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Genetic Diversity and Sequences Analysis from *Elaeis guineensis* Jacq. Infected with *Ganoderma boninense*

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Abstract*— Early screening tests on the seedling phase of *Elaeis guineensis* would have a significant effect on diminishing the time and the resources needed to increase variations in the oil palm breeding program to resistance Ganoderma. This research was conducted to evaluate the genetic diversity and heterozygosity among eight populations of *E. guineensis* Jacq. Seedlings and matures using the molecular of variant loci analysis to an early screening. PCR and GenAlEx studied the method of genetic diversity and oil palm resistance genes. The results showed the microsatellite markers (*EgIFR*, *Eg001*, *Eg002*, and *Eg003*) pattern in the DNA and protein-encoding in the oil palm seedlings' root tissue to Ganoderma resistance played a potential early screening role in material planting. The polymorphic information content (PIC) values were identified as 0.655-0.998. The means number of *allele* distribution in each population and *loci* showed Ho = 0.48. The mean of heterozygosity was found in 0.67 level. The analysis molecular of variance (AMOVA) showed a significant variability by 45% among individuals and 53% within individuals in the population. A total of 92 data sequences were identified and available on the NCBI database. They have a similarity from proteins, i.e., isoflavone reductase, polyadenylated, heat shock cognate, and thaumatin. The highest total score was showed 117, and the similarity was identified at 100%. Furthermore, the phylogenetic clusters were distinct between the seedling and mature based on the same gene and protein. This study suggested the markers and proteins important to *E. guineensis* against *Ganoderma boninense*.

***Keywords*— Genetic diversity; sequences; *Elaeis guineensis*; screening, microsatellite.**

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I. INTRODUCTION

Currently, crude palm oil (CPO) is the only highly productive oil yield. When Indonesia was experiencing an economic crisis, the oil palm industry showed the best yield to increase agroindustries for foreign exchange. The oil palm has excellent potential to develop as a business area. However, the oil palm plantations in Asia, especially Indonesia and Malaysia, challenge the severe Ganoderma pathogen, a fungus attack caused by dead stems in the root or basal stem rot [1-3]. This pathogen cannot be seen in plain sight, but the plants' damage was quite remarkable. Finally, that pathogen was known as destruction fungi [4].

Early screening tests (seedling phase) would have a significant effect on diminishing the time and the resources needed to increase variations in the oil palm breeding programs [5]. Simple sequence repeat (SSR) were obtained from the transcriptome of *Elaeis guineensis* sequence have a great significance to development among molecular markers for the highest throughput, genetic distinct of plants types, and progressed program assisted solution for plants by a marker-assisted [6-8]. As a specific marker to codominant loci, SSR was used as a tool in genotype identification and genetic population study in plant diseases such as *Ganoderma boninense* and *G. sinense* isolates [9].

The SSR methods have been reported for various purposes such as QTL mapping, linkage map development, association mapping, and genetic diversity [10]. Finding functional markers from sequence cDNA-associated tags expressed as an effective first step for the detection of the genetic markers. Moreover, the genetic diversity analyses revealed high variability within plant origins [11]. Developed for high-throughput DNA extraction in molecular marker techniques, including isozyme marker as protein-based, depends on the genetic material's specific structure [12]. This study aims to analyze the microsatellite data from

E. guineensis based on frequency and distribution genetic structure, polymorphism information content, and genetic diversity of oil palm type seedling and mature using the SSR markers among four loci for sequence analysis.

II. Materials and Method

The materials of the genetic diversity were used two types of plant crossings in *Elaeis guineensis* Jacq. It belonged to Socfin Indonesia, derived from Bangun Bandar-Serdang Bedagai located at 3˚15'N 99˚41'E, namely one series of plant crossing from Ganoderma experimental genetic material in the field (2002 planting year), and the one new crossing to early detection for seedling phase resistance (2017, planting year). The plant types were used resistant and susceptible to the previous study. The mature plants consisted of asymptomatic (healthy plants) and symptomatic plants, while the seedlings consisted of the normal plants, and plants inoculated with *Ganoderma boninense* fungi on 8 weeks after the seedlings were planted and it harvested at 16 weeks.

