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Genetic Diversity of *Exobasidium vexans*, the Causal Agent of Blister Blight on Tea in Pagilaran, Central Java, Indonesia Using PCR-RAPD

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ABSTRACT

Indonesia is one of the ten largest tea-producing countries in the world, with a plantation area of 104,420 hectares and a production of 139,285 thousand tons in 2018. Blister blight can cause massive crop losses across tea-growing regions of Asia, particularly in India, Sri Lanka, Indonesia, and Japan. The infection causes a 40% yield loss. The study aimed to determine the genetic diversity in *Exobasidium vexans* that cause blister blight based on polymerase chain reaction-random amplified polymorphic DNA (PCR-RAPD). Sampling was conducted at Pagilaran, a tea plantation located in Central Java, Indonesia, with sampling based on altitude, Andongsili (>1,000 meters above sea level [masl]), Kayulandak (±1,000 masl), and Pagilaran (<1,000 masl) with clones TRI 2024, TRI 2025, Gambung 3, Gambung 7, Gambung 9, and Pagilaran 15. This study used the PCR method using internal transcribed spacers (ITS) 1F and ITS 4 primers. Four primers used in PCR-RAPD were OPA-02, OPA-03, OPA-05, and OPB-17. The characteristics of *E. vexans* observed were ellipse-shaped basidiospore, hyaline, unicellular with one septate, formed at the tip of the sterigma with hyaline and elliptical shapes, with a range size of 7–15.5 µm x 2.3–4.5 µm. PCR-RAPD method was able to show the diversity of *E. vexans* samples between clones, in which three clusters were formed at a coefficient of 0.63. Cluster I consisted of TRI 2024 Andongsili and PGL 15 Pagilaran; Cluster II consisted of TRI 2025 Andongsili and Gambung 3 Andongsili; Cluster III consisted of Gambung 7 Andongsili, Gambung 7 Kayulandak, and Gambung 9 Andongsili.

Keywords: Altitude, blister blight, *Exobasidium vexans*, genetic diversity

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INTRODUCTION

Global tea production increased with an average annual growth of 4.7% over the last decade, reaching 5.98 million tons in 2018. Continued global volume growth generated by China's tea production has nearly doubled since 2009, reaching 2.616 million tons in 2018, which is 44.4% of the world's tea. This massive expansion meets unprecedented domestic demand (Dufrene, 2020). Indonesia is one of the ten largest tea-producing countries globally, with a plantation area of 104,420 ha and a production of 139,285 thousand tons in 2018 (Sub Directorate of Estate Crops Statistics, 2019). Climate change has a major impact on the growth and development of tea plants. According to Ochieng et al. (2016), tea is a plant easily affected by climate change. Significant temperature changes and extreme weather pose a threat to the tea production system. Blister blight (*Exobasidium vexans* Massee) is a major disease in various countries. This disease becomes more prevalent during the rainy season and may become more severe in regions where the monsoon season lasts longer. Microorganisms have also been demonstrated to impact tea quality, with pathogen infection lowering the levels of some secondary metabolites (Ahmed et al., 2018). *Exobasidium vexans* is a biotroph pathogenic fungus that infects shoots. The infection develops by forming a shiny concave lesion on the upper surface and a white powdered lower surface. As the disease progresses, the leaves are malformed and roll (Ahuja et al., 2013). Blister blight can cause massive crop losses across Asia's tea-growing regions, particularly in India, Sri Lanka, Indonesia, and Japan. The infection causes a 40% yield loss (Basu et al., 2010).

Disease management recommendation for blister blight is the application of copper (Cu)-based fungicides directly to the leaves before infection occurs. Nevertheless, the maximum residue level of pesticides causes the tea to be less desirable to consumers (Barooahi et al., 2002). A viable and effective option for the management of blister blight can be made through the genetic improvement of tea resistance (Karunarathna et al., 2020). Molecular marker technology is currently used in tea genetic diversity studies to acquire population structures and uncover the history of domestication (Meegahakumbura et al., 2018). Molecular marker technology provides a rapid set of polymorphic DNA-based markers with advantages of a wide range of applications in plant genetics and breeding (Lee et al., 2019).

Morphological observations of *E. vexans* spores to detect and distinguish the sequences can help compare the variability and similarity of *E. vexans* in different tea-growing areas. The molecular characteristics of *E. vexans* needed to be documented for further characterization studies. Information on the genetic and morphological diversity of *E. vexans* is necessary to support the development of effective control for blister blight (Abeysinghe et al., 2015). Limited information about genetic diversity in the *E. vexans* causes the need for molecular

study, especially using RAPD. This technique is an easy, simple, and economical technique for the population's genetic diversity analysis and does not require any sequence information (Joshi et al., 2009). RAPD analysis is performed to determine intraspecific variability in a species. Similarity measurements using unweighted pair group with arithmetic mean (UPGMA) program in numerical taxonomy system (NTsys) (version 2.1) and continued cluster analysis generally reflect trends between genotypes. RAPD markers are dominant, unable to distinguish amplified DNA from heterophile or homozygous loci. The quality and concentration of DNA templates, PCR component concentrations, and PCR cycle conditions affect results (Nandani & Thakur, 2014). The study aimed to determine the genetic diversity in *E. vexans* that cause blister blight based on PCR-RAPD.

