Indonesian Journal of Biotechnology

VOLUME 29(1), 2024, 33-39 | RESEARCH ARTICLE

Easy extraction of *Ganoderma boninense* liquid sample using portable on-chip device

Adella Josephin[1,4], Yudan Whulanza[2,4,*], Siti Fauziyah Rahman[1,4], Kenny Lischer[3,4], Muhammad Imam Surya[5], Irfan Martiansyah[5], Wiguna Rahman[5] , Uda Hashim[6]

[1]Department of Electrical Engineering, Faculty of Engineering, Universitas Indonesia, Depok 16424, West Java, Indonesia
[2]Department of Mechanical Engineering, Faculty of Engineering, Universitas Indonesia, Depok 16424, West Java, Indonesia
[3]Department of Chemical Engineering, Faculty of Engineering, Universitas Indonesia, Depok 16424, West Java, Indonesia
[4]Research Center of Biomedical Engineering, Universitas Indonesia, Depok 16424, West Java, Indonesia
[5]Research Center for Applied Botany, National Research and Innovation Agency, Cibinong, Bogor 16911, West Java, Indonesia
[6]Institute of Nano Electronic Engineering, Universiti Malaysia Perlis, Seriab 01000 Kangar, Perlis, Malaysia
*Corresponding author: yudan.whulanza@ui.ac.id

SUBMITTED 5 April 2023 **REVISED** 18 January 2024 **ACCEPTED** 20 January 2024

ABSTRACT Detecting *Ganoderma boninense* in Indonesia is crucial for effectively controlling and mitigating the spread of basal stem disease in oil palm fields. While there is ongoing development of tolerant plants, no such plant has been successfully created yet. Consequently, researchers are actively studying detection methods for *Ganoderma boninense*. One established and highly accurate approach is the use of polymerase chain reaction (PCR) techniques for molecular detection. However, this method requires time-consuming sample preparation, which can pose challenges in plantation settings. To address this problem, a portable lab-on-chip device has been introduced. This technology enables easy and automatic DNA retrieval from liquid samples by absorbing lysed DNA using magnetic beads. An efficient mechanism for manipulating the magnetic bead within the semiconductor has been successfully implemented. The extraction process typically takes around 15 minutes using a modified methodology on the chip device approach. The chip facilitates the retrieval of two samples with a capacity of 120 µL for each sample. The PCR method was utilized to validate the equivalence of the lab-on-chip device extraction to the standard extraction method. This represents a promising alternative for expedited and simplified detection of *Ganoderma boninense* in field conditions.

KEYWORDS *Ganoderma boninense*; Infected oil palms; Lab-on-chip; Molecular detection; Polymerase chain reaction

1. Introduction

Oil palm is a vital crop for numerous countries across Southeast Asia, Africa, and Latin America (Godswill et al. 2016; Liew et al. 2015; Siddiqui et al. 2021; Soh 2012). It is the most productive oil crop, providing yields 3–8 times greater than other oil producers, including canola, sunflower, soybean, and rapeseed; very profitable because of its high output; low production costs; and relatively easy-to-grow (Barcelos et al. 2015; Dislich et al. 2017; Soh 2012). The oil palm sector is estimated to be worth USD 65.73 billion as of 2015 and USD 92.84 billion by 2021. Indonesia and Malaysia are the primary producers, accounting for 85% of global production (Bentivoglio et al. 2018; Fathana 2018; USDA 2012). This crop is a significant export and contributes significantly to the economies of many countries (Soh 2012).

Basal stem rot (BSR) is a severe disease in oil palm plantations, notably in Southeast Asia, particularly in Malaysia and Indonesia (Idris et al. 2004; Siddiqui et al. 2021). *Ganoderma boninense*, a basidiomycetes fungus, is the primary cause of BSR putting the oil palm sector in grave danger. Although BSR was initially found in palms older than 25–30 years, it has recently affected younger palms, including those as young as one year old (Zakaria 2023). The oil palm industry suffers significant losses due to this disease, with annual losses in Indonesia and Malaysia reaching up to US$500 million. BSR disease brought on by *Ganoderma* in oil palms still lacks an efficient management method (Ommelna et al. 2012; Tahir et al. 2023).

