Bioma: Berkala Ilmiah Biologi, Desember 2024
Vol. 26, No. 2, Hal. 120-127

p ISSN: 1410-8801
e ISSN: 2598-2370

Electrophoresis-Based DNA Evaluation of Robusta Coffee in Lampung: An Initial Step Toward Germplasm Database Development

Elly Lestari Rustiati[1], Priyambodo Priyambodo[1*], Dian Neli Pratiwi[2], Eko Agus Srihanto[3], Septi Wahyu Lestari[1], Shifa Sandra[1], Winarno[1], Natasya Thesalonika[1]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Lampung, Jl. Prof. Dr. Ir. Soemantri Brojonegoro, Gedong Meneng, Bandar Lampung, Lampung, Indonesia, 35145

[2]Akar Lestari Indonesia, Perum Bumi Puspa Kencana Blok H.20, Bandar Lampung, Indonesia, 35145

[3]Lampung Disease Investigation Centre, Jl. Untung Suropati No.2, Labuhan ratu, Bandar Lampung, Indonesia, 35142

.

*Coresponding author: priyambodo@fmipa.unila.ac.id

Abstract

In response to environmental challenges and the urgent need for sustainability, rural areas, including agriculture, play a key role in ecological resilience. This resilience can be achieved through a synergy between rural development and biological sciences. Ongoing research focuses on the management of robusta coffee (*Coffea canephora*), collecting large-scale data in collaboration with the Lampung Disease Investigation Centre and local farmers in Pesawaran, Lampung. Supported by DIPA BLU Universitas Lampung 2024, the study includes the collection of coffee germplasm data from April to September 2024. Robusta coffee leaves were sampled from two traditional plantations in Wiyono and Bogorejo, Pesawaran. DNA extraction was performed on 18 samples, with electrophoresis results showing that 77% (n=14/18) contained high-quality DNA. Amplification was conducted using n-Methyltransferase encoding primers for species confirmation. This was followed by annealing temperature optimization using the Polymerase Chain Reaction (PCR) method, identifying optimal temperatures of 62°C and 63°C within a 60°C–65°C range. The amplification results will be followed by sequencing for further analysis. This research is expected to contribute to inclusive and sustainable rural development through a bottom-up approach at the local level.

Keywords: *robusta coffee, amplification, Lampung, Pesawaran*

INTRODUCTION

Indonesia's strategic geographic position makes it rich in diverse flora and fauna, including coffee, both Arabica and Robusta (Yunita et al., 2020). Coffee, known for its high economic value, is widely cultivated in Lampung, where it thrives in tropical climates (Gnapi et al., 2022), particularly robusta coffee (*Coffea canephora*).

As one of the world's largest coffee-exporting countries, Indonesia, including Lampung, requires comprehensive data collection on robusta coffee for national and regional germplasm conservation. A biological approach using molecular markers can facilitate the documentation of coffee diversity, serving as fundamental data for Indonesian germplasm. Coffee plays a vital economic and social role in Indonesia, particularly in Lampung.

Molecular analysis of robusta coffee germplasm in Lampung is crucial for conservation and biodiversity development. One essential strategy for preserving germplasm is molecular identification and characterization. Genetic material serves as the foundation of all living organisms, enabling the identification of individual coffee plants with desirable natural traits. Conservation efforts must align with improving community welfare and promoting national economic development in the agricultural and plantation sectors, directly or indirectly supporting food security and local livelihoods.

Pesawaran is a key robusta coffee production center in Lampung, with plantations predominantly managed by local and traditional farmers. Identifying genetic traits is essential for biodiversity conservation and provides valuable insights into species characteristics. DNA sequence analysis is currently the most reliable method for determining genetic similarities among organisms. However, farmers often lack access to genetic diversity data on cultivated coffee, despite its potential to enhance

yields and optimize cultivation techniques. Various methods have been employed to analyze robusta coffee diversity, including molecular analysis through biological approaches.

