

Assessing the Utility of Partial Chloroplast *matK* Sequences for DNA Barcoding of Indonesian Grapevine (*Vitis vinifera* L.) Cultivar from Magelang, Central Java

Amelia Halim¹, Wiwit Probowati^{2*}, Adyatma Irawan Santosa³

¹Department of Biotechnology, School of Health and Life Science, i3L University, Jl. Pulomas Barat Kav 88, Jakarta Timur 13210, Indonesia

²Department of Biotechnology, Faculty of Science and Technology, Universitas 'Aisyiyah Yogyakarta, Jl. Siliwangi No.63, Sleman, Yogyakarta, 55592, Indonesia

³Department of Plant Protection, Faculty of Agriculture, Universitas Gadjah Mada, Jl. Flora No. 1, Sleman, Yogyakarta 55281, Indonesia

*Corresponding author: wiwitprobo@unisayogya.ac.id

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HIGHLIGHTS

- ❖ *matK* was too conserved to resolve ancestry or subspecies in *V. vinifera*
- ❖ The 131-bp *matK* fragment was insufficient for ABGD barcode-gap analysis
- ❖ *V. vinifera* showed limited genetic variation, consistent with clonal propagation
- ❖ Multiple loci and longer DNA barcodes are recommended for future studies

ABSTRACT

Grapevine (*Vitis vinifera*) is an important fruit crop in Indonesia, but its local cultivars remain poorly characterized at the molecular level. This study evaluated the utility of a partial chloroplast maturase K (*matK*) gene for preliminary DNA barcoding of nine grapevine samples from Magelang, Indonesia. DNA was extracted and amplified using conventional PCR, but only five samples yielded usable *matK* sequences. Phylogenetic and Automatic Barcode Gap Discovery (ABGD) analyses showed that the 131 bp *matK* fragment was highly conserved, with no nucleotide variation among the Indonesian samples. The sequences formed a single clade, while ABGD detected no clear barcode gap or cultivar differentiation. These findings indicate that the short partial *matK* fragment has limited utility for discriminating Indonesian grapevine cultivars. Future studies should use longer or multiple barcode regions and higher-resolution molecular markers to improve cultivar identification and genetic characterization.



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INTRODUCTION

Grapevine (*Vitis vinifera*) is a major fruit crop used for fresh fruit, dried fruit, juice, and wine. *V. vinifera* cultivars have heterozygous alleles, which means that it has different alleles at certain loci (Torregrosa et al., 2015). To maximize the quality and production of *V. vinifera*, desirable traits such as resistance to biological and abiotic stresses need to be inherited (Pei et al., 2023). However, grapevine genomes are highly heterozygous, with cultivars such as Chardonnay and Cabernet Sauvignon having 10% heterozygous sites. Across cultivars, only ~7% of genes are shared, while ~8% are unique to each individual. This results in high phenotypic variability (Liu et al., 2024). This variability causes different grapevine cultivars to be cultivated in different environments, leading to increased biodiversity in grapevine genotypes as well as phenotypes.

Currently, genotypic studies on grapevine cultivars locally cultivated in Indonesia remain limited. Grapevine studies have been limited to morphological characterization, grapevine chemical compounds, or productivity based on its environment (Damayanti & Antari, 2025; Kamila et al., 2024; Widyastuti & Dewi, 2023). These aspects could be used to study the cultivars and diversity of *V. vinifera*, but is heavily influenced by the environment. Morphological characteristics of the plant could be distorted by viruses and weather conditions. Species identification also faces mislabeling of cultivars, as different regions have their own synonyms or homonyms of *V. vinifera* species (Martínez et al., 2018; Wu et al., 2018). Therefore, a DNA-based approach provides a more accurate assessment of *V. vinifera* cultivars and diversity in Indonesia through sequencing.

