subcellular determinations (Basyuni and Wati 2017).

Phylogenetic analysis

Phylogenetic analysis was done based on the location and grouping analysis of the phylogenetic tree of *G. boninense* strains NJ3. To extend our knowledge on the relationship of Ganoderma, a total of 12 locations and 49 samples were selected. Furthermore, they were analyzed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) by MVSP ver. 3.22 software (Basyuni *et al.* 2018c). Fig. 2 was constructed based on the length of the base pair nucleotide of the location Ganoderma isolates. On the other hand, Fig. 3 was constructed based on the DNA sequences that were obtained using the FASTA ver 3.4t26 software (Pearson and Lipman 1988) from the Bank of Japan Data DNA (Mishima, Shizuoka, Japan); this was carried out by CLUSTAL W ver 1.83 program (Thompson *et al.* 1994) based on the neighbor-join method. The bootstrap analysis with 1000 replications was used to assess the strength of nodes (Felsenstein 1985).

Result

Genetic structure

Forty nine samples were investigated for their locus (KT124397, KT124403, KT124399 and KT124394), and the additional descriptions of microsatellite loci have reported the success rate of the specific primers. After an interpretation of the genetic variation and the interaction of locus frequency between alleles, the mean of allele frequency in collaboration with individual alleles was found to be 0.57. The mean frequencies of the entire *Ganoderma* population differing in alleles were suspected to be Fit 0.66.

On the other hand, the test aimed at the relationship between heterogeneity per locus. The summary of the genetic differentiation between groups obtained was Fst 0.21. It has been suggested that the value depends on the allele frequency at loci, and it exhibited a variety of properties related to genetic diversity. The estimated number of migrants obtained on average was Nm 0.97. The mean value of the polymorphic information content (PIC) was 0.441, which was the lowest value of the polymorphism allele on the loci KT124394 (0.172) and the loci KT124399

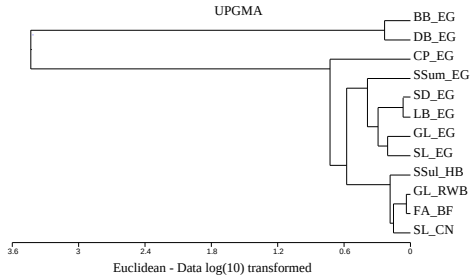

Fig. 2: Cluster analysis of *Ganoderma* pathogen from 12 locations. BB = Bangun Bandar

DB = Dolok-Batu Bara, CP = Central Tapanuli, SSum = South Sumatera, SD = Sei Dadap-Asahan, LB = Labuhan Batu, GL = Gambus Land, SL = Bah Lias Simalungun, SSul = South Sulawesi, FA = Faculty of Agricultural USU. BF = *Borassus flabellifer*, EG = *Elaeis guineensis*, HB = *Hevea brasiliensis*, RWB = rubber wood block

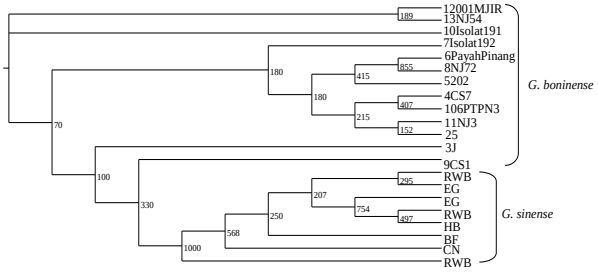

Fig. 3: Phylogenetic tree of *Ganoderma* isolates. DNA sequences were collected by the neighbor-joining method, the Clustel W, with scale indication corresponding to 0.1; the DNA sequences substitution were per site and indicated bootstrap 1000 replicates. BF = *Borassus flabellifer*, EG = *Elaeis guineensis*, HB = *Hevea brasiliensis*, CN = *Cocos nucifera*. RWB = rubber wood block. *G. boninense* data of sequence obtained from our previous research (Purba *et al.*, 2019)

(0.372), while the other two loci have PIC values more than 50% (Table 3).