Molecular marker analysis on coffee plants has been extensively conducted both domestically and internationally. Hendre and Aggarwal (2014) developed a simple sequence repeat (SSR)-based genomic marker for robusta coffee labeling in Karnataka, India. In Indonesia, multiple labeling methods have been applied, including the sequence-related amplified polymorphism (SRAP) method used to analyze Arabica coffee genetic diversity in Solok (Yunita et al., 2020). Additionally, Fatimah et al. (2014) applied microsatellite molecular markers to assess the diversity of coffee fruits and beans in East Kalimantan. SSR markers, similar to those used in India, were also employed to examine genetic diversity among local robusta coffee clones in Pagar Alam (Syafaruddin et al., 2017). Pangestika et al. (2021) analyzed robusta coffee diversity in Temanggung using the random amplified polymorphic DNA (RAPD) method, which was also utilized by Ramadiana et al. (2021) to assess diversity among 24 robusta coffee clones in Lampung. Molecular tests based on DNA sequencing and phylogenetic analysis have recently been initiated at the Gapoktanhut Lestari Sejahtera Sedayu coffee plantation in Tanggamus (Rustiati et al., 2024; Rustiati et al., 2023).

Addressing increasing environmental concerns and the urgent need for sustainable development, rural areas play a crucial role in ecological resilience. Through the integration of rural sustainability, biological sciences, research, and coffee species data management, ongoing studies aim to support environmental stewardship. Genetic diversity mapping can serve as a scientific foundation for developing coffee plantation policies based on technological advancements. Until recently, molecular-based research had not been conducted at the Gunung Betung traditional coffee plantation, located in Wiyono and Bogorejo Villages, Gedong Tataan District, Pesawaran Regency, Lampung. Given its potential, genetic analysis of robusta coffee cultivated in this plantation is essential.

MATERIAL AND METHODS

Research project under DIPA BLU Universitas Lampung year 2024, consisted of site survey, sampling, preparation and analysis.

Leaves Sampling and Sample Preparations.

Sampling of coffee leaves was carried out in two locations, in Wiyono Village (Block 1) and Bogorejo Village (Block 2), Gedong Tataan District, Pesawaran Regency, Lampung Province. Samples were taken from leaves that were still young and clean from pests. Its twigs had 5-7 leaves. The bottom of the twig was covered with damp cotton to maintain freshness (Rustiati et al., 2024). The samples were put into a large envelope containing silica gel, and stored in ziplock plastic. Collected samples were labeled with the collector's name, date, and sampling location. Samples were transported and stored to the Biotechnology Laboratory, Lampung Disease Investigation Center to be stored in a refrigerator at 4ºC.

DNA Extraction

Sample preparation for molecular analysis was done by cutting the leaves into smaller parts (200 mg), grounded using a mortar containing 1 ml phosphate buffer saline (PBS) to destroy the plant's cell wall. Then, each of the prepared samples was transferred into a 1.5 ml microtube.

Plant DNA analysis techniques include DNA extraction, DNA purity and concentration analysis, Polymerase Chain Reaction (PCR). DNA extraction is a process to obtain pure DNA with high concentrations so that it can be used for advanced molecular analysis (Fatchiyah et al., 2011). There are three main principles in DNA isolation, namely cell breakdown (lysis), separation of DNA from solid materials such as cellulose and proteins, and DNA purification (Corkill and Rapley, 2008). DNA extraction can be done using Robusta coffee plant leaf samples in accordance with the DNeasy Plant Mini Kits protocol.

DNA extraction was performed based on the Geneaid Genomic DNA Mini Kit (Plant) protocol (ISO 9001: 2008 QMS). There are four stages e.g. lysis (the process of cell structure destruction), binding process using silica gel, washing or purification, and elution in which the DNA bound to silica gel is dissolved accordingly (Rustiati et al., 2024).

Electrophoresis

The extracted DNA was tested for its quality by flowing it on the 1% agarose gel. Agarose gel was made by dissolving 100 grams of agarose gel powder in 100 ml of TAE buffer while heating in the microwave for 3,5 minutes. A 10 µl SYBR®safe DNA gel stain was added and homogenized with the solution. Then, the solution was transferred to a casting tray completed with a comb that will form the wells. After the agar hardened, it was put into a chamber and a 6 µl sample was inserted into the well. The chamber was connected to a power supply and electrophoresis was carried out for 25 mins with a voltage of 100 V and a current of 300 A. During the process, DNA molecules will be separated based on their size and shown in the form of a band when visualized under blue light. The molecule size is indicated by the distance between the band and the well. The Further band shows smaller molecular size and vice versa. A marker (100 bp DNA ladder) was also included for comparison.