A. DNA Extraction

The roots from oil palm seedlings and matures were extracted using the *cetyl trimethyl ammonium bromide* (CTAB) method with minor modification as previously described [13]. The DNA quantity test was carried out using Nanophotometer [14], and the quality of DNA was verified with agarose gel (1%). Then, the molecular weight using the UVTec-1D Cambridge software [15].

B. Polymerase Chain Reaction

A specific primer of single sequence repeats (SSR) was selected to refer in Table I. The amplification reaction for PCR product was carried out in total volume of 10 µl, containing 3 µl of DNA templates with 2.50 µl mixed GoTaq® Green Master, 0.50 µl forward primer (F), 0.50 µl reverse primer (R) and 3.50 µl double-distilled water (ddH_2O). For the amplification of polymerase chain reaction (PCR) was carried out in 35 cycles (for 30 sec at 95°C, 30 sec at 60°C, and 40 sec at 78°C), then the extensions at 72°C for 8 min. The PCR product was analyzed using electrophoresis with agarose gel stained and GelRed® to the visualization with UV-Transillumination [16].

TABLE I
PRIMER SEQUENCES USED FOR PCR

Primer	Product size (bp)	Protein	Tm (°C)	Primer Sequence (5'-3')	Source
EgIFR	240-534	Isoflavone reductase	55	AAGCTCCTGGACGACTTCAA-ATGGGAGGAAGTAACCAGCA	[17]
Eg001	340-390	Heat shock cognate	55	TTGCTGGAGGAATGCTCTCT-TCCAGCAGATGCAACACTTC	Present study
Eg002	383-398	Thaumatin	56	AGGCCTACTTGGGTTCCACT-GCCCTTTCACATGATGTCCT	Present study
Eg003	380-395	Polyadenylate	55	TCTTCTTCCCCTTCCTCCTC-GGCAGTGCATTGGATGTCTA	Present study

C. Microsatellite Analysis

Each population and locus was analyzed for the allele frequency and correlations among individuals in the subpopulation (Fis), allele frequency correlations among subpopulations (Fst), allele frequency in the population among both factors (Fit), the number of different alleles of frequency > 0.5% (Na), the total of migrant (Nm), Number of the active allele (Ne), and Shannon of the information Index (I).

The genetic polymorphisms were calculated for the heterozygosity expected (He), heterozygosity observed (Ho), fixation index (F), and Polymorphic Information Content (PIC) [18]. Genetic structures were identified by the statistical analysis molecular of variance (AMOVA) in GenAlEx ver 6.502 [19].

D. DNA Sequencing Analysis

The PCR products were purified and sequenced with ABI PRISM® 7700 sequence detection tools (Applied Biosystem). The nucleotides were used to confirm the microsatellite data to compared for sequence similarity in the online databases from the National Center for Biotechnology Information (NCBI) with BLASTX online software (*https://blast.ncbi.nlm.nih.gov/*) [20].

E. Phylogenetic Tree

The phylogenetic analysis was performed based on a mature and seedling population with each locus selected from the sequence a comparable database. Furthermore, the BLASTX score in *e-value* $<10^{-4}$ was considered to be a significant similarity [9]. The trichotomy was analyzed using FASTA ver 3.4t26 software from the Japan bank data of DNA (Mishima, Shizuoka, Japan) by CLUSTAL W ver 1.83 with Neighbor-Joining (NJ) methods. The bootstrap was used in 1000 replications for measured the nodes' strength [21].

