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Phylogenetic Analysis Cantigi Ungu (Vaccinium Varingifolium (Blume) Miq.) Plant Growing in the Highlands of West Java Based on Internal Transcribed Spacer (ITS) Gene

Muhammad Haidar Amrullah[1]*, Andi Salamah[2]
Universitas Islam Negeri Sultan Aji Muhammad Idris, Indonesia[1]
Universitas Indonesia, Indonesia[2]
Email: m.haidar.a@uinsi.ac.id1*

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Abstract

Cantigi Ungu (Vaccinium varingifolium (Blume) Miq.) is an endemic plant of Java that is found in mountainous areas at altitudes ranging from 1,800 to 2,000 m above sea level. It is known by several local names, including Cantigi Ungu, Mentigi, Manis Rejo, and Delima Manda. To date, genetic data reports on this plant remain limited. Genetic conservation efforts are necessary to ensure the sustainability, availability, and preservation of this plant's germplasm, as well as to determine its conservation status. This study aims to analyze the phylogenetic relationships of Cantigi Ungu plants growing in the highlands of West Java based on Internal Transcribed Spacer (ITS) gene sequences. The research was conducted on three Cantigi Ungu plants collected from the highlands of West Java, specifically within the Mount Gede Pangrango National Park area, Mount Tangkuban Parahu Nature Park, and Kawah Putih, and their ITS gene sequences were compared. ITS gene amplification was performed using the forward primer ITS_u1 (5′-GGA AGK ARA AGT CGT AAC AAG G-3′) and the reverse primer ITS_u4 (5′-RGT TTC TTT TCC TCT GCT TA-3′). Phylogenetic analysis was conducted using MEGA XI software with the Maximum Likelihood (ML), Minimum Evolution (ME), Neighbor-Joining (NJ), and Maximum Parsimony (MP) methods. The results showed that the Cantigi Ungu samples analyzed in this study belong to the same species as the reference species, Vaccinium varingifolium, with accession number AY274564.1.

INTRODUCTION

Cantigi Ungu or Vaccinium varingifolium is an endemic Javanese plant found in mountainous areas (Forney et al., 2012). This plant belongs to the family Ericaceae and has various local names, including Cantigi Ungu, Mentigi, Manis Rejo, and Delima Manda. People in West Java commonly refer to it as Cantigi Ungu (Sholikhah et al., 2017), whereas in East Java, particularly in Ijen, Banyuwangi, it is known as Delima Manda (Hapsari & Novitasiah, 2014). The Vaccinium genus is mainly utilized as a food source and grows naturally in the wild. This plant is abundantly found in high-altitude areas ranging from 1,800 to 3,340 m above sea level (Setiawan, 2018). In the Ijen Crater area, for example, this species can be found at approximately 2,000 m above sea level (Ministry for Primary Industries of New Zealand, 2014). Only a few reports have documented the genetic data of Cantigi Ungu in Indonesia, indicating

that available genetic information remains limited (National Center for Biotechnology Information, 2023). Therefore, conservation efforts are needed to determine the existence, genetic status, and sustainability of this plant.

Many limitations exist in identifying certain plant species based solely on morphological characteristics, as this process requires experienced taxonomists due to the presence of species with similar morphological traits, which may lead to subjective identification results (Putri, 2015). DNA barcoding can expand knowledge of various species rapidly and efficiently. Genetic analysis can provide more detailed information and facilitate the identification of reliable species targets (Amrullah et al., 2020; Roziaty et al., 2023).