Polymerase Chain Reaction

PCR was performed using primers encoding the n-methyltransferase gene with the sequence of forward primer that has been used has the following nucleotide base sequence (Rustiati *et al*., 2024) 5‘-ACCTTTCCTTGAACAATGCATA CG-3’ and reverse primer that has been used has the following nucleotide base sequence 5‘-AATCCCCAATTCAATCACCAAACC-3’. In PCR, 0.8µl forward and 0.8µl reverse primers from dilution of primary working stock (Yuanleni, 2019), 4.15 µl of NFW, 10.25 µl of MyTaq HS Red Mix (Bioline: BIO-25047) and 5 µl of two DNA samples of robusta coffee were used respectively. The thermocycler machine was set up with denaturation conditions at 94 °C for 1 minute, annealing at a temperature range of 60°C to 65°C for 2 minutes and extension at a temperature of 72°C for 2 minutes. All stages in the process amplification process was carried out for 35 cycles.

RESULTS AND DISCUSSION

Communication and discussions were held with the coffee plantation selector, Legino and Suhada in Block 1 (Wiyono) and Block 2 (Bogorejo), Gedung Tataan, Pesawaran, Lampung, (**Figure 1**). Discussions were held about data and information related to the coffee plantation and the history of coffee cultivation in the area (**Figure 1**). At this stage, information about the location has been obtained, which enables us to assist in determining which individuals will be used as a representative sample of all population members of the coffee plants in the plantation.

Figure 1. Survey and discussion with the coffee plantation owners on site

DNA source samples have been taken from coffee plant leaves (**Figure 2**a). Sampling has been carried out aseptically. The branch base of the sample was wrapped in such a way as to maintain the freshness of the sample (**Figure 2**b). Furthermore, the samples that have been obtained are then transported to the Biotechnology Laboratory, Lampung Disease Invertigation Center, for further analysis.

(a) (b)

Figure 2. Sampling of coffee leaves/twigs in Gedung Tataan, Pesawaran, Lampung, (a) has been carried out aseptically, and (b) packed the samples that have been taken.

The first stage of the molecular analysis was DNA extraction from coffee leaf samples using the Genomic DNA Mini Kit (Plant) (Rustiati et al., 2024; Geneaid 2017). A total of 18 coffee leaf samples went through a three-stage DNA extraction process: binding, washing, and elution (Qiagen, 2020). DNA extraction from plants is generally more difficult when compared to DNA extraction in animals. The presence of large amounts of contaminants, especially phenolic compounds, polysaccharides, and secondary metabolites can inhibit the DNA extraction process (Pirttilä et al., 2001). Therefore, sample preparation and storage also affect sample conditions and play an important role in the success of the DNA extraction process.

The quality of DNA is indicated by the fluorescent of the DNA band, the thicker the fluorescent of the DNA band, the better the quality of the DNA in the sample. The thin fluorescent quality of the DNA band can be caused by too little amount of DNA extracted in the sample (Rustiati *et al*., 2020). The absence of DNA band fluorescence, as a sign of the presence of DNA in the sample can also be affected by several factors such as the DNA extraction process (method, incubation time, sedimentation process), DNA damage and sample contamination. At the DNA extraction stage, a suboptimal precipitation and binding process can result in not enough DNA being extracted (Emilia *et al*., 2021). Incubation temperature optimization in DNA extraction is highly recommended to ensure the DNA material in the sample, too high a temperature can damage DNA while too low a temperature can cause failure in the cell membrane destruction/lysis process. Too long a DNA incubation time can also cause DNA damage (Haris *et al*., 2003).

Contamination of plant DNA samples in addition to bacterial/viral contamination at the time of sampling, preparation and sample extraction process, can also be in the form of polysaccharide contaminants and secondary metabolites from the plant itself. The presence of polysaccharides and secondary metabolites in plant cells can result in unsuccessful DNA extraction (Maftuchah and Zainuddin, 2013). The success of the DNA extraction process in plant samples is influenced by several factors, one of which is the sampling and extraction process. Each type of plant has different conditions, polysaccharide content and secondary metabolites, so each plant needs optimal uptake, preparation and extraction procedures to obtain good genomic DNA for use in the molecular analysis process.