III. Result and Discussion

A. Genetic Structure

The seedlings and matures from *E. guineensis* was predicted in four loci (*EgIFR, Eg001, Eg002,* and *Eg003*). In case, the microsatellite locus was performed a successful appraisal from a specific primer. In the genetic variation interpretation and interaction among the locus frequency of allele, Fis was showed 0.21–0.34, whereas Fit value was showed 0.44 within the locus. Furthermore, the relationship among heterogeneity per locus showed that the genetic differentiation was showed at a level of 0.16–0.27 (Fst) depending on the frequency allele in loci related to genetic

diversity. The number of migrants (Nm) was predicted at 0.88. Thus, the polymorphic information content (PIC) was showed from 0.65 to 0.99 (Table II). The PIC has been reported in 0.44 using Rgen_Pto primer to predict the potential molecular from the mature of the oil palm plant for selection of resistance against *G. boninense* [22]. On the other hand, the maximum PIC in the African and American oil palm genetic material has been reported a maximum value of 0.79 [23].

The number of different alleles (Na) and the number of allele active (Ne) values were showed 3–4 and 2–4, respectively. The highest population variation index (I) was found in resistance mature asymptomatic (Res-Mat-Asym) at 3.43 (Table III). Furthermore, the mean of the heterozygosity observed (Ho) was described as the number of allele distribution in each population and loci. Ho values were lowest than the heterozygosity expected (He). The Ho was showed 0.48, but He was showed 0.67. The expected heterozygosity in American oil palm has been reported larger than the respective observed. Wherein the highest Ho value was found at 0.314, and He 0.273 [24].

TABLE II
F-STATISTICS, NM ESTIMATED AND PIC IN LOCUS

Loci	*fis*	*fit*	*fst*	*Nm*	*PIC*
EgIFR	0.32	0.49	0.26	0.72	0.65
Eg001	0.34	0.52	0.27	0.67	0.92
Eg002	0.27	0.45	0.25	0.77	0.99
Eg003	0.21	0.33	0.16	1.34	0.99
Mean	0.28±0.03	0.44±0.04	0.23±0.23	0.88±0.15	0.89

Noted: *fis*: Allele frequency correlations between individuals in the subpopulation; *fst*: Allele frequency correlations between subpopulations; *fit*: allele frequency in the population caused by both factors; *Nm*: a total of migrant; and Polymorphic Information Content (*PIC*).

TABLE III
THE GENETIC STRUCTURE FROM OIL PALM GENES

Population	*Na*	*Ne*	*I*	*Ho*	*He*	*F*
Res-Seed	3.00±0.40	2.85±0.37	1.04±0.14	0.08	0.63±0.06	0.88±0.12
Res-Mat-Sym	3.50±0.50	3.00±0.43	1.14±0.17	0.66±0.13	0.64±0.66	0.88±0.23
Susc-Seed	4.00±0.57	3.39±0.65	1.25±0.18	0.50±0.09	0.67±0.07	0.27±0.08
Res-Mat-Asym	4.00±0.71	3.68±0.81	1.29±0.19	0.50±0.17	0.69±0.05	0.31±0.17
Res-Seed-Ino	4.00±0.41	3.13±0.52	1.23±0.14	0.67±0.14	0.65±0.06	0.01±0.18
Susc-Mat-Sym	4.25±0.63	3.80±0.83	1.33±0.19	0.50±0.17	0.69±0.07	0.30±0.17
Susc-Seed-Ino	4.00±0.71	3.60±0.64	1.27±0.23	0.50±0.18	0.68±0.08	0.34±0.22
Susc-Mat-Asym	4.00±0.41	4.00±0.41	3.43±0.52	0.42±0.08	0.68±0.06	0.39±0.09
Mean	3.84±0.18	3.36±0.20	1.22±0.06	0.48	0.67	0.30

Noted: The number of different allele frequencies > 0.5% (*Na*); the number of effective allele (*Ne*); shannon of information index *(I)*; heterozygosity observed *(Ho)*; heterozygosity expected *(He)*; the fixation index *(F)*. Populations: Resistance Seedling (Res-Seed); Resistance Mature Symptomatic (Res-Mat-Sym); Susceptible Seedling (Susc-Seed); Resistance Mature Asymptomatic (Res-Mat-Asym); Resistance Seedling Inoculation (Res-Seed-Ino); Susceptible Mature Symptomatic (Susc-Mat-Sym); Susceptible Seedling Inoculation (Susc-Seed-Ino); Susceptible Mature Asymptomatic (Susc-Mat-Asym).