DNA barcoding identification in plants commonly utilizes several genetic markers, including the Internal Transcribed Spacer (ITS), matK (maturase K), psbA-trnH, rbcL, rpoC1, rpoB, and trnL regions (Osman, 2024; Perveen et al., 2026). DNA barcoding generally employs genes from the plastid genome regions (matK, rbcL, rpoB, rpoC1, trnH-psbA, and ycf1) and the nuclear genome (ITS region). Genetic variations in plant genes, such as rbcL, matK, ycf1, and ITS, have been widely used for terrestrial plant identification (Dong et al., 2015; Hollingsworth, 2011; Li et al., 2011; Yang et al., 2012). Several studies have reported that ITS sequences are useful for phylogenetic studies and investigating genomic relationships at the species taxonomic level (Yao et al., 2010). The ITS region is commonly applied in DNA barcoding, molecular phylogenetics, and biodiversity studies (Cheng et al., 2016).

In plants, ITS sequences vary in length from approximately 500–700 base pairs (bp) in angiosperms. ITS is divided into two types: Specific ITS (ITS-s), which is a DNA marker designed for DNA barcode analysis in specific organisms, particularly certain fungi, algae, or plants, and Universal ITS (ITS-u), which is generally applied to plants, especially terrestrial plants (White et al., 2020). ITS regions are plant DNA fragments that are frequently used in molecular systematic studies up to the species level due to their high potential for evaluating interspecific and intraspecific relationships (Buchheim et al., 2011; Staggemeier et al., 2015; Yuan et al., 2015).

The objective of this research is to study the phylogenetic relationships of Cantigi Ungu (Vaccinium varingifolium) plants growing in the highlands of West Java, collected from the Mount Gede Pangrango National Park area, Mount Tangkuban Parahu Nature Park, and Kawah Putih, and compared based on their ITS gene sequences. This research provides essential baseline genetic data to support conservation strategies and sustainable management of Cantigi Ungu in Indonesia. Practically, the findings support ex situ and in situ conservation programs through accurate species identification, assist the Ministry of Environment and Forestry in determining conservation status, and contribute to the development of a DNA barcode database for Indonesian flora. Theoretically, this study enriches the limited knowledge of molecular phylogenetics of Vaccinium species in Southeast Asia and provides a foundation for further research on the genetic diversity and phylogeography of this endemic species.

METHOD

. This research employed a descriptive-analytic approach with a molecular phylogenetic method to examine the genetic relationships and species identity of Cantigi Ungu (Vaccinium varingifolium) populations growing in the highlands of West Java. The study was laboratory-

based and involved DNA isolation, amplification, sequencing, and phylogenetic analysis to obtain empirical genetic data.

Sampling and Isolation DNA Total

The subject of this study is Cantigi Ungu (Vaccinium varingifolium) collected from ount Gede Pangrango National Park Area, Mount Tangkuban Perahu Nature Park, and Ciwidey White Crater in June-November 2022. Total DNA obtained from 100 mg young leaves. The samples ground with mortar and pistil in liquid nitrogen until homogenous. The isolation DNA total using High Pure DNA Plant Mini-Kit VIOGENE with several modifications. The quantity of DNA sample determined by using Thermo Scientific Nanophotometer.

Amplification DNA

The target genes using primer specification ITS_F 5'-GGAAGKARAAGTCGTAACAAGG -3' as the forward primer, and reverse primer ITS_R 5'- RGTTTCTTTTCC TCCGCTTA -3' and were amplified with PCR Mix (Intron) (Cheng et al., 2016).

Electrophoresis

The 0,2 g Agarose dissolved in 20 mL TAE 50x + 2µl Greensafe to make Agarose 1%. The electrophoresis performed using Mupid-eXu in 100V for 25 minutes. The agarose then examined under UV Lightning using UV Transluminator in Biology Laboratory of Universitas Indonesia.

Sequencing DNA and Phylogenetic Analysis

In First BASE Laboratories, Malaysia, the amplicons samples are then sequenced in this study. The chromatogram sequence data using FinchTV software to display, the contig analysis of the forward and reverse consensus sequence using DNA Baser software. The data compares collected from the National Center for Biotechnology Information (NCBI). Finally, the methods are Maximum Likelihood (ML), Neighbor Joining (NJ), Minimum Evolution (ME), and Maximum Parsimony (MP) to analysis the samples consensus to make topology phylogenetic using MEGAXI software (Tamura et al., 2021).