DNA quality tests were carried out on 18 DNA samples extracted through electrophoresis based on 1% agarose gel. The success of the DNA extraction process can be known through the visualization of electrophoresis results (Rustiati *et al*., 2019). The DNA molecules contained in the DNA extracted sample were separated based on their molecular size and visible in the form of fluorescent bands when the agarose gel is visualized with the help of blue light. The location of the DNA

band is based on the size of the molecule. The farther the band is located from the well, the smaller the molecular size and vice versa. The DNA marker (M) is also included in electrophoresis as a comparison.

Fourteen of the 18 DNA samples extracted showed DNA band fluorescent, while 4 samples showed no DNA band fluorescence (**Figure 3**). DNA band fluorescence indicates the presence of DNA in the sample. Eight out of 14 samples of DNA extraction of coffee leaves showed the presence of DNA in the samples of good quality, while 6 of the other 14 samples showed very thin DNA bands caused by too little amount of DNA in the samples. The absence of DNA band fluorescent in the sample can be caused by several things such as DNA damage in the sample, the success of the extraction process and the amount of DNA in the sample (Rustiati *et al*., 2020).

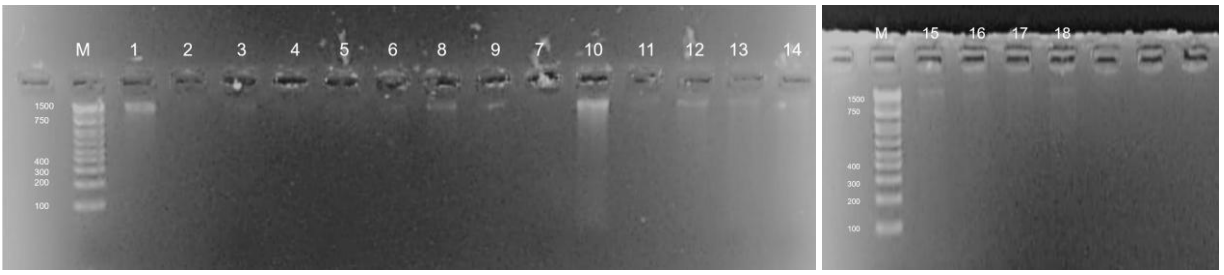

Figure 3. Electrophoresis results of DNA's coffee leaves (M: marker, 1 to 18: sample number).

The PCR optimization stage is carried out with a thermal cycler. Primary temperature optimization is carried out to get optimal PCR conditions, so that the DNA bands formed are cleared. To obtain the result of a good DNA band, the annealing temperature optimization in the primer to be used (Aulia *et al*., 2021). This stage includes the addition of the number of DNA templates in the PCR formula, variations in DNA cutting temperatures, additional DNA cutting time and DNA elongation time (Deniariasih *et al*., 2013). The PCR component was covered by a 0.8µl forward and 0.8µl reverse primer from dilution of primary working stock (Yuanleni, 2019), 4.15 µl of NFW, 10.25 µl of MyTaq HS Red Mix (Bioline: BIO-25047) and 5 µl two DNA sample of robusta coffee. The success of amplification has to do with the primer used to determine its specificity and sensitivity influence. DNA polymerase enzymes can be catalysts in the process of DNA sequence extension. It is because the polymerase enzyme is resistant to repeated heating (Amanda *et al*., 2019).

Determining the proper annealing temperature in the PCR process is crucial for achieving specific and efficient amplification of target DNA sequences. The annealing temperature influences primer binding, specificity, and overall PCR success, making its optimization a key step in various applications, from disease detection to genetic characterization. An optimal annealing temperature ensures that primers bind specifically to the target DNA, reducing non-specific amplification. For instance, in the detection of African Swine Fever virus, a temperature of 55°C was found to yield clear bands corresponding to the target, while lower temperatures resulted in non-specific products (Anggreni et al., 2024). In studies involving extremophilic fish, varying annealing temperatures led to different DNA band appearances, highlighting the need for precise temperature settings to achieve successful identification (Kurniawan et al., 2022).