Our data for the expected heterozygosity was showed at 0.667; it was similar reported previously [25]. However, heterozygosity values were found distinct at 0.09–0.58 [26]. The fixation index value from resistance seedling (Res-Seed) and resistance mature symptomatic (Res-Mat-Sym) was showed (F) 0.01–0.88. In order to identify statistically significant differences among each population variation, the expression differences between individual and population oil palm were analyzed using the molecular of variance analysis (AMOVA). The AMOVA result was showed the characteristics of genetic diversity 2% among the population, 45% among individuals, and 53% within the individual in the population (Table IV).

TABEL IV
SUMMARY OF THE AMOVA

Sources	*df*	*SS*	*MS*	*Est. Var*	*%Var*
Among Pops	7	19.46	2.78	0.04	2
Among Individuals	16	41.00	2.56	0.80	45
Within Individuals	24	23.00	0.96	0.96	53
Total	47	83.46	6.30	1.79	100

Noted: Degree of freedom (*df)*; source of variation (*SS)*; mean squares (*MS*); estimation of variant (*Est.Var*); variance (*Var*).

B. Sequences similarity

The gene expression without sequencing will establish troubled to distinguish clearly between Ganoderma resistance groups [27]. The genes of oil palm sequences and NCBI datasets have been reported and available, including the GenBank, DNA Data Bank of Japan, and protein information Resource [28].

A total of 92 sequences of data were identified and available on the NCBI database. Therein, they have been a similarity to proteins, i.e., isoflavone reductase, polyadenylated, heat shock cognate, and thaumatin. Twenty-two sequences were identified as isoflavone reductase-like protein with 72–100% identity. One sequence was found as a polyadenylated binding protein with two isoforms X1 (with 100% similarity). One sequence was found as a polyadenylated binding protein with eight isoform X2 (100% identity). Twenty sequences were found as a polyadenylated binding protein with two isoforms X3 with 55–100% identity. Twenty-four sequences were found as heat shock cognate protein 2-like 70 kDa (54–97% identity). Twenty four sequences were found as thaumatin-like protein

1 (58–89% homology). Four sequences had no similarity (Table V).

E-value variation range was showed from 1e-17 to 8e-21. The highest total score has been reported in polyadenylated binding protein 2 isoform X1 (117). However, the lowest of total score identity was found as isoflavone reductase-like protein and the start value at 58.5. A total protein available from 10 accessions numbers, namely XP_010940088.1; XP_010940091.2; XP_010940092.1; XP_010911620.1; XP_010928349.1; XP_010928350.1; XP_019707943.1; XP_010937646.1; XP_010906112.1 and XP_010906123.1.

Metallothionein protein has been reported in general stress or response protein as an important function in the oil palm plants. That protein plays a role in regulating normal development and adaptation to changing the environmental condition of plants. That protein also has an active role in oil palms to abiotic stress such as disease resistance to Ganoderma pathogen [29]. This promoter can be obtained from several plants, such as Arabidopsis thaliana and Oryza sativa [30]. In other cases, Isoflavone reductase protein has been reported a role in plant resistance to fungus and virus [31]. Thus, thaumatin (sweetener protein) was provided with a resistance response to pathogens (fungi), and abiotic stress in plants [32]. Furthermore, polyadenylate was found a positive protein resistant to the stimulation related to fungal pathogens [33].