Once the fungus has infected the tree, it will soon colonize the tissues of the trunk and cause a progressive deterioration of the internal structures, leading to the eventual death of the tree (Siddiqui et al. 2021). The disease reduces the profitability and sustainability of oil palm plantations in Southeast Asia by causing significant yield losses, shortening tree life, and increasing management costs for

farmers (Abubakar et al. 2022; Paterson 2007, 2019; Paterson et al. 2013). There have been numerous efforts to control *Ganoderma* in oil palm plantations. Several control methods include environmental sanitation (Naher et al. 2013; Paterson 2007), such as the fungus *Trichoderma* sp. as an antagonistic agent (Priwiratama and Susanto 2014) and endomycorrhizae (Kartika et al. 2010), as well as synthetic chemical control using several fungicide active ingredients. However, the control results are still considered ineffective.

The ideal control for overcoming diseases is using resistant plants against *Ganoderma boninense* (Idris et al. 2004; Durand-Gasselin et al. 2005). Gene knockout using CRISPR/Cas9 of the *EgEMLP* gene, whose expression is known to increase in oil palm plants infected with *G. boninense*, is one of the efforts to obtain a resistant plant. However, further studies are needed to determine how well-modified oil palm clones grow when infected by *G. boninense* (Budiani et al. 2019). Therefore, early detection of the emergence of *G. boninense* in plantations is crucial. Several methods have been explored to detect the disease using the shifting properties in electrochemical (Fowotade et al. 2019), electrical (Ayoib et al. 2020), and spectroscopy (Mohd Hilmi Tan et al. 2023). Those methods demonstrated good results, but they need relatively specific instruments to be applied or a close system, which becomes hard to modify for a different strain of *G. boninense*.

A molecular technology, polymerase chain reaction (PCR), has also been developed to detect *G. boninense* and other antagonistic agent applications have been more precise to control than other methods (Hushiarian et al. 2013). With molecular technology, early detection of *Ganoderma* and other antagonistic agents is expected to help control the primary disease in oil palm plantations. However, the use of PCR instruments is requiring a specialized person to perform molecular biology tasks, costly equipment, and storage of certain reagents at specific temperatures. Furthermore, DNA extraction from liquid samples, a crucial step for the PCR process, is challenging to perform in plantations. Therefore, a lab-on-chip device has been introduced to enable easy and automated extraction of purified DNA from a small, liquid sample.

The use of lab-on-chip technology has been explored in our previous study, such as for various applications in cell culture, neurotransmitter identification, and stem cell encapsulation (Nadhif et al. 2017; Nathani et al. 2022; Whulanza et al. 2014, 2022a,b). Our other studies also showed that a PCR-based chip has significantly increased the portability of the apparatus (Lischer et al. 2021; Whulanza et al. 2017). This paper aims to compare the product of DNA extraction by conventional lab-based protocol and on-chip device. It also aims to compare the standard protocol and its modification of the on-chip device method. This study offers a promising, easier method for DNA extraction of *Ganoderma boninense* and its detection in the field.

2. Materials and Methods

2.1. Conventional method

In this study, the oil palm leaf samples were obtained from PT Socfin, Medan, North Sumatra (Figure 1a). To dissociate plant tissue, 50 mg of fresh or frozen tissue or 10 mg of dried sample were subjected to grinding and put into a 1.5 mL microcentrifuge tube (Figure 1b). DNA extraction was performed using Genomic DNA Mini Kit for plant (GeneAid, Taiwan).

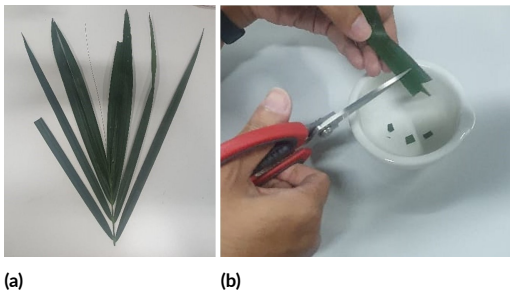

FIGURE 1 Samples of oil palm leaves. (a) The fresh oil palm leaves samples were obtained from PT. Socfin, Medan, North Sumatra. (b) 50 mg of fresh samples were prepared to grind using mortar.

