Microsatellite analysis on oil palm (*Elaeis guineensis*) tolerance to *Ganoderma boninense*

M Basyuni¹, ²*, D Afandi³, R Hayati¹, Y Bimantara¹, D Arifiyanto⁴, I Syahputra⁴

¹Department of Forestry, Faculty of Forestry, Universitas Sumatera Utara, Jl. Tri Dharma Ujung No. 1 Medan, North Sumatera 20155, Indonesia
²Mangrove and Bio-Resources Group, Center of Excellence for Natural Resources Based Technology, Universitas Sumatera Utara, Medan North Sumatera 20155, Indonesia
³Graduate School of Agrotechnology, Faculty of Agriculture, Universitas Sumatera Utara, Medan, North Sumatera 20155, Indonesia
⁴Laboratory of Biomolecular of PT Socfin Indonesia, Martebing, Dolok Masihul, Tebing Tinggi 20991, North Sumatera, Indonesia

*Email: m.basyuni@usu.ac.id*

**Abstract.** Palm oil plants (*Elaeis guineensis*) are nowadays an economically significant crop. Oil palm plantations in Asia, especially in Indonesia and Malaysia encounter the threat of basal stem root (SBR) diseases caused by *Ganoderma boninense*. The research aimed to analyze the microsatellite markers of oil palm tolerance to *G. boninense*. The plant material used in this observation was two cross-series of genetic material belonging to PT Socfin Indonesia. The materials have been known as the level of resistance to *Ganoderma*, the first is cross-series of 15-year-old oil palm in the field, and the second is a new cross-series for early detection in the nursery stage. The quality and quantity test showed that DNA was successfully isolated and can then be used for DNA amplification. The seven primers with DNA banding patterns clearly detected visible in one band. The target genes associated with resistance to SBR disease are found in almost all individual oil palm plants. Furthermore, the low PIC (Polymorphic Informative Content) value of the seven primers except for the Rgen_Pto primer showing the high PIC in the leaf tissue and mature plant roots, suggested the potential molecular marker of this primer for selection of tolerant palm against *G. boninense*.

1. **Introduction**

Palm oil plants (*Elaeis guineensis*) are now an economically important crop and have become one of the primary sources of world vegetable oil which has the potential as a biodiesel feedstock. Indonesia is currently the world's first palm oil producer with palm oil production of 36.5 million metric tons or 53.5% of world palm oil production [1]. Inappropriately, oil palm plantations in Asia, especially in Indonesia and Malaysia challenge the threat of diseases caused by the *Ganoderma boninense* pathogen. Economic losses due to this disease in the two largest palm oil producing countries reached 500 million USD per year [2]. For Indonesian palm oil, the losses arising from every 1% of *G. boninense* attacks range from 256 million USD per year [3].

Disease caused by the fungus *G. boninense* known as basal stem rot (BSR). BSR is also known in Africa and Latin America, but with a low incidence. Initially this disease was found in old oil palm
planted that were grown more than 25 years old, but lately, it often occurs in young plants that have undergone rejuvenation [4-5]. BSR is the most common and most destructive disease in oil palm plantations in Indonesia and Malaysia, resulting in the loss of more than 80% of plants in a certain period which causes oil palm to become uneconomical [2, 4]. It has been shown the damage in PT Socfin Indonesia genetic experiment garden, namely in block 45 of the Bangun Bandar plantation (Dolok Masihul Subdistrict, Serdang Bedagai Regency, North Sumatra Province) which was planted in 1986 and is second generation plant, observed in 2006 there was only 39.83 % damage. After 20 years, the palm tree population has decreased by 60% or 3% per year.

Numerous approaches have been carried out to select oil palm plants that are tolerance to *G. boninense*. The methods were derived from direct observation in the field (census method) in the parent garden and descent testing plantations (progeny trial), resilience selection at the nursery stage (early screening test) to selection at the molecular level [6-7]. The technique of direct observation in the field takes a long time, large land area and costs are quite expensive. Likewise, the screening method in nurseries requires at least one year for one test and requires a relatively large nursery area [6-7].