TABLE V
DISTRIBUTION OF THE SEQUENCE GENES

No	Protein	*Seq*	*Ident (%)*	*Total Score*	*E-Value*	*Accession*
1	Isoflavone reductase-like protein	22	72-100	58.5-104	(5e-13) - (4e-04)	XP_010940088.1; XP_010940091.2; XP_010940092.1; XP_010911620.1
2	Polyadenylate binding protein two isoforms X1	1	100	117	1e-17	XP_010928349.1
3	Polyadenylate binding protein 8 isoforms X2	1	100	115	7e-17	XP_010928350.1
4	Polyadenylate-binding protein 2 isoforms X3	20	55-100	86.3-124	(8e-21)-(7e-03)	XP_019707943.1
5	Heat-shock cognate protein 2-like 70 kDa	24	54-97	86.3-245	(7e-75)-(6e-17)	XP_010937646.1
6	Thaumatin-like protein 1	24	58-89	90.9-226	(5e-73)-(1e-09)	XP_010906112.1; XP_010906123.1
7	No detection	4	-	-	-	-
	Total sequences	96				

C. Phylogenetic Tree

The identification of the genetic variation was derived from eight oil palm type seedlings and matured. The phylogeny constructed based on loci in Fig. 1 shows in three groups from *EgIFR* loci of seedlings and matured. The oil palm type susceptible seedlings (Fig. 1A) shows distinct between the susceptible seedling inoculation and the resistant seedling. Moreover, the mature symptomatic oil palm type resistance included the resistance mature asymptomatic was found in similar clusters.

However, the susceptible mature symptomatic was separated from the susceptible mature asymptomatic (Fig. 1B). Fig. 2 shows the phylogenetic from *Eg001* loci. Whereas Fig. 2A consisted of the resistance seedling inoculation, and the susceptible seedling (one cluster). Furthermore, the susceptible seedling inoculation and the resistance seedling were separated clearly. Additionally, the mature clusters were distinguished between the susceptible mature symptomatic and susceptible mature asymptomatic. Hence, the resistance mature symptomatic was performed in a different cluster with the resistance mature asymptomatic (Fig. 2B). The oil palm resistance against Ganoderma in the seedling stage from root tissue was separated clusters using biochemistry markers that have been reported based on the carbon-chain lengths of *polyprenol* and *dolichol* [34].

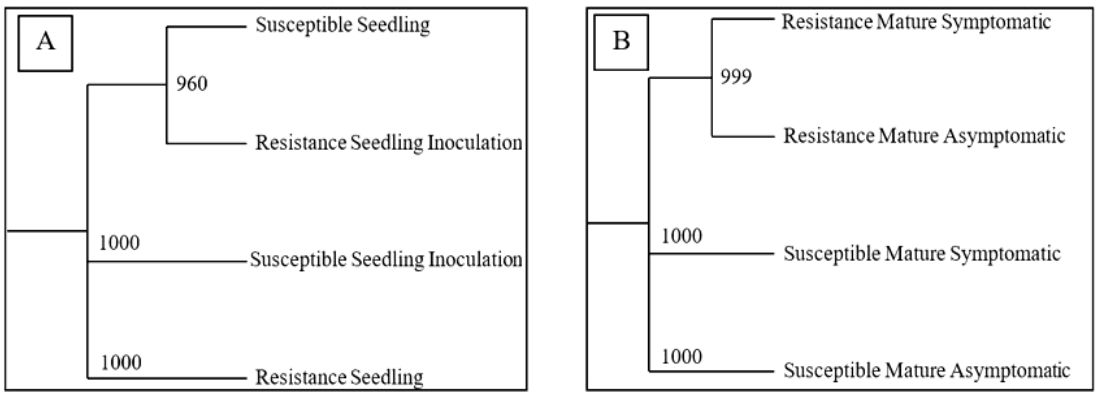

Fig. 1 The phylogenetic from *EgIFR* loci. The DNA sequences were collected by neighbour-joining through the Cluster W method, with a scale indication correspondence of 0.1. The substitution per site number and indicate bootstrap was 1000 replicates. A= Seedlings of *E. guineensis*; B= Matures of *E. guineensis*.