During the lysis procedure, a solution of 400 µL GP1 or GPX1 buffer and 5 µL RNase A was added to the sample tube. The tube was then incubated at 60 °C for 10 min, being flipped every 5 min. The elution buffer was pre-heated to 60 °C, and 100 µL GP2 buffer was added to the lysate, followed by incubation on ice for 3 min. The mixture was transferred to a filter column inside a 2 mL collection tube and centrifuged for 1 minute at 1,000 × g. The supernatant was collected in a new 1.5 mL microcentrifuge tube. DNA binding was facilitated by adding 1.5 volumes of GP3 buffer to the lysate and vortexed. The mixture was added to a GD column and centrifuged at 14–16,000 × g for 2 min. The column was then centrifuged at 14–16,000 × g for 30 s after being washed with 400 µL W1 and 600 µL Wash buffer (ethanol added). The GD column was centrifuged for 3 minutes at 14-16,000 × g to remove residual liquid. The dried GD column was moved to a microcentrifuge tube, and the column matrix was filled with 100 µL of pre-heated elution buffer to elute the DNA. After 3–5 minutes, the column was centrifuged at 14-16,000 × g for 30 seconds to collect the eluted DNA. Figure 2 illustrates the regular DNA extraction method.

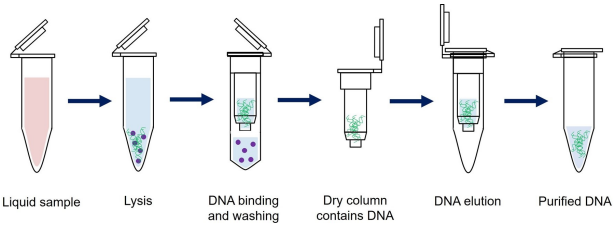

FIGURE 2 DNA extraction process using conventional methods (adapted from Park et al. (2014)).

2.2. DNA extraction of oil palm leaves using portable extraction unit

The on-chip extraction was done using an extraction kit from microfluidic Chipshop GmbH (Jena, Germany). The extraction process employs a microfluidic chip placed in a simple extraction apparatus to execute lysis, washing, and elution processes. Figure 3a depicts magnetic beads for shearing the liquid in the extraction unit with a predetermined time and temperature. Each time the process is completed, the liquid is substituted with a new solution specific to that process, as depicted in Figure 3b. During the lysis process, the lysate binds to the magnetic bead. The elution process retracts the DNA from the magnetic beads to the liquid sample the washing process is introduced in between to ensure that the lysis and elution process succeed.

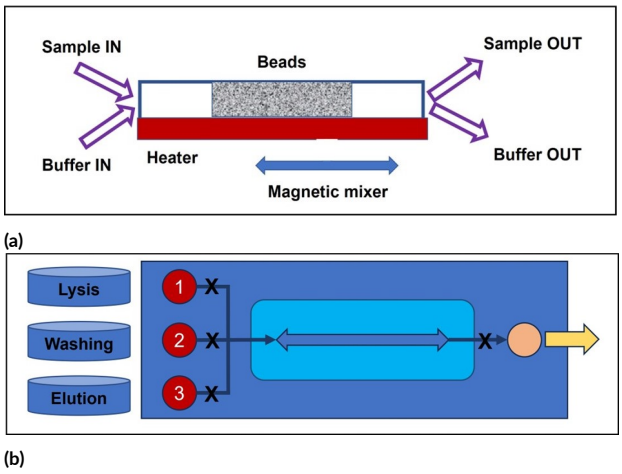

FIGURE 3 (a) Schematic of the extraction process in a chip and (b) solution replacement between each process.

The chip was loaded with 30 µL of the liquid sample from the conventional lysis method and 100 µL of lysis and binding buffer to initiate the lysis and binding process. Four µL of magnetic beads were introduced into the chip and mixed at 55 °C for 15 min. The washing process was then done by removing the solution in the chip and adding 130 µL of washing buffer 1, which was mixed for 30 s and repeated three times. Subsequently, the solution was removed again, and 130 µL of washing buffer 2 was added, mixed for 30 s, and repeated three times. After this, the solution was removed, 130 µL of washing buffer 3/elution buffer was added, mixed for 10 s, and removed for DNA elution. The elution step was performed by adding 125 µL of elution buffer to the chip and stirring for 5 min at a temperature of 55 °C. Different reagents, ChipGenie bacterial buffer, and GeneAid plant buffer were utilized in the extraction process using a portable on-chip device as a comparison with different temperatures for lysis-binding and elution (55 °C for ChipGenie bacterial buffer and 60 °C for GeneAid plant buffer), and PCR confirmed the DNA extraction.