The amplification of specific gene regions, such as the HPPD gene in sunflowers, demonstrated that varying temperatures (e.g., 53°C for one fragment) directly affected the success of PCR, emphasizing the importance of temperature optimization (Yunita et al., 2023). While optimizing annealing temperatures is essential for PCR success, it is also important to consider that variations in other factors, such as primer design and DNA quality, can significantly impact results. Therefore, a holistic approach to PCR optimization is necessary to ensure reliable outcomes across

different applications (Kartika, 2022; Martati et al., 2024).

Primer temperature optimisation uses the PCR method with three main stages including denaturation, annealing, and extension. The pre-denaturation stage is carried out at a temperature of 94 degrees Celsius for 4 minutes and 55 seconds. Denaturation stage at 94°C for 1 minute. This stage uses 35 cycles. The denaturation stage is a stage carried out to decompose double-stranded DNA into a single strand of DNA at high temperatures, namely temperatures of 94°C to 98°C (Herman *et al*., 2018).

The stage after denaturation that has been carried out is the annealing stage. In this study, the most optimal temperature for the implementation of this amplification procedure was determined. Manual calculation of the annealing temperature was done based on Wallace's formula, stated:

$$Tmw\ P = nG + nC * 4 + nA + nT * 2$$

The Wallace formula, which estimates Ta based on the melting temperatures of the primers, serves as a foundational guideline for optimizing this parameter. A well-calibrated Ta enhances specificity and yield, minimizing non-specific amplification. Both sub-optimal and super-optimal Ta can lead to reduced yields. Research indicates that optimizing Ta is particularly critical when amplifying longer DNA fragments or using total genomic DNA as a substrate (Rychlik et al., 1990). The Wallace formula can guide initial Ta settings, but experimental validation is often necessary to fine-tune conditions for specific primer-template pairs.

The temperature specified for the annealing will affect the successful amplification. Too high a temperature will result in no primer attachment, but a temperature that is too low causes the primer to stick on the other side of the genome, so that DNA will be formed with low specificity and DNA products will not be formed (Saputri *et al*., 2023). Then, the extension stage as the stage of elongation of the DNA strand is carried out at a temperature of 72°C for 7 minutes. The results of PCR activity have then been tested qualitatively using agarose gel electrophoresis (**Figure 4**).

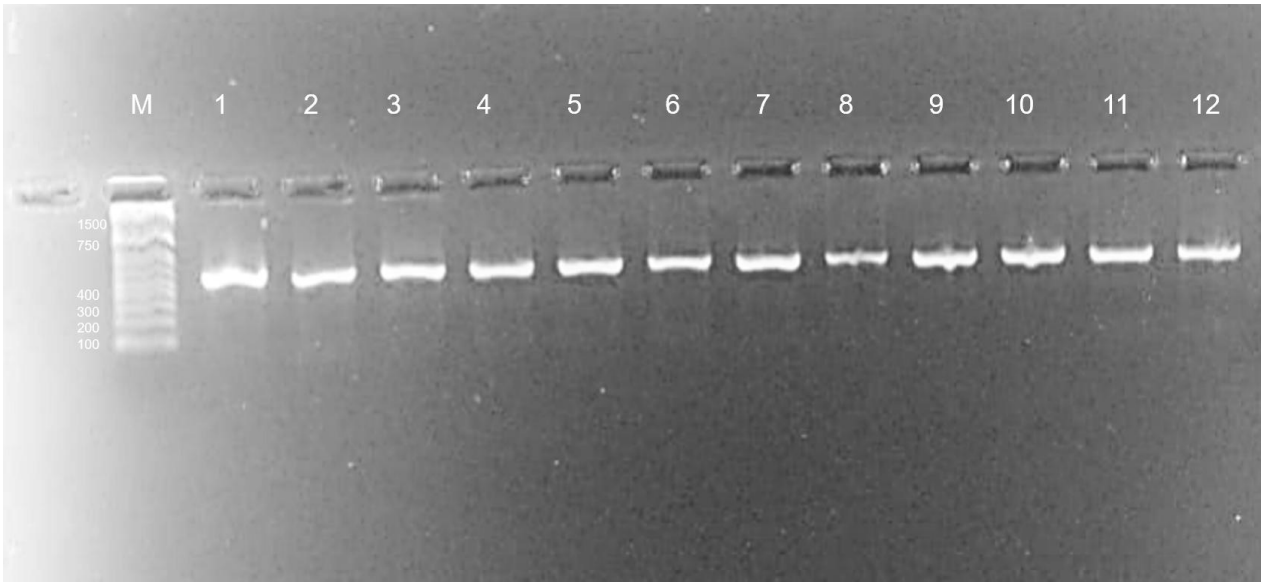

Figure 4. Electrophoresis result of annealing temperature of primer (M: marker, 1 and 2: 60°C, 3 and 4: 61°C, 5 and 6: 62°C, 7 and 8: 63°C, 9 and 10: 54°C, 11 and 12: 55°C).