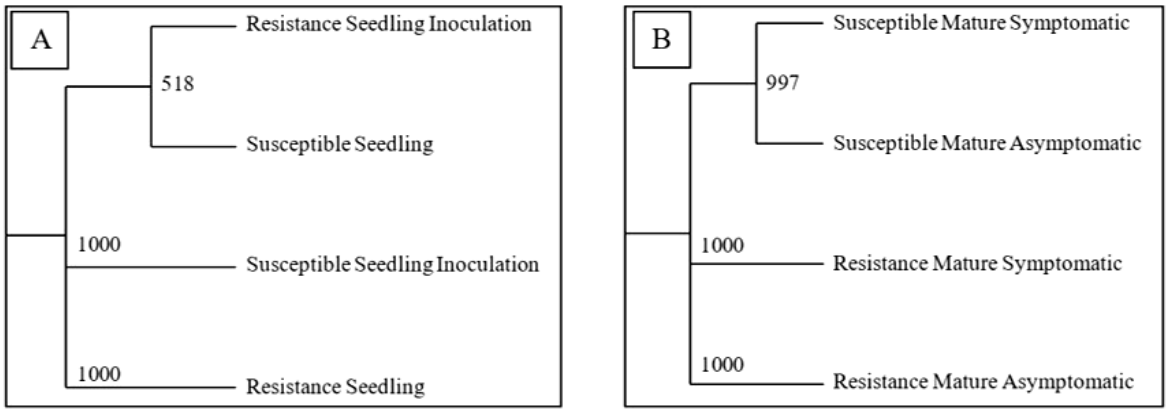

Fig. 2 The phylogenetic from *Eg001* loci. The DNA sequences were collected by neighbor-joining through the Cluster W method, with a scale indication correspondence of 0.1. The substitution per site number and indicate bootstrap was 1000 replicates. A= Seedlings of *E. guineensis*; B= Matures of *E. guineensis*.

Fig. 3 describes three main groups in oil palm type seedlings and matures from *Eg002* loci. One cluster included the resistance seedling and susceptible population. Whereas, the susceptible seedling inoculation and resistance seedling inoculation was performed a distinct cluster (Fig. 3A). On the other hand, Fig. 3B shows the susceptible mature symptomatic and resistance mature asymptomatic to one group. However, the susceptible mature asymptomatic and resistance mature symptomatic were found in a different cluster.

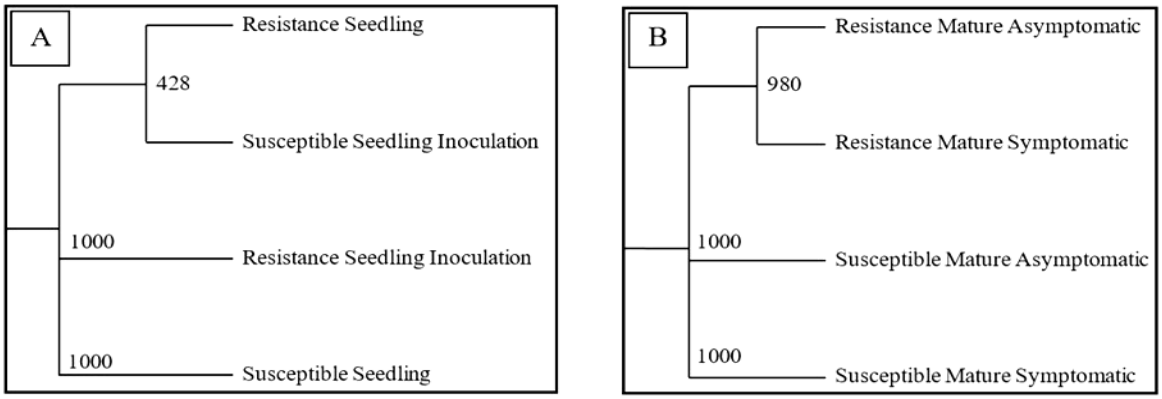

Fig. 3 The phylogenetic from *Eg002* loci. The DNA sequences were collected by neighbour-joining through the Cluster W method, with a scale indication correspondence of 0.1. The substitution per site number and indicated bootstrap were 1000 replicates. A= Seedlings of *E. guineensis*; B= Matures of *E. guineensis*.