Sequencing involves the collection of genetic information from the grapevines that could be used for further analyses through bioinformatic tools (Hu et al., 2021). For cultivar identification and genotyping in *V. vinifera*, nuclear molecular markers with higher levels of polymorphism are generally more appropriate than conserved chloroplast barcode regions. Microsatellite or simple sequence repeat (SSR) markers have been successfully applied to characterize grapevine germplasm and discriminate closely related cultivars based on multilocus allelic profiles (Cretazzo et al., 2022; Tsivelikas et al., 2022). More recently, high-throughput genotyping approaches based on single nucleotide polymorphisms SNPs, including genotyping-by-sequencing (GBS) and targeted SNP genotyping, have provided high-resolution information for assessing genetic diversity, population structure, and relationships among grapevine accessions (Kaya et al., 2023; Yang et al., 2023; Zhang et al., 2025). Genome-wide approaches such as restriction site-associated DNA sequencing (RAD-Seq) can generate large numbers of polymorphic SNP markers and have also demonstrated their utility for high-resolution genetic characterization in plant germplasm (Probowati et al., 2023). Other multilocus marker systems, including ISSR and RAPD, have also been successfully used to detect genetic variation among grapevine genotypes (Çilesiz, 2025). Collectively, these nuclear and genome-wide approaches provide greater resolution for cultivar discrimination and population-level genetic analyses than short, conserved chloroplast barcode sequences.

Commonly used barcoding loci include *matK* and *rbcL*, which the Consortium for the Barcode of Life (CBOL) and the Canadian Center for DNA Barcoding (CCDB) recommended for plant biodiversity analysis. Chloroplast Maturase K (*matK*) encodes maturase K and evolves rapidly at its first and second codon positions, while Ribulose Biphosphate Carboxylase/Oxygenase Large Subunit (*rbcL*) encodes the large subunit of Rubisco and is highly conserved. *matK* performs better for species identification, while *rbcL* excels at resolving family and class levels; therefore, usage of both barcoding regions is recommended for biodiversity analysis (Ho & Nguyen, 2020). Furthermore, barcoding regions could determine a barcoding gap using an Automatic Barcoding Gap Discovery (ABGD), which could be used for further species delimitation (Puillandre et al., 2017).

Despite these limitations, evaluating the performance of commonly used barcode loci in locally cultivated grapevine material remains useful, particularly where molecular information is still limited. Such an evaluation can provide empirical evidence regarding whether a given barcode region contains sufficient variation for the intended level of discrimination and can guide the selection of more appropriate markers for subsequent studies.

Therefore, this preliminary study evaluated the utility of a partial chloroplast *matK* sequence for DNA barcoding of nine grapevine accessions collected from Saung Paribon, Magelang, Indonesia. Specifically, this study assessed whether the obtained partial *matK* sequences contained sufficient variation to differentiate the examined grapevine samples, evaluated their phylogenetic placement, and examined the presence of a barcode gap using ABGD analysis. Rather than assuming *matK* would provide cultivar-level resolution, the study aimed to assess the limitations and potential utility of this marker as a preliminary molecular approach for characterizing Indonesian grapevine germplasm. The findings are expected to provide a basis for selecting more informative molecular markers, including nuclear SSRs, SNP-based approaches, or genome-wide methods, for future grapevine cultivar identification and genetic characterization.

MATERIALS AND METHODS

Materials

The materials used within this research were FavorPrep™ Plant Genomic DNA Extraction Kit, ethanol 96%, liquid nitrogen, microtube, pipette tips, SeaKem® LE Agarose, Tris Borate EDTA (TBE) buffer, 100 bp DNA Ladder from Solis Biotec, *matK* primer by Agrimonti & Marmiroli (2018), WmtF (5'-CGTTGGCTAAAGCGAGATT-3') and WmtR (5'-CCAAAGTTCGAGCACAGA-3'), NS1001 FluoroVue™ gel stain.

The equipment used in this research was micropipettes, oven (Mettler), Centrifuge (Z 206 A, Hermle LaborTechnik), autoclave, mortar and pestle, vortex, icebox, blueGel™ miniPCR, and thermocycler.

Methods

Sample collection

Around nine different cultivars of grapes were collected from Saung Paribon Vineyard in Magelang on 18 August 2025 and 12 September 2025, with the condition of each plant at the early stages of reproduction. Around 2–3 leaves were collected for each variant and then sealed in resealable plastic bags and stored in an ice box before being transported to UNISA. The leaves were stored at 4°C in the refrigerator. The cultivars that were collected were Tamaki (T), Basanti (BS), Early Adora (EA), Vetseedless (V), Jubile (J), Baikonur (BK), Ninel (N), Gos V (G), and Heliodor (H).