MATERIAL AND METHOD

Sampling and Specimen Collection

Sampling was done by exploring the symptomatic leaves with symptom criteria in stages 2 (blister formation stage) and 3 (incipient stage of sporulation). The samples were then collected in an envelope. The basidiospores were scraped using toothpicks and then put into 1.5 ml Eppendorf tubes containing 1 ml 6% sucrose solution. Each Eppendorf tube was filled with basidiospores from 20–30 leaves with blister blight symptoms. The samples were stored at a temperature of -20 °C.

Sampling was conducted in the PT Pagilaran tea plantation, Central Java, Indonesia. The sampling site was divided by altitude (high, middle, and low), which was grouped into three criteria: high >1,000 masl, middle ±1,000 masl, and low <1,000 masl. Selected blocks include Andongsili (high), Kayulandak (middle), and Pagilaran (low). At each site, samples of blister blight were collected from TRI 2024, TRI 2025, Gambung 3, Gambung 7, Gambung 9, PGL 6, PGL 11, and PGL 15 clones.

Morphological Character

The macroscopic characterization in this study was determined by observation with the naked eye, and the symptoms of blister blight were documented at the plantation. The symptomatic tea leaves obtained from each clone were then compared to other clones. For microscopic observation, each stored sample in a 6% sucrose solution was dripped on the object-glass, added with lactophenol cotton blue solution, and then covered with a glass cover. The single spore was observed for each replication at three groups of altitude locations using 40x magnification and measured using Image Raster (version 4.0) (Miconos, Indonesia).

DNA Extraction and Amplification

DNA extraction was done using the Aboul-Maaty et al. (2019) protocol with modifications adjusted to laboratory conditions. DNA extraction of *E. vexans* was carried out using the 3% (w/v) cetyltrimethylammonium bromide (CTAB) method. The 3% (w/v) CTAB (Vivantis, Malaysia) heated at 65 °C and added with 0.3% (v/v) beta-mercaptoethanol (Merck,

Germany). The 50 µl 1× TE buffer (Tris-ethylenediaminetetraacetic acid) (Vivantis, Malaysia) was incubated with 3% (w/v) CTAB. A total of 50 mg of the sample was grounded and then put into a 1.5 ml Eppendorf tube. The sample was then added with 800 ml of 3% (w/v) CTAB that had been reheated. The mixture was incubated in a water bath at 60-65 °C for 1 hour (the sample was turned around every 20 minutes). Chloroform isoamyl alcohol 24:1 (v/v) (Merck, Germany) was added to the sample and shaken until homogeneous. The sample was centrifuged at 10,000 x *g* for 15 minutes, and the top solution was transferred into the new Eppendorf. The 6 M sodium chloride (NaCl) (Vivantis, Malaysia) was added to as many as 0.5 volumes of the supernatant obtained and then shaken until homogeneous. A much of 0.1 M potassium acetate (Merck, Germany) and 500 µl cold isopropanol (Merck, Germany) was added, then shaken until homogeneous. The supernatant was then stored at -20 °C for 30 minutes. The supernatant was centrifuged at 10,000 x *g* for 5 minutes, and the supernatant was discarded. The pellets were washed with 500 µl 70% ethanol and then shaken until homogeneous. The samples were centrifuged again at 10,000 x *g* for 5 minutes. The ethanol was discarded, and the pellets were dried for 15 minutes. The Eppendorf tubes containing pellets were incubated for 1-2 hours and then added with TE buffer. The DNA was stored at -20 °C.

The PCR components consist of 5 µl MyTaq™ HS Red Mix Bioline (Meridian Bioscience, USA), 3 µl ddH_2O, 0.5 µl forward and reverse primers, and 1 µl DNA templates. In this study, the ITS primary pair was ITS-1F and ITS-4, with a target size of about 600 bp (Mohktar & Nagao, 2019). The PCR program was as follows: an initial 3-minute pre-denaturation at 95 °C and 35 cycles of 30 seconds at 95 °C (denaturation), 30 seconds at 45 °C (annealing), and 1 minute at 72 °C (extension), followed by a final 5-minute extension at 72 °C. The amplicons and a 100 bp marker were included in the agarose well and a volume of 1-2 µl each. Next, the agarose gel was run in electrophoresis using Bio-Rad DNA Electrophoresis Cell (USA) at 70 V for 50 minutes with a 1x Tris-borate-EDTA (TBE) (Vivantis Technologies, Malaysia). Next, the 1% agarose gel was soaked in ethidium bromide (EtBr) for 15 minutes for DNA staining, then washed by soaking the gel in aqua dest for 5 minutes. Finally, the visualization was done on the UV transilluminator (Bio-Rad, USA).