RESULTS AND DISCUSSIONS

The visualization of DNA Bands (Figure 1) via UV-Transluminator with band target ± 750 base pairs (bp) using Gel Agarose 1% and DNA Ladder (Marker) 1 kb. The topological reconstruction phylogeny (Figure 1) using different kind of methods. The samples obtained from different areas, KW in Ijen Crater (Control), TP in Tangkuban Parahu, GD in Mount Gede, and CD in White Crater Ciwidey. Before that, the samples to align consensus sequences with the ClustalW method using ClustalX software (Dharmayanti, 2011; Kumar et al., 2016).

Figure 1. **Visualization of DNA bands; M: Marker, KW: Sample from Ijen Crater (Control), TP: Tangkuban Parahu, GD: Mount Gede, and CD: Ciwidey White Crater.**
Source: Author's documentation, 2022

Neighbor Joiningn (NJ) method shows that the KW, CD, GD samples are same species with Vaccinium varingifolium AY274564.1 and BRB, KWI, WL4 Amrullah et al., (2020) with bootstrap value 73 but the TP sample is same species with bootstrap value 99 with Vaccinium varingifolium AY274564.1 and KW, CD, GD, BRB, KWI, WL4 samples (Figure 2); as well as in Minimum Evolution (ME) method shows that the KW, CD, GD samples are same species with Vaccinium varingifolium AY274564.1 and BRB, KWI, WL4 Amrullah et al., (2020) with bootstrap value 74 but the TP sample is same species with bootstrap value 99 with Vaccinium varingifolium AY274564.1 and KW, CD, GD, BRB, KWI, WL4 samples (Figure 3); Maximum Likelihood (ML) method shows that the KW, CD, GD samples are same species with Vaccinium varingifolium AY274564.1 and BRB, KWI, WL4 Amrullah et al., (2020) with bootstrap value 94 but the TP sample is same species with bootstrap value 99 with Vaccinium varingifolium AY274564.1 and KW, CD, GD, BRB, KWI, WL4 samples (Figure 4). Neighbor Joiningn (NJ) method shows that the KW, CD, GD samples are same species with Vaccinium varingifolium AY274564.1 and BRB, KWI, WL4 [33] with bootstrap value 87 but the TP sample is same species with bootstrap value 97 with Vaccinium varingifolium AY274564.1 and KW, CD, GD, BRB, KWI, WL4 samples (Figure 5). NJ and ME analyze based on genetic distance meanwhile ML and MP analysed based on sequence character from barcode gene. From the analysis, the result points out that the species happened because both sequences are at the same clade. It also indicates that the samples and Vaccinium varingifolium accession number AY274564.1 belong to monophyletic which is they have the same ancestor (Aprilyanto & Sembiring, 2016).