2.3. Modified protocol of DNA extraction using portable extraction unit

The GeneAid plant buffer is used to perform the extraction using this protocol. The chip was loaded with 30 µL of the liquid sample from the conventional lysis method and 100 µL of GP3 buffer to initiate the lysis and binding process. Four µL of magnetic beads were introduced into the chip and mixed at 60 °C for 5 min. The washing process was then done by removing the solution in the chip and adding 130 µL of W1 buffer, which was mixed for 30 s. Subsequently, the solution was removed again, and 130 µL of wash buffer was added and mixed for 30 s. Following this, the solution was removed, 130 µL of elution buffer was added, mixed for 10 s, and removed for DNA elution. The elution step was performed by adding 125 µL of elution buffer to the chip and stirring for 5 min at a temperature of 60 °C. PCR then confirmed the DNA extraction.

2.4. Polymerase chain reaction (PCR)

A PCR amplification protocol was conducted to validate DNA extraction in the previous method. The PCR temperature profiles comprised several stages: initial denaturation at 94 °C for 3 min, denaturation at 94 °C for 60 s, annealing at 54 °C for 30 s (depending on the primer used), extension at 72 °C for 1–2 minutes, post-extension at 72 °C for 5 min, and termination at 4 °C for an infinite period. The PCR cycle was reiterated for 35 cycles using a mixture consisting of 2 µL DNA template sample, 1 µL forward primer (ITS 1 CTTGGTCATTTAGAGGAAGTAA), 1 µL reverse primer (Gan 2 GCGTTACATCGCAATACA), 3 µL nuclease-free water, and 6 µL My Taq PCR mix-Bioline (Bioline, US). A 1% agarose gel stained with GelRed was electrophoresed to determine the purity of the isolated DNA. The PCR products were visualized as fluorescent bands with GelDoc (BioRad, US).

3. Results and Discussion

Leaf samples were employed in both the conventional and on-chip methods, as illustrated in Figure 2, which displays the DNA extraction process using the conventional

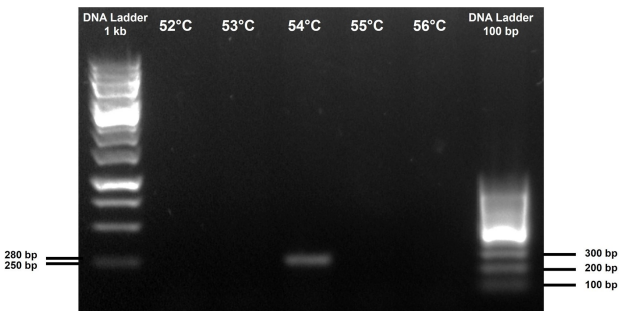

FIGURE 4 PCR result using marker 1 kb and 100 bp of conventional extraction from leaf samples with an annealing temperature variation of 52–56 °C.

method. The PCR was utilized to compare the extraction outcomes of both methods, utilizing Z primers (ITS 1 forward and Gan 2 reverse) since it showed one specific band, which yielded a 280 bp amplicon. Later, the amplification using the conventional DNA extraction method was shown in Figure 4. It revealed visible luminescence bands at annealing temperatures of 54 °C. Thus, the optimal annealing temperature for detecting *G. boninense* using the conventional method ranges from 54 °C. The PCR results are in line with the study conducted by Minarsih et al. (2018), where *Ganoderma* in oil palm plants can be detected by molecular analysis using PCR with specific *Ganoderma* DNA primers (Gan 1-Gan 2 and ITS 1-ITS 4) with certain combinations.

The internal transcribed spacer (ITS) region of rRNA is widely used as a primer for the general identification of fungi, including *Ganoderma* (Minarsih et al. 2018; Moncalvo et al. 1995; Nusaibah et al. 2011; Rees et al. 2009; Utomo and Niepold 2000). In a study conducted by Nusaibah et al. (2011), the combination of ITS 1 and ITS 4 as primers was reported to be accurate for determining genetic variation in *Ganoderma* species. Earlier, Utomo and Niepold (2000), who designed specific primers of Gan 1 and Gan 2 from the ITS region, were also successful in separating accessions of *Ganoderma boninense* from the other species of *Ganoderma*.

Figure 5a depicts the filling of the chip with the master mix and magnetic beads for the first process, which is the lysis. Figure 5b shows the extraction process that involved liquid replacement for washing and elution processes. These figures showed that the magnetic bead moved forth and back for specific temperatures at the initial setup. Lastly, the liquid is collected from the chip to the mini tube for PCR testing, as shown in Figure 5c.

Figure 6a depicts the DNA amplification using ChipGenie buffer (1–2, 5–6) and GeneAid Plant buffer (3–4, 7–8). Table 1 shows an explanation for bands 1–8 in Figure 6a. Both buffers effectively extract *Ganoderma boninense*

FIGURE 5 Extraction process: (a) preparation of master mix together with magnetic beads in the chip; (b) the chip seated in the portable extraction unit with ChipGenie buffer at 60 °C; (c) result of extracted DNA placed in a mini tube to be ready for the amplification process.