PCR-RAPD

The primers used in RAPD analysis to determine the genetic diversity of *E. vexans* (Table 1) were OPA-02, OPA-06 (Joshi et al., 2009), OPB-17, and OPA-03 (Abeysinghe et al., 2015). The RAPD component consists of 7.5 µl MyTaq™ HS Red Mix, 5.7 µl ddH_2O, 1.5 µl DNA, and 0.3 µl primer. The PCR-RAPD program was as follows: an initial 3-minute pre-denaturation at 95 °C and 40 cycles of 30 seconds at 95 °C (denaturation), 30 seconds at 45 °C (annealing), and 1 minute at 72 °C (extension), followed by a final 5-minute extension at 72 °C.

Table 1
RAPD primers sequence

No.	Primer	Sequences	GC content (%)	Tm (°C)	Annealing (°C)
1.	OPA-02	5'- TGC CGA GCT G-3'	70	40.7	45
2.	OPA-03	5'- AGT CAG CCA C -3'	60	34.3	45
3.	OPA-06	5'- AAT CGG GCT G -3'	60	35.1	45
4.	OPB-17	5'- AGG GAA CGA G -3'	60	33.1	45

Note. GC content = Guanine-cytosine content; Tm = Melting temperature

The amplicons and a 100 bp marker were included in the agarose well with a volume of 1-2 µl. The agarose gel was then run-in electrophoresis using Bio-Rad DNA Electrophoresis Cell (USA) at 70 V for 50 minutes with a 1x TBE. The agarose gel was soaked in EtBr for 15 minutes for DNA staining, then washed by soaking the gel in aqua dest for 5 minutes. The visualization was done on the UV transilluminator (Bio-Rad, USA).

Data Analysis

The results of ITS 1F and ITS 4 gene sequences were compared to sequences obtained from National Center for Biotechnology (NCBI)-GenBank by conducting a nucleotide basic local alignment search tool (BLASTn) (https:/blast.ncbi.nlm.nih.gov/Blast.cgi). Phylogenetic trees were generated by sorting the downloaded references region and analyzed by neighbor-joining at 1,000 bootstraps using Molecular Evolutionary Genetics Analysis (MEGA) (version 11). Each RAPD band was considered one putative locus. Only the locus showing a clear band was included in the scoring: band present (1) and no band (0). The matrix binaries phenotype RAPD was then compiled for individual cluster analysis using the UPGMA program in NTsys (version 2.1). Principal Coordinate Analysis (PCoA) was analyzed using paleontological statistics (PAST) software (version 4.03) based on the RAPD data. Similarity analysis based on FASTA DNA sequence data generated pairwise matrix similarity using Sequence Demarcation Tool (SDT) (version 1.2).

RESULT AND DISCUSSION

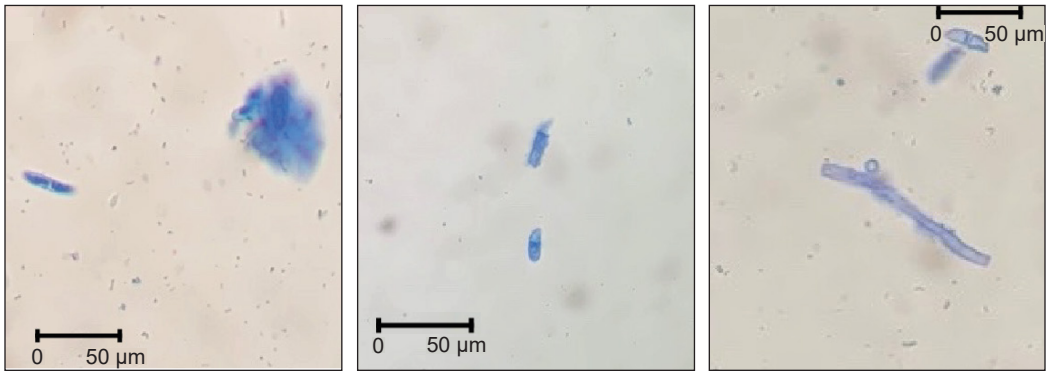
Morphological Character

Tea blister blight is indicated by the presence of white convex blister lesions on the lower surface of leaves, forming the typical symptom of blister blight. The advanced infection will cause necrotic symptoms. High temperature and humidity conditions support the germination of *E. vexans* spores. Symptomatic tea leaves taken from three different altitudes (Figure 1) showed no symptoms differences at different heights. The higher locations are foggy, and this condition will cause an increase in air humidity. High humidity and lack of sunlight are ideal conditions for the development of blister blight (Rezamela et al., 2016).