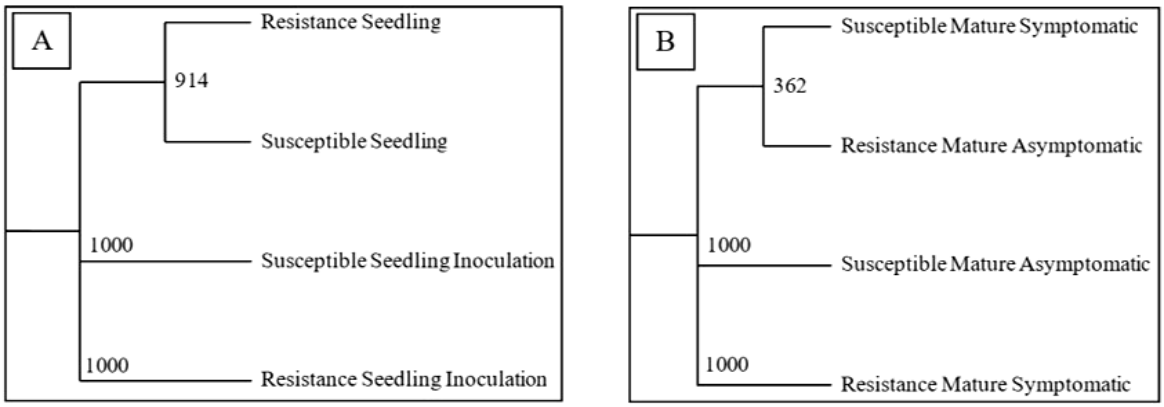

Fig. 4 The phylogenetic from *Eg003* loci. The DNA sequences were collected by neighbour-joining through the Cluster W method, with a scale indication correspondence of 0.1. The substitution per site number and indicate bootstrap was 1000 replicates. A= Seedlings of *E. guineensis*; B= Matures of *E. guineensis*.

Fig. 4 performs three large clusters separated from seedling and mature from *Eg003* loci. The resistance seedling was like susceptible seedling inoculation. However, resistance seedling inoculation was found in different groups (Fig. 4A). Thus, the susceptible mature asymptomatic were scattered into one group with resistance mature symptomatic (Fig. 4B).

The long-term strategies would be to breed genotypes oil palm resistance or tolerance to Basal Stem Root (BSR) [35]. For a long time, Ganoderma disease was tackled using the planting material, which resistant disease [36]. *EgIFR,*

Eg001, Eg002, and *Eg003* markers may be used to perform genetic screening approximation, and the molecular marker as single nucleotide polymorphism (SSR) has been widely applied to the study of genetic structure among *E. guineensis* resistance genes. Screening and breeding were reported as the fundamental method for developing Ganoderma resistance of oil palm. Recently, the material planting from Deli has been reported to emphasize material plant breeding resistance to Ganoderma because of high relativity to susceptible [37]. Two years before, Moderat Tahan Gano (MTG) using RAPD markers to detect polymorphism among various oil palm to genetic analyses was reported [38]. Furthermore, nine oil palms specific SSR primer pairs were found to assess the genetic diversity of a total of 107 alleles from two oil palm species (*E. guineensis* and *E. oleifera*) on markers were polymorphic [39]. The genetic base of existing screening palm cultivars has prompted the oil palm breeders to prioritize germplasm and achieve sustainable development material planting that Ganoderma resistance [40].

IV. Conclusions

The high genetic diversity was identified within the seedling and matures oil palm derived by the Socfindo.ltd collections. Four SSR markers *EgIFR*, *Eg001*, *Eg002*, and *Eg003* could be recommended as a great marker for resistance Ganoderma pathogen. The clustering result from seedlings and matures were separated clearly among asymptomatic and symptomatic plants, thus a potential early screening in the oil palm plant breeding.

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