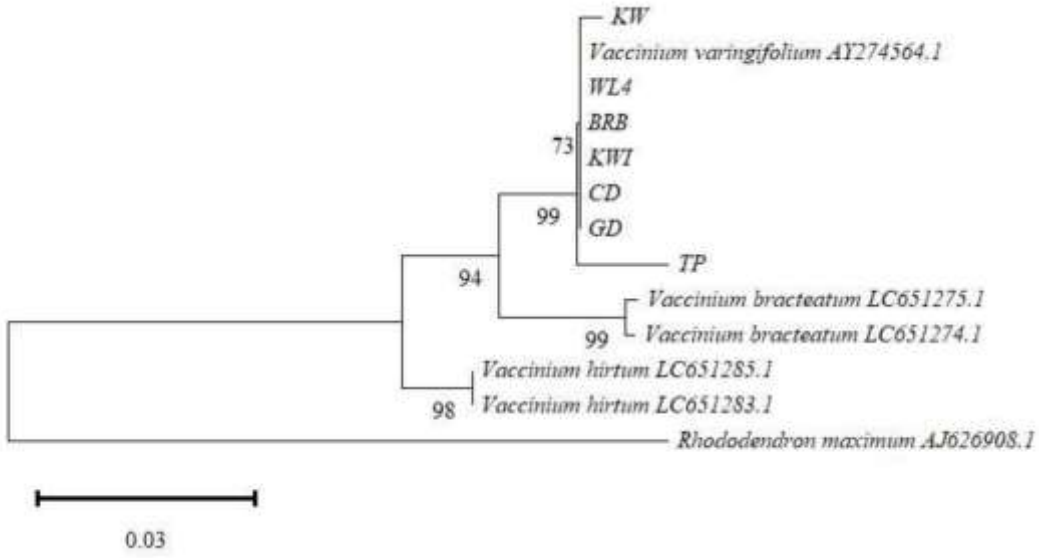


Figure 2. Phylogenetic topology using method: Neighbor-Joinning.
Source: Author's analysis using MEGA XI software, 2022

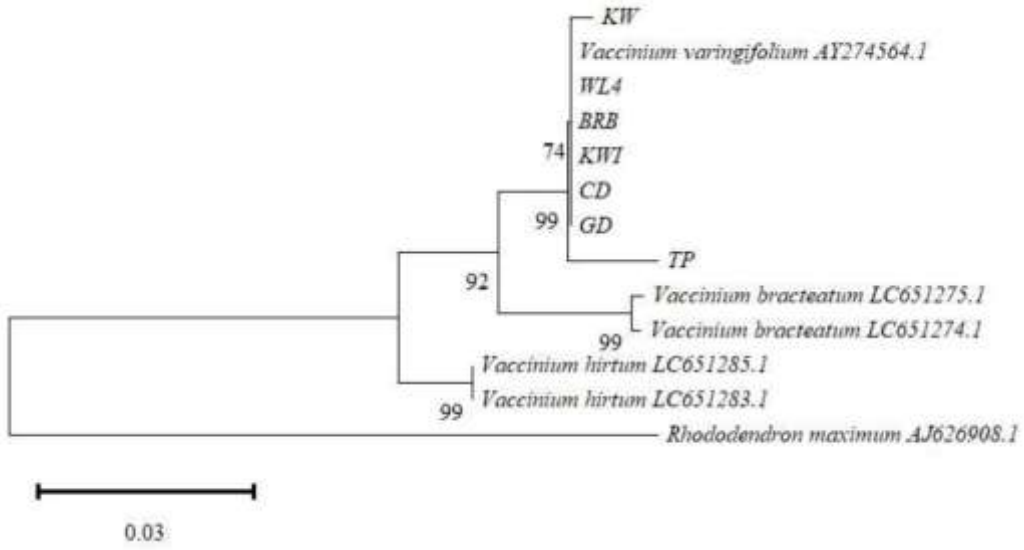


Figure 3. Phylogenetic topology using method: Minimum Evolution.
Source: Author's analysis using MEGA XI software, 2022