Based on Figure 2, the characteristics of *E. vexans* basidiospore were ellipses-shaped, hyaline, unicellular, and one septate. Spores

(a) (b) (c)

Figure 1. Macroscopic symptoms of blister blight: (a) TRI-2024 Andongsili, (b) Gambung 7 Kayulandak, and (c) PGL 15 Pagilaran, respectively



(a) (b) (c)

Figure 2. Microscopic observation of *Exobasidium vexans* spores: (a) TRI-2024 Andongsili, (b) Gambung 7 Kayulandak, and (c) PGL 15 Pagilaran, respectively

E. vexans are 15 x 5.5 µm. Basidiospores of *E. vexans* are formed at the top of the sterigma with hyaline and elliptical shapes measuring 7-15.5 µm x 2.3-4.5 µm. In the latest study by Chaliha and Kalita (2020), the germination of basidiospores can be performed *in vitro* on the agar surface after 4 hours post-incubation. After 8 hours post-incubation, hyphae formation differentiates into branches forming complex hyphae.

PCR Amplification

The use of pathogen nucleotide sequences was widely developed in line with PCR technique development as it has advantages of convenient use, speed, specificity, and sensitivity. Molecular technology is important for obligate pathogens that are difficult to grow in artificial media. Analysis of DNA sequences can be utilized to understand the evolution of pathogens and epidemics over time. PCR can be used as a genetic identifier for pathogens. Nilsson et al. (2014) explain that species identification is often difficult because fungi have complex life cycles and a large and diverse collection of eukaryotes. They also mentioned that DNA sequence is an important tool in identifying fungal plant pathogens by internal transcribed spacers (ITS). Its region is widely used to date for the amplification of fungal DNA. The primer pair ITS-1F and ITS-4 used in this study can amplify Ascomycota and Basidiomycota. *Exobasidium vexans* is a species categorized

into the Basidiomycota phylum. Mohktar and Nagao (2019) also used this primer pair for DNA amplification of *E. vexans*. Based on sequencing results, the species *E. vexans* is identified at 612-614 bp (Figure 3 and Table 2).

The phylogenetic tree (Figure 4) was constructed based on the sequence data of *E. vexans* in this study and other *Exobasidium* species in Genbank. Sequences are arranged using the maximum likelihood tree (MLE) on MEGA 11. Based on the data above, the sequence of seven samples used has a 100% similarity with *E. vexans* isolate MC32016 with accession number MG827276.1. The phylogenetic tree was divided into two clades based on the host group *E. vexans*, in this study based on the *Camellia* clade.

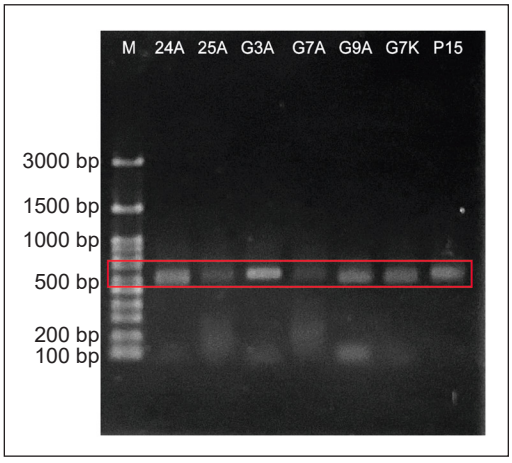


Figure 3. PCR amplification based on ITS sequences: 24A (TRI-2024 Andongsili), 25A (TRI-2025 Andongsili), G3A (Gambung 3 Andongsili), G7A (Gambung 7 Andongsili), G9A (Gambung 9 Andongsili), G7K (Gambung 7 Kayulandak), and P15 (PGL 15 Pagilaran), respectively

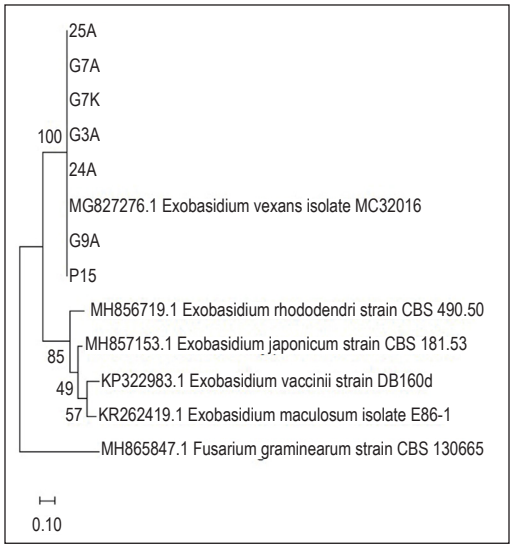


Figure 4. Phylogenetic tree of *Exobasidium vexans* based on the maximum likelihood of ITS sequence analysis