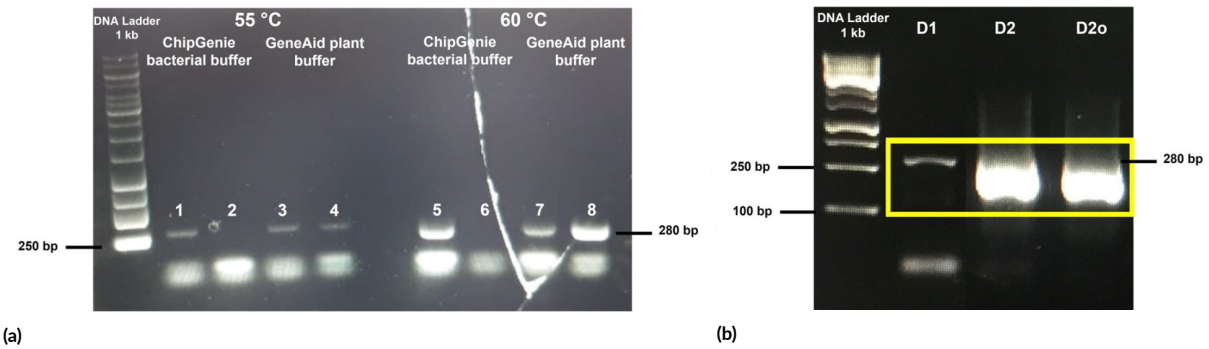

FIGURE 6 (a) PCR result of on-chip extraction at 55 and 60 °C and (b) PCR result using marker 1 kb (M) of on-chip extraction with ChipGenie bacterial buffer (D1), GeneAid plant buffer (D2) based on standard protocol and GeneAid plant buffer based on modified protocol (D2o).

TABLE 1 The BOD removal of tofu wastewater after 48 h.

Band	Sample Description	Protocol	Additional Note
1	Oil palm leaves with ChipGenie bacterial buffer	Standard on-chip extraction using 55 °C	Sample a
2	Oil palm leaves with ChipGenie bacterial buffer	Standard on-chip extraction using 55 °C	Sample b (repetition)
3	Oil palm leaves with GeneAid plant buffer	Standard on-chip extraction using 55 °C	Sample a
4	Oil palm leaves with GeneAid plant buffer	Standard on-chip extraction using 55 °C	Sample b (repetition)
5	Oil palm leaves with ChipGenie bacterial buffer	Standard on-chip extraction using 60 °C	Sample a
6	Oil palm leaves with ChipGenie bacterial buffer	Standard on-chip extraction using 60 °C	Sample b (repetition)
7	Oil palm leaves with GeneAid plant buffer	Standard on-chip extraction using 60 °C	Sample a
8	Oil palm leaves with GeneAid plant buffer	Standard on-chip extraction using 60 °C	Sample b (repetition)

DNA, with the invisibility of the band in Figure 6a (2 and 6) related to the composition of the master mix used. The bacterial buffer used was successful in extracting DNA from plant samples. This shows that the buffer used for DNA extraction is universal. It has been observed that a change to a higher temperature (60 °C) results in stronger luminescence bands, indicating that more DNA was extracted at 60 °C (5–8) rather than 55 °C (1–4). Therefore, the optimum temperature for extracting DNA from plant samples is 60 °C. This amplification process confirmed that the lab-on-chip extraction is comparable to the conventional extraction method, as in Figure 4.

An evaluation was conducted to compare the standard and modified protocols of the on-chip extraction method. The experimental findings demonstrated that the altered techniques were capable of expediting the isolation of DNA in oil palms. Our testing found that the conventional method required 30 minutes for all the extraction processes, while the chip approach was 15 minutes faster. However, the user must become familiar with the process of moving in and out of a liquid using a syringe in a more limited pathway. Furthermore, the chip offers two channels that allow for the simultaneous extraction of two specimens. Additionally, there is a chip that offers four channels, which theoretically increases the number of specimens available for our study.

Our proposed method was validated by the presence of DNA bands on PCR product visualization by electrophoresis on agarose gel, as in Figure 6b during the amplification phase. However, there are still many secondary metabolites that reduce the concentration and purity of DNA. The use of bacterial and plant lysis buffers generally showed differences in DNA luminescence in the gel. The luminescence band located below the marker is the primer dimer.