DNA extraction

DNA extraction followed the FavorPrep™ Plant Genomic DNA Extraction Kit. Around 1000 mg of leaves from each grapevine cultivar were cut into small pieces and were frozen at -20°C next to dry ice for 1–2 hours, and then ground using a cold mortar and pestle with liquid nitrogen. During DNA extraction, centrifugation was done at 12000 rpm, and the centrifugation time was doubled from the original protocol, from 1 minute to 2 minutes. Extracted DNA was kept at -20°C. Gel electrophoresis was performed in Tris-Borate-EDTA (TBE) buffer to visualize the extraction results, using BlueGel™ miniPCR at 60V. The ladder used was the 100 bp DNA Ladder from Solis Biotec, and the gel stain was sourced from NS1001 FluoroVue™ gel stain. 1% agarose gel was made for the analysis using SeaKem® LE Agarose.

Polymerase Chain Reaction (PCR) and sequencing

Genes *matK* and *rbcl* were chosen to design the primers. The *matK* primer by Agrimonti & Marmioli (2018) targeted *V. vinifera* (accession number AJ429274). Meanwhile, NCBI Primer-BLAST was used to design the *rbcl* primer (accession number GCF_030704535.1). PCR product size was set to be 700-1200 bp, with a 1°C T_m difference. The database used was nt and limited to *V. vinifera* (taxid:29760). The results were VvrbclF (5'-TGGGGTAAGCAGGAGCCATA-3') and VvrbclR (5'-GCACTTGGCGTTATTGTCG-3').

PCR amplification used the WmtF and WmtR primers under the following conditions: Predenaturation at 95°C for 10 min; 35 cycles of PCR at 95°C for 1 min, 56°C for 30s, 72°C for 1 min; final extension at 72°C for 8 min. Meanwhile, PCR amplification for Vvrbcl_GCF_030704535.1 primers was 95°C for 4 minutes; 35 cycles of PCR at 95°C for 30s, 60–61°C for 30s, 72°C for 1 min; final extension at 72°C for 8 min. Its purity was confirmed using 1% gel electrophoresis. The results were sequenced by PT Genetika Science Indonesia. The chromatogram was cleaned up using MEGA 12 to remove low-signal nucleotides.

The read sequences were collected and aligned using ClustalW 1.6 in MEGA 12, and were analyzed for the best DNA/Protein model, and the models with the lowest BIC & AICc were used for phylogenetic analysis, which was the Tamura 3-Parameter (T92) model. The phylogenetic analysis was done with 500 bootstrap values on Maximum-Likelihood Tree, with all sites, Nearest-Neighbor-Interchange, and a Neighbor-Joining initial tree with no branch-swap filter was constructed in MEGA 12.

The dataset was collected using the NCBI nucleotide database by filtering *V. vinifera* chloroplast genomes with selection criteria requiring a complete chloroplast genome. Incomplete *matK* fragments were not included. The list of datasets can be found in **Table S1**.

Secondary analysis of the phylogenetic tree and ancestry relationships was performed using SplitsTree4 and ABGD. The ABGD program was run on Galaxy of ITaxoWebTools (<http://galaxy.itaxotoolsweb.org/>) to analyze (N) datasets. The analysis used a minimum gap width of 1.5 and utilized the K2P model; the prior limit of intraspecific divergence was set at 0.001 for the minimum and 0.1 for the maximum. The steps were set at 10, with the K2P transition/transversion value set at 2.

RESULTS

Primers

The primers that were produced within this study were filtered based on the criteria previously established. The *rbcl* primers amplified the purified *V. vinifera* genome, as shown in **Figure 1**, where bands were detected around the 800 bp ladder, indicating the correct size. However, the PCR conditions should be further optimized, as additional bands indicated nonspecific amplification.