Data from observations in the field and testing in nurseries have shown different and inherited endurance properties from oil palm plants [8]. The phenotypic data were then used by researchers to look at the molecular relationships of oil palm plants that have resistance to BSR disease caused by the fungus *G. boninense*. In addition to explaining the mechanisms of strength possessed by tolerant plants, these results will be molecular markers to select oil palm plants that are tolerant of BSR disease [6-7]. Molecular markers will further strengthen and enhance the effects of selection conducted in the field and nurseries. With molecular markers, it is also possible to make an early selection of oil palm crosses that have genes associated with disease resistance caused by *G. boninense*. The proper initial screening will make the selection process faster, easier and cheaper. Thus, this study aimed to determine the microsatellite markers of oil palm tolerance to *G. boninense*.

2. Materials and Method

2.1. *G. boninense* sample and DNA extraction

Sampling and crossing of plants were carried out in the experimental garden and the parent garden of PT. Socfin Indonesia (Socfindo), Socfindo Seed Production Laboratory located in Martebing village, Dolok Masihul district, Serdang Bedagai Regency, North Sumatra, Indonesia. The genetic material used in this study was two series of plant crosses, namely: a set of plant crosses taken from the genetic material of the *G. boninense* experiment in a field that is now 15 years old, and a series of new crosses for early detection of resistance in the nursery stage. For genetic material in nurseries, plant samples consist of healthy plants (seeds without *G. boninense* infection treatment) and plant samples infected with the fungus *G. boninense*. Inoculation is carried out when the plant is eight weeks after the sprouts are sown.

DNA extraction of samples on leaves and root tissue was performed using the CTAB method [9]. The quality and quantity of the DNA were evaluated using 1% agarose gels and measured by UV-Spectrophotometer. All the DNA samples were kept at -20 °C before applying for the next analysis.

2.2. Amplification of DNA with microsatellite primers

Seven specific primers were selected and synthesized as shown in Table 1 from the designated oil palm tolerance genes. For each reaction, 2.5 ng DNA templates were mixed with 5 µl PCR buffer (10 mmol/L Tris-HCl, 50 mmol/L KCl, 2 mmol/L MgCl₂, 0.001 g/µl glycerol), 200 µmol/L dNTPs, 0.10 µmol/L primer, 0.1 Unit Taq DNA polymerase. PCR amplification was carried out at Eppendorf Mastercycler ep 384 (Eppendorf, Westbury, New York, USA). The amplification program consisted of an initial 4 min denaturation cycle at 94°C, followed by 35 cycles of denaturation 94°C for 30 sec, a step of 52°C for 1 min 15 sec, an extension of 72°C for 1 min 30 sec, and final elongation at 72°C for 8 min.
2.3. Visualization of DNA amplification
The results of DNA amplification were checked by electrophoresis with 2% agarose gel. The gel is made by dissolving 0.9 g of agarose with 60 ml of TAE 1x and then heated into a microwave at medium temperature for 2 minutes. Then the solution was removed and added 0.5 µl Gel Red (Biotium) and stirred slowly until homogeneous. Then the agarose solution is poured into electrophoresis gel, fitted with a comb and left for about 30 min. The next process is pouring 300 ml of TAE 1x into electrophoresis. DNA ladder marker 50bp–1500 bp which has been mixed with loading dye (2 µl) injected into the agarose gel well in the first column as a comparison using a micropipette. Furthermore, PCR products from each sample are applied to the next electrophoresis well. Electrophoresis is carried out with 50 watts of power with 110 volts of electricity and 25 mA for 30 min [11]. The results of electrophoresis were then documented with the UV-transilluminator (UV-Doc-its) and Gel-Doc (U-Doc-its) [11].