Chemical extraction procedures, such as those involving cetyltrimethylammonium bromide (CTAB) or phenol-chloroform, are recognized for their time-consuming nature (Elkins 2013; Green and Sambrook 2017). Thus, (Siegel et al. 2017) performed a straightforward DNA extraction method that involved using cellulose filter paper to separate DNA from the source. Although the filter was inexpensive, it posed challenges in the recovery of concentrated DNA. This highlights another advantage of employing magnetic beads for the extraction in our technique.

4. Conclusions

Our findings demonstrated that employing on-chip portable devices provides a viable and encouraging approach for collecting *Ganoderma boninense* DNA from liquid samples. This technology offers a substitute for performing the lysis, washing, and elution procedures that are comparable to traditional methods, with the added capability of working with up to four specimens simultaneously. The efficacy of the lab-on-chip device extraction was validated by a polymerase chain reaction and determined to be comparable to that of the conventional approach. By adjusting temperatures and time duration, it is possible to expedite the extraction process in on-chip protocols.

Acknowledgments

We gratefully acknowledge the funding from Badan Pengelola Dana Perkebunan Kelapa Sawit (BPDPKS) Grand Research Sawit No: NKB-667/UN2.RST/HKP.05.00/2021. We thank the support from PT Socfin Indonesia Plantations, Medan, Indonesia, for validating the idea.

Authors' contributions

AJ: Writing-Original Draft Preparation and Investigation; YW: Resources, Project Administration and Funding Acquisition; SFR: Supervision; KL: Data Curation; MIS: Writing-Review and Editing; IM: Methodology and Investigation; WR: Formal Analysis and Investigation; UA: Conceptualization. All authors read and approved the final version of the manuscript.

Competing interests

All authors declare that there are not any conflicts of interest.

References

Abubakar A, Ishak MY, Bakar AA, Uddin MK. 2022. *Ganoderma boninense* basal stem rot induced by climate change and its effect on oil palm. Environ. Sustain. 5(3):289–303. doi:10.1007/s42398-022-00244-7.

Ayoib A, Hashim U, Gopinath SC. 2020. Automated, high-throughput DNA extraction protocol for disposable label free, microfluidics integrating DNA biosensor for oil palm pathogen, *Ganoderma boninense*. Process Biochem. 92:447–456. doi:10.1016/j.procbio.2020.02.003.

Barcelos E, De Almeida Rios S, Cunha RN, Lopes R, Motoike SY, Babiychuk E, Skirycz A, Kushnir S. 2015. Oil palm natural diversity and the potential for yield improvement. Front. Plant Sci. 6:190. doi:10.3389/fpls.2015.00190.

Bentivoglio D, Finco A, Bucci G. 2018. Factors affecting the Indonesian palm oil market in food and fuel industry: Evidence from a time series analysis. Int. J. Energy Econ. Policy 8(5):49–57.

Budiani A, Nugroho IB, Sari DA, Palupi I, Putranto RA. 2019. CRISPR/Cas9-mediated knockout of an oil palm defense-related gene to the pathogenic fungus *Ganoderma boninense*. Indones. J. Biotechnol. 24(2):101–105. doi:10.22146/ijbiotech.52170.

Dislich C, Keyel AC, Salecker J, Kisel Y, Meyer KM, Auliya M, Barnes AD, Corre MD, Darras K, Faust

H, Hess B, Klasen S, Knohl A, Kreft H, Meijide A, Nurdiansyah F, Otten F, Pe'er G, Steinebach S, Tarigan S, Tölle MH, Tscharntke T, Wiegand K. 2017. A review of the ecosystem functions in oil palm plantations, using forests as a reference system. Biol. Rev. 92(3):1539–1569. doi:10.1111/brv.12295.

Durand-Gasselin T, Asmady H, Flori A, Jacquemard JC, Hayun Z, Breton F, De Franqueville H. 2005. Possible sources of genetic resistance in oil palm (*Elaeis guineensis* Jacq.) to basal stem rot caused by *Ganoderma boninense* - Prospects for future breeding. In: Mycopathologia, volume 159. p. 93–100. doi:10.1007/s11046-004-4429-1.

Elkins K. 2013. DNA Extraction. Academic Press. p. 39–52. doi:10.1016/B978-0-12-394585-3.00004-3.