Table 2
Visualization of DNA sequence

Species	Isolate code	Amplification (bp)	Origin
Exobasidium vexans	24A	612	Andongsili
Exobasidium vexans	25A	613	Andongsili
Exobasidium vexans	G3A	612	Andongsili
Exobasidium vexans	G7A	612	Andongsili
Exobasidium vexans	G9A	614	Andongsili
Exobasidium vexans	G7K	612	Kayulandak
Exobasidium vexans	P15	613	Pagilaran

Note. 24A = TRI-2024 Andongsili; 25A = TRI-2025 Andongsili; G3A = Gambung 3 Andongsili; G7A = Gambung 7 Andongsili; G9A = Gambung 9 Andongsili; G7K = Gambung 7 Kayulandak; and P15 = PGL 15 Pagilaran

The separation of clade *E. vexans* with other species (*rhododendri*, *japonicum*, *vaccini*, *and maculosum*) is supported by an 85% bootstrap maximum likelihood value.

Molecular identification of *E. vexans* faces a major challenge where only a few ITS region sequences are stored in NCBI. Chaliha and Kalita (2020) said that this challenge encourages the development of specific molecular barcodes to identify *E. vexans*. In addition, the other challenge is the extraction of biotroph fungi to avoid the presence of secondary pathogens that can cause DNA contamination.

Based on the matrix data (Figure 5), the seven samples in the study had high similarity with *E. vexans* MC32016 isolate shown in red. It also confirmed that the *E. vexans* isolates in the study had a distance similarity with other *Exobasidium* sequences shown in blue. Matrix pairwise similarity confirms the similarity of *E. vexans* samples closed to *E. vexans* MC32016 isolate that corresponds with the phylogenetic tree in the study.

PCR-RAPD

The selection of four primers (Figure 6) showed that all of them produced polymorphic bands. These primers produce a clear, relatively stable, and easy-to-read polymorphic band. Primer selection for RAPD analysis determines the polymorphic bands' results as each primer