Optimization was carried out on sample number 1 and 10 from the DNA extraction results with temperature range (60°C to 65°C). It showed a clear visualization of DNA band fluorescent (**Figure 4**). The optimum temperature recorded is 62°C and 63°C. Based on this visualisation, the

optimal annealing temperatures for the next PCR process are 62°C and 63°C. These results can be used for further analysis, including sequencing and phylogenetic construction in building comprehensive molecular data.

CONCLUSION

In a study involving 18 samples of robusta leaves from traditional coffee plantations in Wiyono and Bogorejo, Pesawaran, Lampung, DNA extraction yielded successful results in 14 samples following electrophoresis analysis. The optimal annealing temperatures in PCR procedure were determined to be 62 °C and 63 °C. Future steps include species verification procedures. This research project is to provide a grassroots contribution towards the establishment of inclusive and sustainable rural development at a local scale.

ACKNOWLEDGMENT

Our gratitude to LPPM University of Lampung for research grant DIPA BLU Universitas Lampung year 2024.

REFERENCES

Anggreni, L. D., Dewi, N. M. R. K., Mahardika, I. G. N. K., & Putra, I. G. N. N. 2024. Optimisation of primer concentration and annealing temperature in pcr test method for african swine fever virus detection. *Buletin Veteriner Udayana*. https://doi.org/10.24843/bvu.v16i1.88

Amanda K., Rafika., S., dan Pratiwi, A. 2019. Optimasi Suhu Annealing Proses PCR Amplifikasi Gen shv Bakteri Escherichia coli Pasien Ulkus Diabetik. *Jurnal Mahasiswa Farmasi Fakultas Kedokteran UNTAN*. 4(1): 1-6.

Asy'ari, M., & Noer, A. S. 2005. Optimasi konsentrasi MgCl2 dan suhu annealing pada proses amplifikasi multifragmens mtDNA dengan metoda PCR. *Jurnal Kimia Sains dan Aplikasi*, *8*(1), 23-27.

Aulia, S. L., Suwignyo, R. A., & Hasmeda, M. 2021. Optimasi Suhu Annealing untuk Amplifikasi Dna Padi Hasil Persilangan Varietas Tahan Terendam dengan Metode Polymerase Chain Reaction. *Sainmatika: Jurnal Ilmiah Matematika dan Ilmu Pengetahuan Alam*, 18(1), 44-54. https:// DOI 10.31851/sainmatika.v18i1.5805.

Deniariasih, N. W., Ratnayani, K., & Yowani, S. C. 2013. Optimasi PCR (Polymerase Chain Reaction) fragmen 724 pb gen katG multi drug resistance tuberculosis untuk meningkatkan produk amplifikasi. *Jurnal Farmasi Udayana*, 2(3), 279743. https://doi.org/10.33627/oz.v12i1.1057.

Fatimah, Urnemi, Mustopa, A.Z., Syahrumsyahm H. 2014. Aplikasi Marka Molekuler pada Buah dan Biji Kopi asal Kalimantan Timur. *Berita Biologi* 13(1): 13 22. https:// 10.14203/beritabiologi.v13i1.649.

Gnapi, D.E., D.N. Pokon, H. Legnate, Z. Dapeng, D.S. Akaffon, K.C. Koffi, B. Bertrand, C. Montagnon, A.S. N'Gnetta. 2022. Genetic structuring of parental populations of coffee (Coffea canephora Pierre ex A. Froehner) breeding in Cote d'Ivoire using SNP markers. *Biotechnol. Agron. Soc. Environm*. 26 (3): 178-190.