Fathana H. 2018. Palm oil politics in Malaysia and Indonesia: Competition or collaboration? J. Southeast Asian Stud. 23(2):47–64. doi:10.22452/jati.vol23no2.3.

Fowotade SA, Yusof NA, Abdullah J, Sulaiman Y, Abd Rahman SF. 2019. Enhanced electrochemical sensing of secondary metabolites in oil palms for early detection of *Ganoderma boninense* based on novel nanoparticle-chitosan functionalized multiwalled carbon nanotube platform. Sens. Bio-Sensing Res. 23:100274. doi:10.1016/j.sbsr.2019.100274.

Godswill NN, Frank NEG, Walter AN, Edson MYJ, Kingsley TM, Arondel V, Martin BJ, Emmanuel Y. 2016. Chapter 10 - Oil Palm. San Diego: Academic Press. p. 217–273. doi:10.1016/B978-0-12-801309-0.00010-0.

Green MR, Sambrook J. 2017. Isolation of high-molecular-weight DNA using organic solvents. Cold Spring Harb. Protoc. 2017(4):pdb.prot093450. doi:10.1101/pdb.prot093450.

Hushiarian R, Yusof NA, Dutse SW. 2013. Detection and control of *Ganoderma boninense*: Strategies and perspectives. Springerplus 2(1):555. doi:10.1186/2193-1801-2-555.

Idris AS, Ahmad Kushairi D, Ismail S, Ariffin D. 2004. Selection for partial resistance in oil palm progenies to *Ganoderma* basal stem rot. J. Oil Palm Res. 16:12–18.

Kartika E, Lizawati L, Hamzah H. 2010. Isolasi, karakterisasi dan pemurnian cendawan mikoriza arbuskular dari tanah bekas tambang batu bara [Isolation, identification and purification of arbuscular mycorrhiza fungi (AMF) from coal post mining soil]. In: Seminar Nasional MKTI. p. 3–257.

Liew WL, Kassim MA, Muda K, Loh SK, Affam AC. 2015. Conventional methods and emerging wastewater polishing technologies for palm oil mill effluent treatment: A review. J. Environ. Manage. 149:22–235. doi:10.1016/j.jenvman.2014.10.016.

Lischer K, Avila F, Sahlan M, Whulanza Y. 2021. Assessment of cost-efficient thermocycler prototype for polymerase chain reaction and loop-mediated isothermal amplification. Int. J. Technol. 12(6):1207–1216. doi:10.14716/IJTECH.V12I6.5207.

Minarsih H, Widiastuti H, Santoso D. 2018. Deteksi *Ganoderma* secara molekuler pada kebun kelapa sawit yang diberi perlakuan biofungisida Ganor [Molecular detection of *Ganoderma* on oil palm plantation treated with Ganor biofungicide]. E-Journal Menara Perkeb. 86(1):21–28. doi:10.22302/iribb.jur.mp.v1i1.289.

Mohd Hilmi Tan MIS, Jamlos MF, Omar AF, Kamarudin K, Jamlos MA. 2023. *Ganoderma boninense* classification based on near-infrared spectral data using machine learning techniques. Chemom. Intell. Lab. Syst. 232:104718. doi:10.1016/j.chemolab.2022.104718.

Moncalvo JM, Wang Hsi-Hua, Hseu Ruey-Shyang. 1995. Phylogenetic relationships in *Ganoderma* inferred from the internal transcribed spacers and 25S ribosomal DNA sequences. Mycologia 87(2):223. doi:10.2307/3760908.

Nadhif MH, Whulanza Y, Istiyanto J, Bachtiar BM. 2017. Delivery of Amphotericin B to *Candida albicans* by using biomachined lab-on-A-chip. J. Biomimetics, Biomater. Biomed. Eng. 30:24–30. doi:10.4028/www.scientific.net/JBBBE.30.24.

Naher L, Yusuf U, Tan S, Ismail A. 2013. Ecological status of *Ganoderma* and basal stem rot disease of oil palms (*Elaeis guineensis* Jacq). Aust. J. Crop Sci. 7:1723–1727.

Nathani RC, Irwansyah R, Nurhayati RW, Whulanza Y. 2022. Analysis of droplet size control for stem cells encapsulation using lab-on-a-chip. In: AIP Conf. Proc., volume 2537. p. 040006. doi:10.1063/5.0097945.

Nusaibah SA, Latiffah Z, Hassaan AR. 2011. ITS-PCR-RFLP analysis of *Ganoderma* sp. infecting industrial crops. Pertanika J. Trop. Agric. Sci. 34(1):83–91.