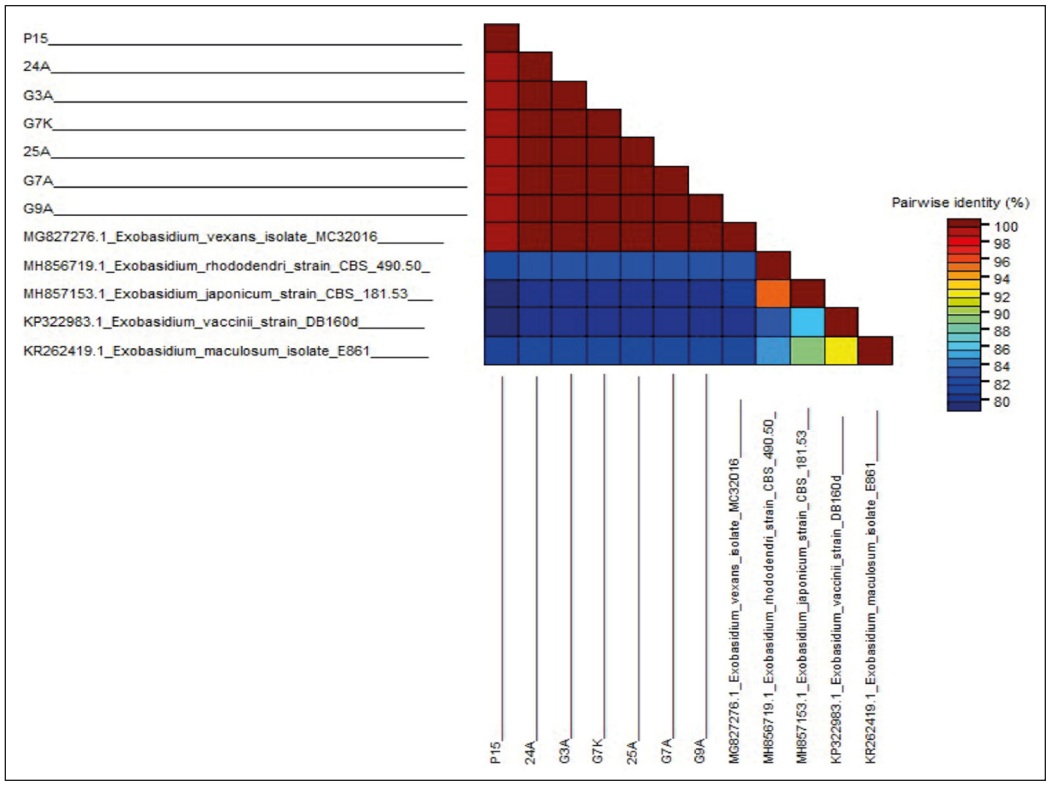


Figure 5. Pairwise similarity DNA of *Exobasidium*

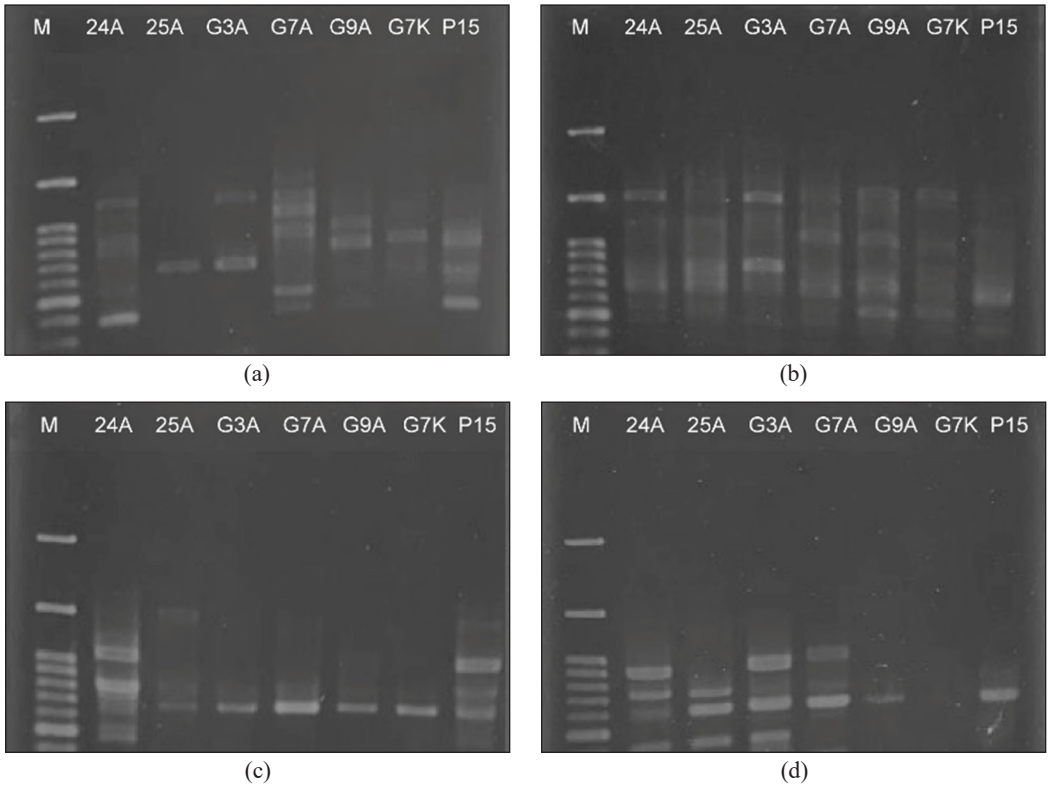


Figure 6. Visualization of PCR-RAPD results on 2% agarose gel, (a) OPA-02, (b) OPA-04, (c) OPA-06, and (d) OPB-17

has its attachment site. This feature results in polymorphic DNA produced differently, both the size and the number of DNA bands. The intensity of the amplified DNA bands is influenced by the purity and concentration of the DNA template (Gusmiaty et al., 2016).

The Pearson correlation test (Table 3) was used to determine the relationship between polymorphic information content (PIC) with effective multiplex ratio (EMR), PCR with marker index (MI), and EMR with MI. There is a positive correlation between PIC and EMR ($r = 0.935$; $p = 0.002 < 0.05$. A positive correlation was also shown between PIC and MI ($r = 0.940$ and $p = 0.001 < 0.05$). A positive correlation of $p = 0.997$ and a significant $r = <0.0001<0.05$ are also shown in the relationship between EMR and MI. PIC, EMR, and MI are relevant to use as markers in genetic mapping and phylogenetic studies. It is in line with a study by Kolade et al. (2016), which reported that the RAPD method could determine genetic diversity and a population and detect plant mutation.