Table 1. Specific primers used in this study

| Gene      | Putative identity               | Primer sequence (5'-3')                       | Product (bp) | References |
|-----------|--------------------------------|-----------------------------------------------|--------------|------------|
| EgPRP     | Pathogenesis-related protein   | -TCTCTCAGTGCGATTTTGTCG-AGAGTGGCATATTGGCATCC- | 161          | This study |
| EgMT      | Metallothionein-like protein   | -CTGTTGATTCTGTGCGGCTTT-CTTGGATGCTTGGAGAGACC- | 100          | [6]        |
| EgIFR     | Isoflavone reductase           | -ACCTTCGCCTTGTGAGAGAAGCGATGTCTTGGTCTGAT-     | 346          | [6-7]      |
| Rgen Pto  | Kinase                         | -GCATTGAGAACAGGTGAAAGGGGACCACCCACGTAAG       | 100          | [10]       |
| Eg001     | Polyadenylate-binding protein2 | -TTGCTGGAGAATGCTTCTC-TCCAGACATGCAACACTTCTC-  | 372          | This study |
| Eg002     | Heat shock cognate            | -AGGCCTACTTGGTCCACTG-CCCTTCACATGATGTCCTCT-  | 421          | This study |
| Eg003     | Thaumatin                     | -TCTTCTCCCTTCCTTCCTCCTC-                      | 379          | This study |

2.4. Data analysis
Data analysis was based on the results of DNA band scoring on an agarose gel. The banding is manually scanned as binary data with a present (1) or none (0) band. Data analysis to find out the allele length range was done by semi-quantitative with standard curves using UVITEC Cambridge software [11]. Then the data is imported based on the request of the software used. The banding pattern was detected to compare between tolerant, vulnerable and moderate plants.

3. Results and Discussion

3.1. Quantity and quality of DNA isolation
DNA isolation was carried out on the roots and leaves of mature plants and seedlings from all samples in this study. The isolated DNA was then tested qualitatively and quantitatively. Qualitative tests were carried out by looking at the isolated DNA in the agarose gel 2%. The quality test results showed there was DNA in the results of isolation of roots and leaves of mature plants and seeds with varying quality. The visualization of DNA quality in leaves of adult plants from samples 1 to 24 with the UV-transilluminator and Gel-Doc is shown in Figure 1.
Quantity tests were also carried out to see the level of purity and concentration of the isolated DNA. The quantity test is carried out using a Nanodrop spectrophotometer. Quantity test of DNA from isolation was measured at a wavelength of 260 nm for measurement of DNA concentration and purity ratio of DNA at wavelength A260/280. Absorbance values between 1.80 to 2.00 indicate the results of a good quantity test [9, 11]. The absorbance values above 2.00 indicated that the isolated DNA contains more RNA while the absorbance value below 1.80 shows that the DNA that has been isolated contains protein, lipids, and carbohydrates [11].

From the results of DNA isolation on leaf and root tissue in the sample of seedlings and mature plants using the CTAB method, the values of varying purity and DNA concentration were obtained as shown in Table 2. In general, the isolation of DNA purity was dominant in the criteria of excellent purity and high concentration. The results of this quality and quantity test show that DNA was successfully isolated and can then be used for DNA amplification.

| Development | Tissue | Purity (A260/280) | Concentration (ng/µl) | Sample |
|-------------|--------|------------------|-----------------------|--------|
| Mature      | Leaves | < 1.80           | 92.49 – 122.77        | 2      |
|             |        | 1.80 – 2.00      | 45.35 – 366.44        | 12     |
|             |        | > 2.00           | 32.22 – 279.71        | 10     |
|             | Roots  | < 1.80           | 11.06 - 1.303.37      | 11     |
|             |        | 1.80 – 2.00      | 105.45 – 1.157.25     | 13     |
|             |        | > 2.00           | -                     | 0      |
| Seedling    | Leaves | < 1.80           | -                     | 0      |
|             |        | 1.80 – 2.00      | 119 - 525             | 24     |
|             |        | > 2.00           | -                     | 0      |
|             | Roots  | < 1.80           | 151                   | 1      |
|             |        | 1.80 – 2.00      | 95 – 272              | 23     |
|             |        | > 2.00           | -                     | 0      |