The microsatellites observed in six populations showed the value of alleles (N) to be 6.75 with differences in alleles in the frequency of 5%. This was found to be 7.21 (Na), and the number of active alleles (Ne) was found to be 6.38. The highest population variation index found in basal stem rot, *E. guineensis* (BSR – EG), was 2.82 (I). Furthermore, the lowest value in basal stem rot, *H. brasiliensis* (BSR – HB), was 1.03. This value was the same as the genetic diversity (He). The value of heterozygosity (Ho) showed the number of genes or allele distribution in the population and locus, and this value was lower than the expected heterozygosity (He). The mean of Ho was found to be 0.75, higher than that of Ho 0.32. The fixation index value in the upper stem rot, *E. guineensis* (USR – EG) was found to be 0.92 (F), and the lowest value in the population of rubber wood block (RWB) was found to be 0.19; however, the value of heterozygosity had different comparisons (Table 2).

We observed statistically significant differences in variation between individual isolates, and the expression differences between individuals increased the variation. Molecular of variance analysis (AMOVA) summarized based on the characteristics of the genetic diversity revealed 85% among individuals and 15% within the individual (Table 4). It was not the genetic diversity detection in the population.

Distribution of the BLASTX

The BLASTX of the 40 sequences analysis indicated previously as in *G. boninense* had a closing similarity as Transcriptional factor proteins to *G. sinense*. However, only eight sequences were identified as *G. sinense* strain ZZ0214-1 (Table 5). The overall results of that species with varied E-value ranges, and the total scores obtained and identified were 49.3–97.4 and 64–96%, respectively. The predictive approaches were other fungal proteins such as hypothetical proteins for *Hebeloma cylindrosporum, Stylophora pistillata, Bostrobasidium botryosum, Aspergillus cristatus, Mixia osmundae, Pseudogymnoascus sp, Lomentospora prolificans, Trametes versicolor, Trichoderma gamsii,* and *Acidomyces richmondensis*, with different strains. However, an identified hypothetical protein, *G. sinense*, only had a total score of 33.9 with a large E-value of seven despite identifying a 68% similarity. On the other hand, the proteins were other sequences, including Alpha/Beta hydrolase for *Leucosporidium creatinivorum*, Probable to GDP/GTP for *Ustilago hordei*, Utp-14 domain for *Trametes coccinea*, putative glicyne-tRNA ligase for *Tolypocladium paradoxum*, Neurexin-3b for *Rhinocerous sinocyclocheilus*, and non-identity of sequence analysis for 11 samples.

Physical and chemical properties of the *G. sinense*

The bioinformatics information of the Ganoderma sequence genes for *G. sinense*, including several physical and chemical parameters are described in Table 6. The database identified eight population sequences of *G. sinense*. It has the value of the length from 252 – 264 bp. The molecular weight was shown in the range of value 21,118 to 22,508. The theoretical isoelectric point values were 5.09 to 5.24, suggesting it has a minor variation. *B. flabellifer* (BF) and *H. brasiliensis* (HB) have a stable total number of atoms. The highest extinction coefficient in *E. guineensis* (EG) was 6000, with the smallest half-life period (1.2 h). Instability coefficient has been established for isolates of *G. sinense* from 59.29 to 78.11. The aliphatic index EG was significantly higher than that of BF, HB, CN, and RWB. The grand average of hydropathicity was unstable from 0.900 to 1.085.