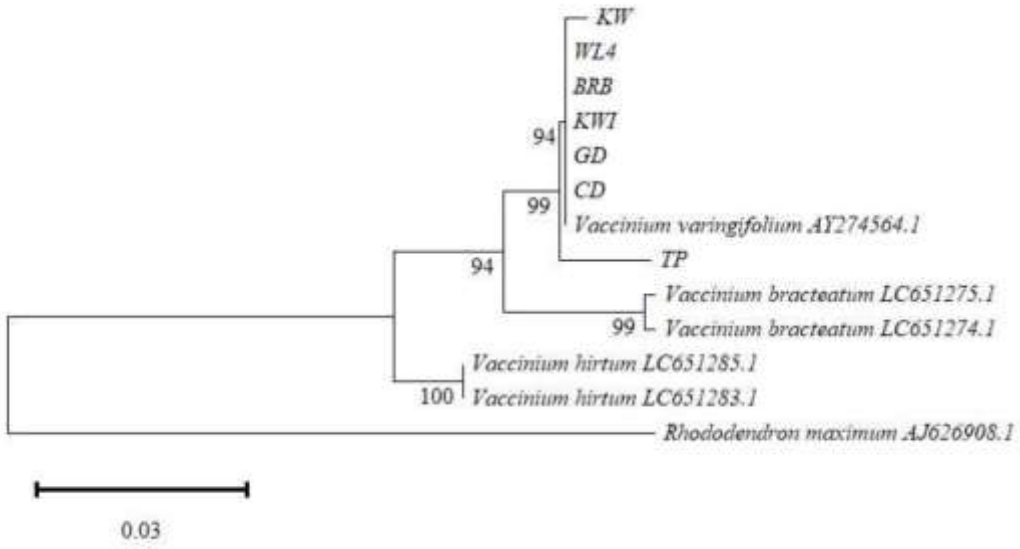


Figure 4. Phylogenetic topology using method: Maximum Likelihood.