3.2. Amplification of palm oil DNA
Amplification of DNA samples from mature and seedlings oil palm to validate the character of BSR disease resistance was carried out using seven specific primers, 

\[
\text{EgPRP, EgMT, EgIFR, Rgen_Pto, Eg001, Eg002, and Eg003.}
\]

The amplification pattern of the PCR product was carried out using agarose gel. The seven primers used included specific primers, with DNA banding patterns visible in only one band (Figure 2). For example, in the primary \(\text{EgPRP}\) with a sample of the root tissue of a mature oil palm plant, there is only one band almost in all samples, in which some samples are undetectable. The number of bands appearance shows the allele conditions of the target gene, whether
homozygous or heterozygous [12]. In this study, all genes thought to be related to resistance to \textit{G. boninense} were homozygous in all samples.

The results of visualization and band readings with seven primers on the character of resistance to root stem disease were obtained by the number of primary alleles with varying range and allele length per primer but still within the scope of the band length of each target. The target DNA from all (primary) markers generally appears in all groups of plant samples. Therefore the presence of alleles (bands) cannot be used as unique markers to identify the resistance properties of plants to \textit{G. boninense}. This finding also was indicated by the low PIC (Polymorphic Informative Content) value of the seven primers (Tables 3 and 4). This exception for the \textit{Rgen_Pto} primer showing high PIC values in leaf and root tissue both mature and seedling plants so that it has the potential primer to be used as a molecular marker for selection of tolerant plants against \textit{G. boninense}. It is noteworthy that \textit{EgIFR} primer had a high PIC in the root tissues of mature plants (Table 3).

Similarly, the relatively high PIC belonging to primers of \textit{EgPRP}, \textit{EgIFR}, \textit{Eg001}, and \textit{Eg003} for roots of oil palm seedlings as displayed in Table 4. The results of DNA amplification using seven special primers also suggested that the target genes associated with resistance to SBR disease are found in almost all individual oil palm plants, but may have different expressions or have SNP (Single Nucleotide Polymorphism) in sequence the DNA (data not shown). The information obtained in this study is valuable additional information from much of the information collected in our previous

\begin{table}
\centering
\begin{tabular}{|c|c|c|c|c|}
\hline
\textbf{No.} & \textbf{Primer} & \textbf{Tissue} & \textbf{Detected allele} & \textbf{Allele length(bp)} & \textbf{Polymorphic level (PIC)} \\
\hline
1. & \textit{EgPRP} & Leaves & 24 & 123 – 156 & 0.00  \\
 & & Roots & 23 & 136 – 162 & 0.08  \\
2. & \textit{EgMT} & Leaves & 24 & 100 – 115 & 0.00  \\
 & & Roots & 24 & 95 – 109 & 0.00  \\
3. & \textit{EgIFR} & Leaves & 24 & 238 – 310 & 0.00  \\
 & & Roots & 14 & 276 – 317 & 0.49  \\
4. & \textit{Rgen_Pto} & Leaves & 16 & 90 – 114 & 0.44  \\
 & & Roots & 16 & 91 – 112 & 0.44  \\
5. & \textit{Eg001} & Leaves & 24 & 250 – 394 & 0.00  \\
 & & Roots & 24 & 221 – 370 & 0.00  \\
6. & \textit{Eg002} & Leaves & 24 & 378 – 558 & 0.00  \\
 & & Roots & 24 & 325 – 400 & 0.00  \\
7. & \textit{Eg003} & Leaves & 23 & 400 – 515 & 0.08  \\
 & & Roots & 24 & 297 – 394 & 0.00  \\
\hline
\textbf{Average} & 22 (14 - 24) & - & 0.11 (0 – 0.49) \\
\hline
\end{tabular}
\end{table}

The results of visualization and band readings with seven primers on the character of resistance to root stem disease were obtained by the number of primary alleles with varying range and allele length per primer but still within the scope of the band length of each target. The target DNA from all (primary) markers generally appears in all groups of plant samples. Therefore the presence of alleles (bands) cannot be used as unique markers to identify the resistance properties of plants to \textit{G. boninense}. This finding also was indicated by the low PIC (Polymorphic Informative Content) value of the seven primers (Tables 3 and 4). This exception for the \textit{Rgen_Pto} primer showing high PIC values in leaf and root tissue both mature and seedling plants so that it has the potential primer to be used as a molecular marker for selection of tolerant plants against \textit{G. boninense}. It is noteworthy that \textit{EgIFR} primer had a high PIC in the root tissues of mature plants (Table 3).