Potential transit of peptide and subcellular localization of *G. sinense*

The DNA spread into the chloroplast transit peptide (cTP)

Table 2: The profile of microsatellite loci for all population of *Ganoderma* isolates

Population	N	Na	Ne	I	Ho	He	F
BSR-CN	2.25 ± 0.25	3.25 ± 0.25	2.90 ± 0.23	1.11 ± 0.07	0.46 ± 0.04	0.65 ± 0.02	0.29 ± 0.09
BSR-BF	2.75 ± 0.25	3.25 ± 0.25	3.07 ± 0.19	1.14 ± 0.06	0.21 ± 0.13	0.67 ± 0.02	0.69 ± 0.20
BSR-EG	19.50 ± 1.85	18.25 ± 1.31	15.44 ± 0.91	2.82 ± 0.06	0.10 ± 0.05	0.94 ± 0.00	0.90 ± 0.05
USR-EG	10.75 ± 0.25	11.00 ± 0.41	10.22 ± 0.44	2.36 ± 0.04	0.07 ± 0.02	0.90 ± 0.00	0.92 ± 0.03
BSR-HB	2.25 ± 0.25	3.00 ± 0.41	2.73 ± 0.33	1.03 ± 0.13	0.33 ± 0.12	0.62 ± 0.05	0.49 ± 0.19
RWB	3.00 ± 0.00	4.50 ± 0.29	3.90 ± 0.37	1.42 ± 0.08	0.75 ± 0.16	0.74 ± 0.03	0.00 ± 0.19
Mean	6.75 ± 1.37	7.21 ± 1.20	6.38 ± 1.02	1.65 ± 0.15	0.32 ± 0.06	0.75 ± 0.03	0.54 ± 0.09

Number of different alleles (N), number of different alleles frequency > 0.5% (Na), number of effectife alleles (Ne) and shannon of information index (I), observed heterozigosity (Ho), expected heterozigosity (He), fixation index (F). BSR – CN (basal stem rot – *Cocos nucifera),* BSR – BF (basal stem rot – *Borassus flabellifer),* BSR – EG (basal stem rot – *Elaeis guineensis),* USR – EG (upper stem rot – *E. guineensis),* BSR – HB (basal stem rot – *Hevea brasiliensis),* RWB = rubber wood block

Table 3: F-Statistics and Estimates of Nm overall population for each locus

Locus	Fis	Fit	Fst	Nm	PIC
KT124397	0.65	0.71	0.19	1.08	0.61
KT124403	0.57	0.66	0.19	1.07	0.61
KT124399	0.61	0.70	0.23	0.85	0.37
KT124394	0.47	0.59	0.23	0.86	0.17
Mean	0.57	0.66	0.21	0.97	0.44
SE	0.04	0.03	0.01	0.06	

Allele frequency correlations between the individuals in the subpopulation (Fis), allele frequency correlations between subpopulation (Fst), allele frequency in the population caused by both factors above (Fit) and the total number of migran (Nm), polimorfic information content (PIC)

Table 4: Summary analysis of molecular variance (AMOVA)

Source	Df	SS	MS	Est. var.	% Var
Among pops	5	18.00	3.60	0.00	0
Among indiv	43	152.42	3.55	1.63	85
Within indiv	49	14	0.29	0.29	15
Total	97	184.42		1.92	100

Df = degree of freedom, SS = source of variation, MS = mean squares, Est. Var = estimation of variant, Var = varian

and mitochondria transit peptide (mTP), was described in Table 7. Estimation of the total number of different proteins occupant in the chloroplast varied among the *G. sinense* of EG, RWB, and CN except BF and HB, which had a value of 0.119. Table 8 showed the most significant subcellular localization of *G. sinense* found in the cytoplasm (Cyto) and mytochondria (Myto), wherein it reported in Cyto 2.0 to 8.5 with Myto 2.0 to 6.0.