The amplification results of PCR-RAPD (Figure 6) show the thickness level and the number of bands that vary in each primer. Primer series of RAPD OPA-03 and OPB-17 has the highest number of bands compared to the other two primers and have varying thicknesses. The DNA quality of the different

Table 3
Results of polymorphism analysis and effectiveness of RAPD primers were noted

Primer	nB	nPB	% PB	PIC	EMR	MI
OPA 02	6	6	100	0.38	36	13.61
OPA 04	8	8	100	0.32	64	20.22
OPA 06	7	3	43	0.27	21	5.63
OPB17	8	7	88	0.34	56	18.87
Total	29	24	330	1.30	177	58.33
Average	7.25	6	83	0.32	44.25	14.58

Note. nB = Number of bands; nPB = Number of polymorphic bands, % PB = Percentage of the polymorphic bands; PIC = Polymorphic information content; EMR = Effective multiplex ratio; MI = Marker index

extractions in each sample determines the PCR result. The resulting band shows that a difference in thickness can also be affected by higher DNA concentrations, resulting in thick bands (Gusmiaty et al., 2016).

The NTsys analysis showed that the seven samples used in this study were divided into three clusters with a coefficient of 0.63. Cluster I consist of 24A (TRI 2024 Andongsili) and P15 (PGL 15 Pagilaran); Cluster II consists of 25A (TRI 2025 Andongsili) and G3A (Gambung 3 Andongsili); and Cluster III consists of G7A (Gambung 7 Andongsili), G9A (Gambung 3 Andongsili), and G7K (Gambung 7 Kayulandak). The coefficient of genetic similarities between individuals used ranges from 0.59-0.84. At the similarity level, 0.63 produces three clusters. This result suggests that the genetic distance of individuals in clusters is relatively far. Close similarity indicates low genetic distance, and far similarity indicates high genetic distance (Lucic et al., 2011). Far genetic distance indicates the presence of genetic diversity between the samples used in the study. The presence of high genetic diversity is one factor in the assembly of new superior varieties. Increased genetic diversity is done by utilizing germplasm that is available in nature or can be through crosses.

Each clone spreads into several clusters based on dendrogram data (Figure 7). For example, samples of Andongsili origin are distributed in several clusters. This result is probably due to variations in altitude, allowing recombination. Joshi et al. (2009) explain that genetic diversity can occur due to simple mutations and/or recombination during reproduction.

In Table 4, G9A (Gambung 9 Andongsili) and G7K (Gambung 7 Kayulandak) have a genetic similarity coefficient of 0.84, meaning that the two samples have a genetic similarity of 84%. While the samples P15 (PGL 15 Pagilaran) and G3A (Gambung 3 Andongsili), as well as P15 (PGL 15 Pagilaran) and G7A (Gambung 7 Andongsili), showed a coefficient of 0.45, which indicated that both had 45% similarities, respectively. Samples were taken from three locations, Andongsili, Kayulandak, and Pagilaran, with different altitudes. Andongsili is at an altitude of >1,000 masl, Kayulandak is at 1,000 masl, and Pagilaran <1,000 masl. The higher

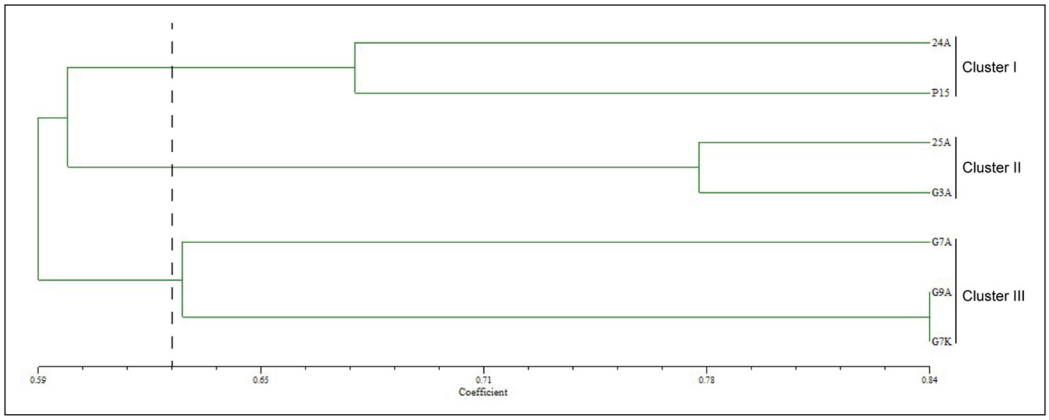


Figure 7. Dendrogram derived using data based on RAPD primers: 24A (TRI-2024 Andongsili), 25A (TRI-2025 Andongsili), G3A (Gambung 3 Andongsili), G7A (Gambung 7 Andongsili), G9A (Gambung 9 Andongsili), G7K (Gambung 7 Kayulandak), and P15 (PGL 15 Pagilaran), respectively