Similarly, the relatively high PIC belonging to primers of \textit{EgPRP}, \textit{EgIFR}, \textit{Eg001}, and \textit{Eg003} for roots of oil palm seedlings as displayed in Table 4. The results of DNA amplification using seven special primers also suggested that the target genes associated with resistance to SBR disease are found in almost all individual oil palm plants, but may have different expressions or have SNP (Single Nucleotide Polymorphism) in sequence the DNA (data not shown). The information obtained in this study is valuable additional information from much of the information collected in our previous
studies of oil palms on biochemical markers for the breeding strategy of plant materials [13-14], especially in relation to resistance genes to *G. boninense*.

Table 4. Profile of seven primers in 24 seedlings oil palm plants

| No. | Primer     | Tissue | Allele detected |Allele length (bp) | Polymorphic level (PIC) |
|-----|------------|--------|-----------------|-------------------|-------------------------|
| 1.  | *EgPRP*   | Leaves | 24              | 147–167           | 0.00                    |
|     |            | Roots  | 21              | 152–165           | 0.22                    |
| 2.  | *EgMT*    | Leaves | 24              | 82–112            | 0.00                    |
|     |            | Roots  | 24              | 90–106            | 0.00                    |
| 3.  | *EgIFR*   | Leaves | 23              | 259–332           | 0.08                    |
|     |            | Roots  | 20              | 291–329           | 0.28                    |
| 4.  | *Rgen Pto*| Leaves | 23              | 98–119            | 0.08                    |
|     |            | Roots  | 22              | 102–108           | 0.15                    |
| 5.  | *Eg001*   | Leaves | 23              | 336–431           | 0.08                    |
|     |            | Roots  | 20              | 383–436           | 0.28                    |
| 6.  | *Eg002*   | Leaves | 24              | 394–450           | 0.00                    |
|     |            | Roots  | 24              | 444–508           | 0.00                    |
| 7.  | *Eg003*   | Leaves | 23              | 416–446           | 0.08                    |
|     |            | Roots  | 21              | 415–469           | 0.22                    |

The classification of genetic material resilience in this study and previous works is based on the results of research at PT Socfindo that has been carried out since the 1970s. Resistance observation was carried out in genetic experiments (genetic trial/progeny trial), parental garden and seed garden, first experiments on oil palm plant resistance in the field (specific *G. boninense* trial) and in early selection testing (initial screening test) at the nursery stage. Tolerant plants are plants which in all observations and experiments above show resistance to *G. boninense*.

From the data from observations and testing of plant resistance to *G. boninense* in nurseries and the field, several Socfindo genetic materials that are tolerant and vulnerable have been identified. The results of the observations also showed the existence of additive properties of resistance to *G. boninense* derived from their parents. Furthermore, offspring from crossing elders tolerant with susceptible elders will produce a moderate offspring population (intermediate). The grouping of resilience in this study therefore based on the results of observations and tests and has been proven from the attack index on crossing samples of mature plants used.

4. Conclusions

The seven primers with DNA banding profiles clearly detected evident in one discrete band. The target genes associated with resistance to SBR disease are found in almost all individual oil palm plants. The low PIC value of the seven primers except for the *Rgen Pto* primer showing the high PIC in the leaf tissue and mature plant roots suggested the potential molecular marker of this primer for selection of tolerant palm against *G. boninense*.