Phylogenetic analysis

We identified the genetic variation from six populations of BSR – CN (basal stem rot – *C. nucifera),* BSR – BF (basal stem rot – *B. flabellifer),* BSR – EG (basal stem rot – *E. guineensis),* USR – EG (upper stem rot – *E. guineensis),* BSR – HB (basal stem rot – *H. Brasiliensis)* and RWB (rubber wood block). It showed 12 locations in Fig. 2. The dendrogram (UPGMA) showed two large groups in the population. The first group consisting of location was Bangun Bandar and Dolok – Batu Bara. The second group consisted mainly only eight sequences from Central Tapanuli, South Sumatra, Sei Dadap – Asahan, Labuhan Batu, Gambus Land, Bah Lias – Simalungun, South Sulawesi and the Faculty of Agriculture. To extend our knowledge of the relationship between *G. boninense* and *G. sinense*, 13 sequences were used to draw the dendrogram. Eight relationships of *G. sinense* (Rubber Wood Block, *E. guineensis*, *C. nucifera*, *H. brasiliensis*, *B. flabellifer*, *C. nucifera*) were selected to be displayed as a dendrogram. Fig. 3 showed two large groups separated from *G. boninense* strain NJ3 with original isolates from the *E. guineensis*, but *G. sinense* strain ZZ0214-1 from various plant sources have a close genetic diversity. They were identified as *G. sinense* scattered over two form the same group.

Discussion

The genetic analysis of the population structure of Indonesian species of *Ganoderma* supported the taxonomic distinction among the isolates studied, and five distinct species were identified. The allele of frequency can be classified according to the Buchert *et al.* (1997), which can be divided into four categories. The alleles with a frequency of ≥ 0.75 at particular loci were categorized as high, and alleles with a frequency of $0.75 > P \geq 0.25$ were categorized as medium. The alleles of frequency $0.25 > P \geq 0.01$ were taken as low alleles and frequencies < 0.01 as rare alleles.

On the other hand, according to Marshall and Brown (1975), the alleles categorized were as general alleles if they had a frequency ≥ 0.05 and special alleles if the frequency was < 0.05. The population and loci of *Ganoderma* have a mean frequency with value 0.54. According to Mercière *et al.* (2015), and our SSR data for the PIC value showed high polymorphic information content (PIC) with only two loci,

Table 5: Distribution of the BLASTX for *Ganoderma* isolates of sequence gene Accession

Accession	Description	Sequences	Identify (%)	Total score	E-value
PIL35715.1	Transcription factor (*Ganoderma sinense* ZZ0214-1)	8	64-96	49.3-97.4	(1e-04)-(9e-23)
KIM39402.1	Hypothetical protein M413DRAFT_29551 (*Hebeloma cylindrosporum* h7)	1	62	33.5	2.5
ORY85318.1	Alpha/Beta hydrolase protein (*Leucosporidium creatinivorum*)	1	33	32	9.4
PFX24695.1	Hypothetical protein AWC38_SpisGene10691 (*Stylophora pistillata*)	2	46	37.7	0.59-0.61
D05693.1	Hypothetical protein BOTBODRAFT_182311 (*Botryobasidium botryosum* FD-172SS1)	1	35	118	9e-28
CCF48258.1	Probable to GDP/GTP exchange factor Rom2p (*Ustilago hordei*)	1	55	32.3	6.8
SD00840.1	Utp14-domain-containing protein (*Trametes coccinea* BRFM310)	1	58	34.3	4.2
ODM20124.1	Hypothetical protein (*Aspergillus cristatus*)	1	33	35.8	1.7
XP_014568041.1	Hypothetical protein L969DRAFT_48519 (*Mixia osmundae* IAM 14324)	1	32	35	2.7
PIL26871.1	Hypothetical protein GSI_11051 (*Ganoderma sinense* ZZ0214-1)	1	68	33.9	7
POR37917.1	Putative glycine tRNA ligase (*Tolypocladium paradoxum*)	1	71	58.2	2e-8
F47594.1	Hypothetical protein 495_01898 (*Pseudogymnoascus sp.* M F 4514 FW-929)	1	72	31.6	6.8
PKS09315.1	Hypothetical protein 003929 (*Lemontospora prolificans*)	1	54	34.7	4.5
XP_008042932.1	Hypothetical protein TRAVEDRAFT 74270 (*Trametes versicolor* FP 101664 SS1)	1	40	32	8.1
XO_024400.1	Hypothetical protein TGAM01_v203499 (*Trichoderma gamsii*)	1	51	35.4	6.9
XP_016393967.1	Neurexin-3b-like (*Sinocyclocheilus rhinocerous*)	1	46	36.6	3.9
KYG40648.1	Hypothetical protein M433DRAFT_160121 (*Acidomyces richmondensis* BFW)	2	48	32	4.4 - 4.6
KFY47594.1	Hypothetical protein V495_01898 (*Pseudogymnoascus sp.* VKM F-4514 FW-929)	2	78	35-37	0.08 - 0.45
KYG406113.1	Hypothetical protein M433DRAFT_8635 (*Acidomyces richmondensis* BFW)	1	48	32	4.4
UNKNOWN	Not Detection	11	nd	nd	nd