Hendre, P.S., dan Aggarwal, R.K. 2014. Development of genic and genomic SSR markers of robusta coffee (Coffea canephora Pierre Ex A. Froehner). PLoS One 9(12).

Kartika, A. I. 2022. Optimization of DCR1 and DCR2 epigenetic annealing temperatures for breast cancer biomarker using in-silico and in-vitro study. *Jurnal Teknologi Laboratorium*, *11*(1), 25–32. https://doi.org/10.29238/teknolabjournal.v11i1.327

Kurniawan, A., Anjani, T. P., Kurniawan, A., Syarif, A. F., & Ichsan, M. 2022. Optimization of annealing temperature for coi gene amplification on extrimophil fish using real-time pcr. *Journal of Aquatropica Asia*, *7*(2), 57–62. https://doi.org/10.33019/joaa.v7i2.3401

Martati, E., Kirana, P. K., & Mahatmanto, T. 2024. Optimization of Real Time-Polymerase Chain Reaction (RT-PCR) Annealing Temperatures for the Detection of Superoxide Dismutase (SOD) in Wistar Rat (Rattus novergicus) Liver. *The Journal of Experimental Life Science*, *14*(2), 50–54.

https://doi.org/10.21776/ub.jels.2024.014.02.01

Pangestika, I.W., Susilowati, A., Purwanto, E. 2021. Genetic diversity of Coffea canephora Pierre ex A. Froehner in Temanggung District, Indonesia based on molecular marker RAPD. Biodiversitas 22(11): 4775 – 4783. https://doi.org/10.13057/biodiv/d221109

Ramadiana, S., Hapsoro, D., Evizal, R., Setiawan, K., Karyanto, A., Yusnita, Y. 2021. Genetic diversity among 24 clones of robusta coffee in Lampung based on RAPD markers. *Biodiversitas*. 22(6): 3122 - 3129. https://doi.org/10.13057/biodiv/d220614

Rychlik, W., Spencer, W. J., & Rhoads, E. 1990. *Optimization oftheannealing temperature forDNA amplification invitro*.

Rustiati, E.L., Priyambodo, D. N. Pratiwi, E. A. Srihanto, M. Febriansyah, A.W. Kusuma, E. Saswiyanti, A. W. Susanto. 2024. Management for Gapoktanhut Lestari Sejahtera: Initial optimization on robusta coffee DNA amplification for molecular species confirmation. *Biovalentia: Biological Research Journal*. 10 (1): 44-51. DOI: https://doi.org10.24233/biov.10.1.2024.429, ISSN Online: 2477-1392.

Rustiati, E.L., Priyambodo, Y. Yulianti, E. A. Srihanto, D. N. Pratiwi, E. Virnarenata, T. Novianasari, E. D. Krismuniarti, E. Saswiyanti. 2020. The Essential Contribution of Captive Sumatran Elephant in Elephant Training Center, Way Kambas National Park for Wildlife Genetics Conservation. Biovalentia: Biological Research Journal 6 (1): 38-42. https://doi.org/10.24233/BIOV.6.1.2020.173

Syafaruddin, Dani, Penyegar, M.B. 2017. Keragaman Genetik Antar Klon Kopi Robusta Pagar Alam Berdasarkan Analisis Marka SSR. *Journal of Industrial and Beverage Crops* 4(3): 133 -144. https://doi.org/10.21082/jtidp.v4n3.2017.p133-144.

Yuanleni, Y., 2019. Langkah-Langkah Optimasi PCR. *Indonesian Journal of Laboratory*; 1(3): 51-56. https://doi.org/10.22146/ijl.v1i3.48723

Yunita, R., M. Oktaviani, I. Chaniago, L. Syukriani., M.A. Setiawan, J. Jamsari. 2020. Analysis of genetic diversity of Arabica coffee (Coffea arabica) in Sulak Regency by SRAP molecular markers. *IOP Conf. Series Earth and Environmental Science* 497.012018: 1-6. https://doi.org/10.1088/1755-1315/497/1/012018

Yunita, R., Rosadi, F. N., Jamsari, J., & Azizah, A. 2023. Optimization of annealing temperature for amplification of the exon one region of the HPPD gene in HA1 accession sunflowers. *IOP Conference Series*, *1160*(1), 012002. https://doi.org/10.1088/1755-1315/1160/1/012002