Acknowledgment

This work was supported in part by a Penelitian Strategis Nasional Institusi (PSN Institusi 2018 to MB) from the Directorate for Research and Community Service, Ministry of Research, Technology and Higher Education, Republic of Indonesia. The authors are grateful to PT. Socfin Indonesia for their full support on this study and the Universitas Sumatera Utara.

References

[1] USDA 2017 Indonesia and product annual report 2017 *USDA Foreign Agricultural Service*. 
[2] Husharian R, Yusof NA, Dutse SW 2013 Detection and control of Ganoderma boninense: strategies and perspectives SpringerPlus 2 555.

[3] Morel A, Friedman R, Tulloch DJ, Caldecott B 2016 Stranded assets in Palm Oil Production: A case study of Indonesia. Sustainable Finance Programme, SSEE, University of Oxford, Working Paper, July 2016.

[4] Susanto A 2009 Basal Stem Rot in Indonesia – Biology, economic importance, epidemiology, detection and control proceedings of International workshop on awareness, detection and control of oil palm devastating diseases, Kuala Lumpur, Malaysia, Paper 2.

[5] de Franqueville H, Asmady H, Jacquemard JC, Hayun Z, and Durand-Gasselin T 2001 Indications on sources of oil palm (Elaeis guineensis Jacq.) genetic resistance and susceptibility to Ganoderma sp., the cause of basal stem rot InCutting-Edge Technologies for sustained Competitiveness, Proceedings of International Palm Oil Congress, Eds Malaysian Palm Oil Board, Kuala Lumpur, Malaysia : 420-431.

[6] Tan YC, Yeoh KA, Wong MY, Ho CL 2013 Expression profiles of putative defense-related proteins in oil palm (Elaeis guineensis) colonised by Ganoderma boninense J. Plant Physiol. 170 1455–1460

[7] Tee SS, Tan YC, Faridah A, Melina OA, Ho CL 2013 Transcriptome of oil palm (Elaeis guineensis Jacq.) roots treated with Ganoderma boninense Tree Genet. Genomes 9 377–386

[8] Basyuni M, Amri N, Putri LA, Syahputra I, Arifiyanto D 2017 Characteristics of fresh fruit bunch yield and the physicochemical qualities of palm oil during storage in North Sumatra, Indonesia Indones. J. Chem. 17 182–190.

[9] Basyuni M, Baba S, Oku H 2017 Microsatellite analysis on genetic variation in two populations of red mangrove Rhizophora mangle L. (Rhizophoraceae) and its implication to conservation IOP Conf. Ser.: Mater. Sci. Eng. 180 012243.

[10] Foan CC, Lee YW, Tan JS, Alwee SSRS 2012 Amplification and sequencing of partial-length disease resistance gene homologues coding for NBS-LRR proteins in oil palm (Elaeis guineensis) Asia Pac J. Mol. Biol. Biotechnol. 20 25-31

[11] Basyuni M, Prayogi H, Putri LAP, Syahputra I, Siregar ES, Risnasari I, Wati R, Arifiyanto D 2018 RAPD markers on genetic diversity in three populations of pisifera type of oil palm (Elaeis guineensis) IOP Conf. Ser.: Earth Environ. Sci. 130 012050.

[12] Basyuni M, Baba S, Oku H 2018 Microsatellite analysis of genetic diversity and structure of Bruguiera gymnorrhiza and Kandelia obovata E3S Web Conf. 52 00027.

[13] Arifiyanto D, Basyuni M, Sumardi, Putri LAP, Siregar ES, Risnasari I, Syahputra I 2017 Occurrence and cluster analysis of palm oil (Elaeis guineensis) fruit type using two-dimensional thin layer chromatography Biodiversitas 18 1487–1492.

[14] Basyuni M, Wati R, Deni I, Tia AR, Slamet B, Siregar ES, Syahputra I 2018 Cluster analysis of polyisoprenoid in oil palm (Elaeis guineensis) leaves in different land-uses to find the possible cause of yield gap from planting materials Biodiversitas 19 1492–1501.