Table 6: Physical and chemical properties of *Ganoderma sinense*

Plant spesies	BF	EG	HB	RWB	RWB	RWB	CN	EG
Length of genes/bp	252	264	257	253	255	252	255	260
Molecular weight	21118	22508	21297	21357	21491	21206	21544	22037
Theoretical isoelectric point values	5.24	5.22	5.23	5.24	5.22	5.24	5.21	5.09
Total number of atoms	2582	2753	2582	2609	2619	2596	2612	2725
Extinction coefficient	5500	6000	5875	5625	5750	5500	6125	5250
Half-life period	4.4h	1.2h	4.4h	4.4h	1.2h	4.4h	1.2h	7.2h
Instability coefficient	62.57	77.17	66.14	64.12	66.15	59.29	78.11	65.82
Aliphatic index	21.83	21.59	20.62	20.55	18.43	21.83	20.78	19.62
Grand average of hydropathicity	1.054	1.085	1.074	1.042	0.998	1.052	1.126	0.900

BF = *Borassus flabellifer*, EG = *Elaeis guineensis*, HB = *Hevea brasiliensis*, RWB = rubber wood block, CN = *Cocos nucifera*

KT124397 and KT12440. Both had a value of 0.61. The specific primers were capable of producing several alleles in several genotypes in specific loci.

The genetic diversity of the *G. sinense* isolates from different sources in this study provided information on *C. nucifera*, *H. brasiliensis*, *E. guineensis*, and *B. flabellifer* plants, as they were similar strains of the *G. sinense*. The nucleotide sequences from specific regions depicted the phylogeny at various taxonomic levels. All populations were detected as randomly grouped to this study, and they do not cause geographical distribution. The isolates from the North Sulawesi province have a genetic relationship close to the North Sumatra Province.

Furthermore, the sequence analysis with BLASTX does not reflect the numerous same on *G. boninense*. However, the conjunction morphology among the different isolates was similar. The comparison of the genes sequences indicated some translocations and duplications among two *G. sinense* and *G. boninense* fungal species and also revealed many non-homology genes. It suggested rapid divergent evolution consequent speciations. Zhu *et al.* (2015) reported the genome sequence of *G. sinense*, one of the most valuable medicinal fungi such as *G. lucidum*.

The newly identified conserved DNA of *G. boninense* in *E. guineensis* have been predicted by the homology with some diverse plant groups (Purba *et al.* 2019). The phylogeny is indicated as *G. boninense*. They did not cluster together with the identified *G. sinense*. The present study implied high intra species diversity or the presence of the gene. The data generated in this study could be helpful for researchers in studying the conservation of both species. Mercière *et al.* (2015) reported that the mean value identified for N was eight for isolates from Indonesia. It was not much different in this study. The phylogenetic analysis of the SSR sequence separated the *Ganoderma* isolates. It showed several isolates maybe had been misclassification (Smith and Sivasithamparam 2000). The bioinformatics method of the DNA identification isolate the family *G. sinense* for the species research community is reported.