Source: Author's analysis using MEGA XI software, 2022

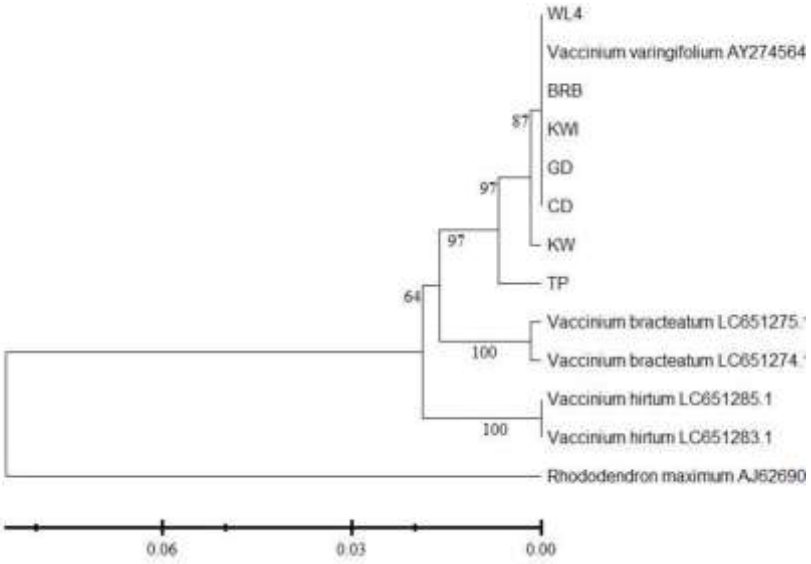
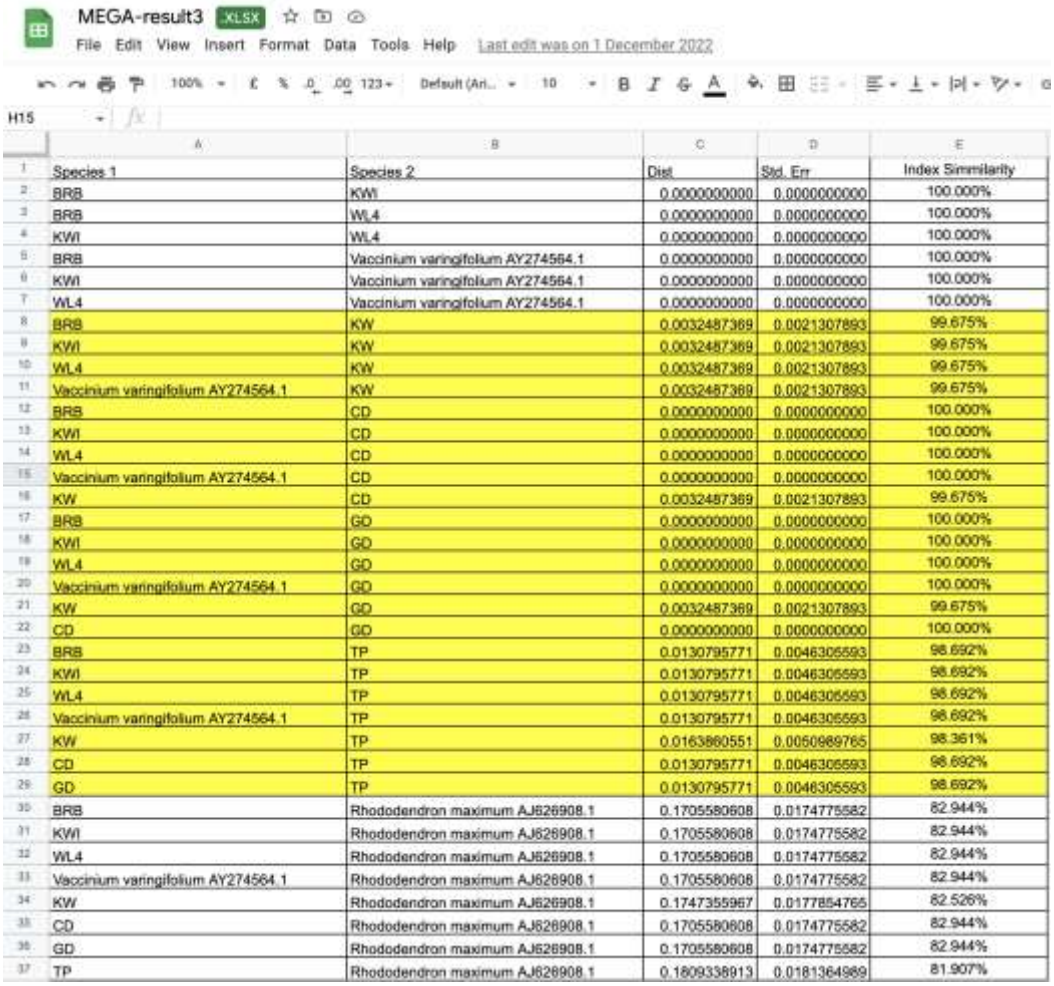