Table 4
Jaccard's coefficient similarity among Exobasidium vexans *samples*

	24A	25A	G3A	G7A	G9A	G7K	P15
24A	1						
25A	0.61	1					
G3A	0.65	0.77	1				
G7A	0.52	0.52	0.55	1			
G9A	0.65	0.65	0.55	0.67	1		
G7K	0.67	0.76	0.65	0.58	0.84	1	
P15	0.67	0.67	0.45	0.45	0.58	0.61	1

Note. 24A = TRI-2024 Andongsili; 25A = TRI-2025 Andongsili; G3A = Gambung 3 Andongsili; G7A = Gambung 7 Andongsili; G9A = Gambung 9 Andongsili; G7K = Gambung 7 Kayulandak; and P15 = PGL 15 Pagilaran

the coefficient value indicates that the two genotypes have a close or uniform correlation. Conversely, the lower the coefficient of genetic tendency has a genetic similarity (Martono & Syafaruddin, 2018). This result is in line with the dendrogram (Figure 7), in which the lowest coefficient value of 0.45 is PGL 15 Pagilaran and Gambung 3 Andongsili; PGL 15 and Gambung 7 Andongsili.

Other mechanisms such as horizontal gene transfer between *E. vexans* and tea plants can lead to evolution and environmental stress. The difference in the height of the place leads to differences in growth and the quality of the tea. The quality of tea is influenced by catechins, L-theanine, caffeine, flanovoles, theaflavins, and thearubigins that are generally obtained from a higher location.

Principal Coordinate Analysis (PCoA) (Figure 8) shows that sample codes P15 (clones PGL 15 Pagilaran) and 24A (TRI 2024 Andongsili clones) are in the same

cluster, sample codes G7A (Gambung 7 Andongsili clones) and G9A (Gambung 9 Andongsili clones) are in the same cluster, as well as sample code G7K (clone Gambung 7 Kayulandak), 25A (TRI 2025 Andongsili clone), and G3A (Gambung 3 Andongsili clone). Three large clusters were identified based on the seven genotypes of *E. vexans* analyzed by PCoA. The results of the PCoA analysis showed that genotypes in the same coordinates and adjacent positions were to be avoided as parents for breeding. Kumar et al. (2015) explain that the genotypes at the coordinates adjacent to its position are not to be crossed to obtain prospective segregation results in certain environments.

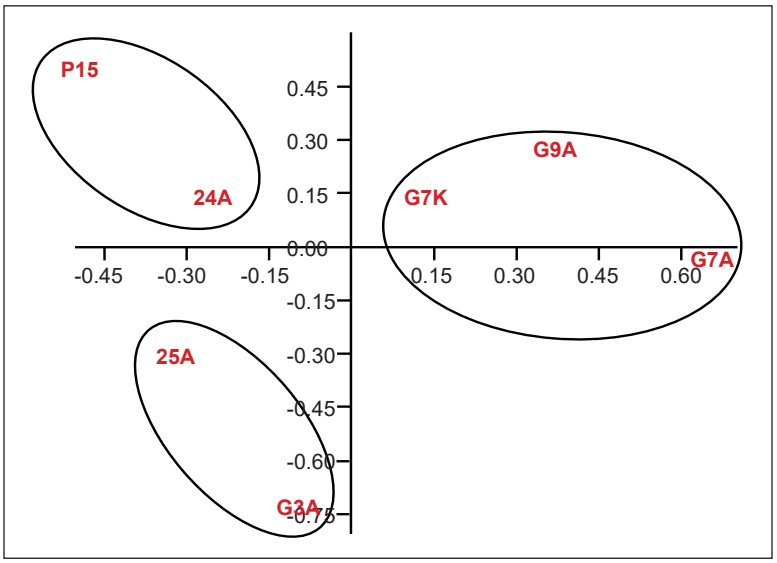


Figure 8. PCoA plot based on RAPD data: 24A (TRI-2024 Andongsili), 25A (TRI-2025 Andongsili), G3A (Gambung 3 Andongsili), G7A (Gambung 7 Andongsili), G9A (Gambung 9 Andongsili), G7K (Gambung 7 Kayulandak), and P15 (PGL 15 Pagilaran), respectively

CONCLUSION

Morphology of blister blight symptoms caused by *E. vexans* in between clones at each height showed no difference. The PCR-RAPD method using primary OPA-02, OPA-04, OPA-06, and OPB-17 can show the diversity of *E. vexans* samples between clones, and at coefficient 0.63 formed three clusters, cluster I consisting of TRI 2024 clones Andongsili and PGL 15 Pagilaran; cluster II clone TRI 2025 Andongsili and Gambung 3 Andongsili; and cluster III clone Gambung 7 Andongsili, Gambung 7 Kayulandak, and Gambung 9 Andongsili.

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