We have identified the physical and chemical properties conserving the DNA belonging to the *G. sinense* sequences (strain ZZ0214-1, GenBank Accession number PIL35715.1; PIL26871.1). Experiments confirm the powerfulness of bioinformatic prediction of DNA. These findings will be helpful to comprehend the DNA life processes in the Ganoderma pathogen. Recently, *G. lucidum*

Table 7: Possibility of the potential transit peptide of *Ganoderma sinense*

Nucleotide ID	Reliability			
	Chloroplast transit peptide	Mitochondrial target peptide	Signal peptide of secretory pathway	Reliability prediction
BF	0.119	0.089	0.057	3
EG	0.074	0.109	0.088	3
HB	0.119	0.116	0.052	3
RWB	0.102	0.080	0.082	3
RWB	0.110	0.075	0.079	4
RWB	0.085	0.090	0.091	3
CN	0.055	0.069	0.379	5
EG	0.096	0.097	0.072	3

BF = *Borassus flabellifer*, EG = *Elaeis guineensis*, HB = *Hevea brasiliensis*, RWB = rubber wood block, CN = *Cocos nucifera*

Table 8: Subcellular localization of the predicted *Ganoderma sinense*

Protein ID	Golg	Cyto	Plasm	Perox	Cyto-Mito	Cyto- Nucl	Myto	Vac	ER	Nucl	Secr
BF	nd	8.5	nd	nd	6.6	12.3	3.5	nd	nd	14	1.0
EG	nd	8.5	nd	2.0	nd	5.5	3.0	2.0	5.0	1.5	5.0
HB	2.0	5.5	1.0	9.0	nd	6.0	4.0	nd	nd	5.5	nd
RWB	nd	8.0	2.0	nd	nd	nd	6.0	nd	nd	4.0	7.0
RWB	nd	6.0	4.0	nd	nd	nd	4.0	nd	nd	9.0	4.0
CN	nd	2.0	1.0	nd	nd	nd	2.0	4.0	1.0	nd	17
EG	nd	6.0	1.0	nd	nd	nd	2.0	3.0	nd	1.0	14

BF = *Borassus flabellifer*, EG = *Elaeis guineensis*, HB = *Hevea brasiliensis*, RWB = rubber wood block, CN = *Cocos nucifera*, Golg = golgi body, Cyto = cytoplasm, Plasm = plasma membrane, Perox = peroxixom, Cyto – Myto = cytoplasm – mythochondria, Cyto – Nucl = cytoplasm – nuclear, Myto = mythochondria, Vac = vacuolar, ER = endoplasmic reticulum, Nucl = nucleolar, Secr = secretary

and *G. sinense* were registered as Lingzhi in Chinese Pharmacopoeia, which are used to relieve cough and dyspnea (Liu *et al.* 2009). Many studies reported *G. lucidum* as an antitumor activity, but few reports were only focused on *G. sinense* (Lin and Zhang 2004; Grace *et al.* 2006; Nonaka *et al.* 2006; Cheng *et al.* 2007; Pang *et al.* 2007).

Eight *G. sinense* genes were assessed using bioinformatics analysis, and the clusters were expected to produce in chloroplast transit peptide (cTP) or mitochondrial transit peptide (mTP) and were identified from this fungus. An upregulation of ferredoxin may reflect its direct participation in pathogen defense wherein the chlorophyll of several photosynthetic proteins is affected by the plant-fungi interaction in the chloroplast (Konishi *et al.* 2001; Castillejo *et al.* 2004). Bioactive compounds such as the Ganoderic Acid T (GA-T) were predicted in the mitochondrial of *G. lucidum*, wherein a triterpenoid exerted cytotoxicity on various human carcinoma cell (Tang *et al.* 2006).

Conclusion

Thirteen *G. boninense* strains were chosen in the present study in order to identify eight *G. sinense* strains clustered into two groups. It was deduced that *G. sinense* had genetic diversity in four loci. The data obtained in this study demonstrated SSR is very sensitive and practical tool to identify the Ganoderma.

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