Table 1. Designed primers for *rbcl*, its expected size, melting temperature, GC content, and self-complementarity

Primer Set	Amplicon Size bp	Name	Sequence 5'→3'	T _m *	GC%	Self complementarity	Self-3' complementarity
1	804	VvrbclF	TGGGGTAAGC AGGAGCCATA	60.33	60.33	60.33	60.33
		VvrbclR	GCACTTGGCG GTTATTGTCG	55.00	55.00	55.00	55.00

*T_m: Melting temperature

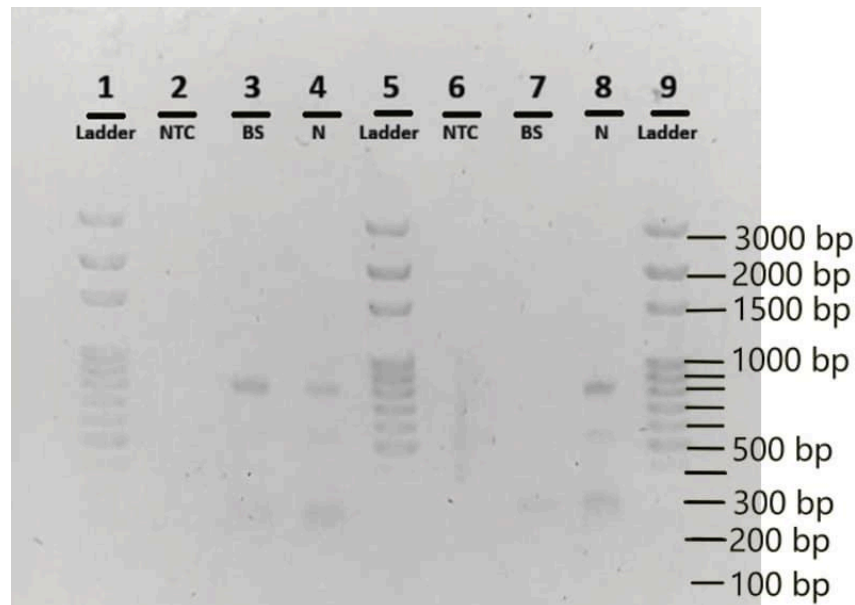


Figure 1. Amplicon of chloroplast *rbcL*. Lanes 2- 4 were annealed at 60°C for 30 seconds, 35 cycles. Lanes 6–8 were annealed at 61°C for 30 seconds, 35 cycles. (1, 5, 9: 100 bp DNA Ladder, Solis BioDyne. 2 & 6: No Template Control. 3 & 7: Basanti. 4 & 8: Ninel).

Polymerase chain reaction

Preliminary amplification using WmtF & WmtR, based on Agrimonti & Marmioli (2018), showed the purity of the DNA extraction. Samples Basanti, Jubile, and Heliodor showed strong bands between 300–200 bp, while Vetseedless, Ninel, and Gos V showed faint bands. Tamaki, Early Adora, and Baikonur showed no bands, possibly due to contaminants such as phenolic compounds. The amplified bands showed the correct base-pair size of 201 bp. However, primer dimers also appeared around 200 bp, making it unable to distinguish successful amplification from primer dimers. Therefore, further research selected Basanti, Jubile, Ninel, Gos V, and Heliodor samples for sequencing.