Ommelna BG, Jennifer AN, Chong KP. 2012. The potential of chitosan in suppressing *Ganoderma boninense* infection in oil-palm seedlings. J. Sustain. Sci. Manag. 7(2):186–192.

Park SY, Jang SH, Oh SO, Kim JA, Hur JS. 2014. An easy, rapid, and cost-effective method for DNA extraction from various lichen taxa and specimens suitable for analysis of fungal and algal strains. Mycobiology 42(4):311. doi:10.5941/MYCO.2014.42.4.311.

Paterson RR. 2007. *Ganoderma* disease of oil palm-A white rot perspective necessary for integrated control. Crop Prot. 26(9):1369–1376. doi:10.1016/j.cropro.2006.11.009.

Paterson RR, Sariah M, Lima N. 2013. How will climate change affect oil palm fungal diseases? Crop Prot. 46:113–120. doi:10.1016/j.cropro.2012.12.023.

Paterson RRM. 2019. *Ganoderma boninense* disease of oil palm to significantly reduce production after 2050 in Sumatra if projected climate change occurs. Microorganisms 7(1):24. doi:10.3390/microorganisms7010024.

Priwiratama H, Susanto A. 2014. Utilization of fungi for the biological control of insect pests and *Ganoderma* disease in the Indonesian oil palm industry. J. Agric. Sci. Technol. A 4:103–111.

Rees RW, Flood J, Hasan Y, Potter U, Cooper RM. 2009. Basal stem rot of oil palm (*Elaeis guineensis*); Mode of root infection and lower stem invasion by *Ganoderma boninense*. Plant Pathol. 58(5):982–989. doi:10.1111/j.1365-3059.2009.02100.x.

Siddiqui Y, Surendran A, Paterson RRM, Ali A, Ahmad K. 2021. Current strategies and perspectives in detection and control of basal stem rot of oil palm. Saudi J. Biol. Sci. 28(5):2840–2849. doi:10.1016/j.sjbs.2021.02.016.

Siegel CS, Stevenson FO, Zimmer EA. 2017. Evaluation and comparison of FTA card and CTAB DNA extraction methods for non agricultural taxa. Appl. Plant Sci. 5(2):apps.1600109. doi:10.3732/apps.1600109.

Soh AC. 2012. Breeding and Genetics of the Oil Palm. AOCS Press. p. 31–58. doi:10.1016/B978-0-9818936-9-3.50005-8.

Tahir S, Putri W, Wardani A, Sunaryanto R. 2023. Fermentation medium optimization of *Streptomyces* sp. as an antifungal agent against the *Ganoderma boninensis* pathogen in oil palm. Indones. J. Biotechnol. 28:216. doi:10.22146/ijbiotech.82396.

USDA. 2012. MALAYSIA : Stagnating Palm Oil Yields Impede Growth. October 2012. United States Dep. Agric. - Foreign Agric. Serv. URL https://ipad.fas.usda.gov/highlights/2012/12/Malaysia/.

Utomo C, Niepold F. 2000. Development of diagnostic methods for detecting *Ganoderma*-infected oil palms. J. Phytopathol. 148(9-10):507–514. doi:10.1046/j.1439-0434.2000.00478.x.

Whulanza Y, Aditya R, Arvialido R, Utomo MS, Bachtiar BM. 2017. Ease fabrication of PCR modular chip for portable DNA detection kit. In: AIP Conf. Proc., volume 1817. p. 040006. doi:10.1063/1.4976791.

Whulanza Y, Antory AD, Warjito, Rahman SF, Gozan M, Utomo MS, Kassegne S. 2022a. Integrated electrochemical dopamine sensing with finger priming pump on a chip. Int. J. Technol. 13(8):1735–1744. doi:10.14716/ijtech.v13i8.6140.

Whulanza Y, Arafat YB, Rahman SF, Utomo MS, Kassegne S. 2022b. On-chip testing of a carbon-based platform for electro-adsorption of glutamate. Heliyon 8(5):e09445. doi:10.1016/j.heliyon.2022.e09445.

Whulanza Y, Widyaratih DS, Istiyanto J, Kiswanto G. 2014. Realization and testing of lab-on-chip for human lung replication. ARPN J. Eng. Appl. Sci. 9(11):2064–2067.

Zakaria L. 2023. Basal stem rot of oil palm: The pathogen, disease incidence, and control methods. Plant Dis. 107(3):603–615. doi:10.1094/PDIS-02-22-0358-FE.