Figure 5. Phylogenetic topology using method: Maximum Parsimony.
Source: Author's analysis using MEGA XI software, 2022

The Samples (Figure 6) has genetic distance 98% until 100% that is mean KW, TP, CD, and GD BRB, and WL4 are belongs to the same species with sequence compare Vaccinium varingifolium accession number AY274564.1 and sequences compare BRB, KWI, WL4. The genetic distance less than 3% would be considered as an intraspecific while the genetic distance more than 0.03 would be considered as an interspecific and the genetic distance more than 5% has a mean most divergent to the others (Bansal et al., 2018; Hall, 2013; Young et al., 2013; Yuan et al., 2015).



MEGA-result3 .XLSX

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H15

	A	B	C	D	E
1	Species 1	Species 2	Dist	Std. Err	Index Simmilarity
2	BRB	KWI	0.0000000000	0.0000000000	100.000%
3	BRB	WL4	0.0000000000	0.0000000000	100.000%
4	KWI	WL4	0.0000000000	0.0000000000	100.000%
5	BRB	Vaccinium varingifolium AY274564.1	0.0000000000	0.0000000000	100.000%
6	KWI	Vaccinium varingifolium AY274564.1	0.0000000000	0.0000000000	100.000%
7	WL4	Vaccinium varingifolium AY274564.1	0.0000000000	0.0000000000	100.000%
8	BRB	KW	0.0032487369	0.0021307893	99.675%
9	KWI	KW	0.0032487369	0.0021307893	99.675%
10	WL4	KW	0.0032487369	0.0021307893	99.675%
11	Vaccinium varingifolium AY274564.1	KW	0.0032487369	0.0021307893	99.675%
12	BRB	CD	0.0000000000	0.0000000000	100.000%
13	KWI	CD	0.0000000000	0.0000000000	100.000%
14	WL4	CD	0.0000000000	0.0000000000	100.000%
15	Vaccinium varingifolium AY274564.1	CD	0.0000000000	0.0000000000	100.000%
16	KW	CD	0.0032487369	0.0021307893	99.675%
17	BRB	GD	0.0000000000	0.0000000000	100.000%
18	KWI	GD	0.0000000000	0.0000000000	100.000%
19	WL4	GD	0.0000000000	0.0000000000	100.000%
20	Vaccinium varingifolium AY274564.1	GD	0.0000000000	0.0000000000	100.000%
21	KW	GD	0.0032487369	0.0021307893	99.675%
22	CD	GD	0.0000000000	0.0000000000	100.000%
23	BRB	TP	0.0130795771	0.0046305593	98.692%
24	KWI	TP	0.0130795771	0.0046305593	98.692%
25	WL4	TP	0.0130795771	0.0046305593	98.692%
26	Vaccinium varingifolium AY274564.1	TP	0.0130795771	0.0046305593	98.692%
27	KW	TP	0.0163860551	0.0050989765	98.361%
28	CD	TP	0.0130795771	0.0046305593	98.692%
29	GD	TP	0.0130795771	0.0046305593	98.692%
30	BRB	Rhododendron maximum AJ626908.1	0.1705580608	0.0174775582	82.944%
31	KWI	Rhododendron maximum AJ626908.1	0.1705580608	0.0174775582	82.944%
32	WL4	Rhododendron maximum AJ626908.1	0.1705580608	0.0174775582	82.944%
33	Vaccinium varingifolium AY274564.1	Rhododendron maximum AJ626908.1	0.1705580608	0.0174775582	82.944%
34	KW	Rhododendron maximum AJ626908.1	0.1747355967	0.0177854765	82.526%
35	CD	Rhododendron maximum AJ626908.1	0.1705580608	0.0174775582	82.944%
36	GD	Rhododendron maximum AJ626908.1	0.1705580608	0.0174775582	82.944%
37	TP	Rhododendron maximum AJ626908.1	0.1809338913	0.0181364989	81.907%

Figure 6. The genetic distance analysis shows that the samples: KW (Control), TP, GD, CD with sequences compare Vaccinium varingifolium with accession number AY274564.1 and KWI, BRB, WL4 is 98% until 100% Similarity.

Source: Author's analysis using MEGA XI software, 2022

According to this study, it can be concluded that Cantigi Ungu in this study (The Sample KW, CD, GD and TP) were same species as existing in the database (Vaccinium varingifolium accession number AY274564.1and BRB, KWI, WL4) and considered as an intraspecific.

CONCLUSION

Based on the results of molecular phylogenetic analysis using the Internal Transcribed Spacer (ITS) gene, Cantigi Ungu (Vaccinium varingifolium) samples collected from Mount Gede Pangrango National Park, Mount Tangkuban Parahu, and Kawah Putih showed high genetic similarity with reference sequences available in the NCBI database (accession number AY274564.1). Phylogenetic reconstruction using the Neighbor-Joining, Minimum Evolution, Maximum Likelihood, and Maximum Parsimony methods consistently placed all studied

samples within the same clade as Vaccinium varingifolium, with strong bootstrap support values, indicating a stable evolutionary relationship.

Genetic distance analysis further confirmed that the similarity among the samples ranged from 98% to 100%, indicating intraspecific variation. This finding suggests that, despite differences in geographical origins, the Cantigi Ungu populations examined belonged to the same species without significant genetic divergence. Therefore, the results support the monophyletic relationship of Vaccinium varingifolium populations in the highlands of West Java.

In conclusion, ITS-based DNA barcoding is an effective molecular approach for confirming species identity and evaluating genetic relationships in Cantigi Ungu. These findings provide important baseline genetic data for future conservation strategies and genetic resource management of this endemic plant species in Indonesia.

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