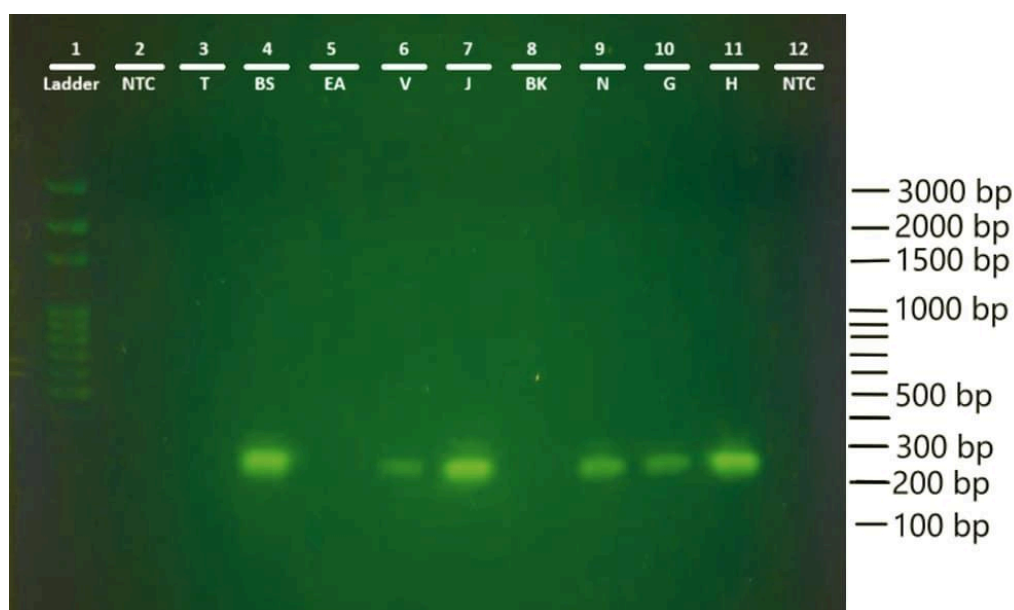


Figure 2. Amplicon of chloroplast *matK*. (1: 100 bp DNA Ladder, Solis BioDyne; 2 & 12: No Template Control; 3: Tamaki; 4: Basanti; 5: Early Adora; 6: Vetseedless; 7: Jubile; 8: Baikonur; 9: Ninel; 10: Gos V; 11: Heliodor).

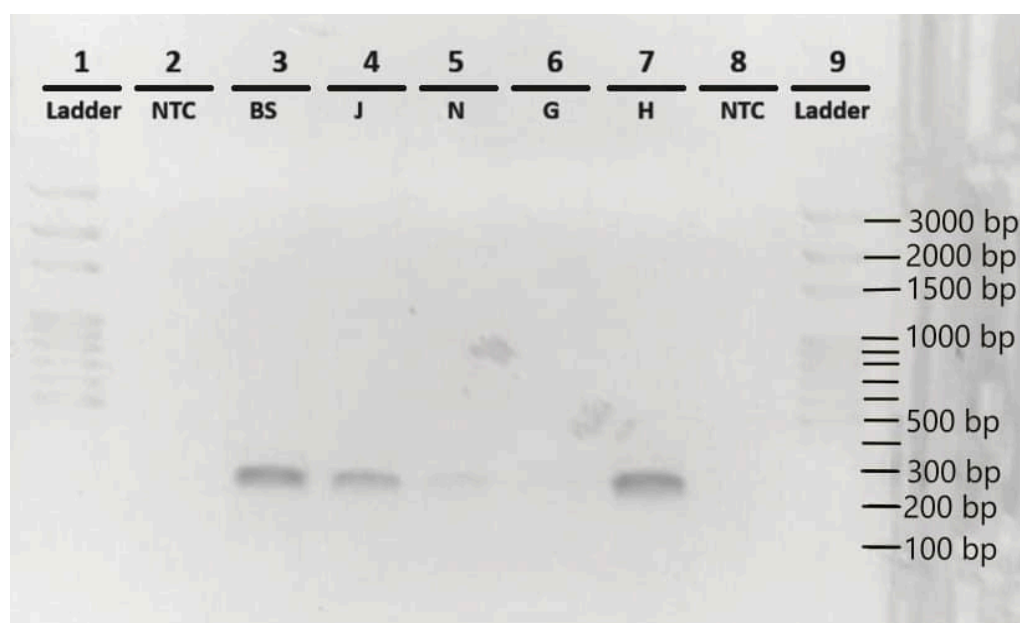


Figure 3. Amplicon of chloroplast *matK*. (1&9: 100 bp DNA Ladder Solisbiodyne, 2&8: No Template Control; 3: Basanti; 4: Jubile; 5: Ninel; 6: Gos V; 7: Heliodor).

Based on **Figure 2**, amplification of Basanti and Heliodor was successful; Jubile showed a lower PCR product concentration, while Ninel and Gos V showed a very low amount of amplification. Amplification in Ninel and Gos V was heavily affected by residual contamination, but overall PCR products varied across samples. Faint bands could also indicate low DNA concentration from the extraction.

Nucleotide composition

All Indonesian samples showed the same nucleotide composition across the sequenced 131 bp *matK* fragment: 32.9% thymine, 19.1% cytosine, 25.9% adenine, and 22.1% guanine. The 131 bp PCR product is located at 1,113–1,243 in the *matK* gene. Compared with NCBI samples, the region shared the same nucleotide sequence and composition. This result was expected, as *matK* is a conserved region, especially as *matK* is primarily used for species delimitation and not cultivar determination. Therefore, the results suggest that 131 bp *matK* PCR fragments could not differentiate between species or cultivars, and that *matK* is not sensitive to evolutionary mutations within species or cultivars.

Table 2. Nucleotide composition of Indonesian *V. vinifera* partial chloroplast *matK* genome

Sample	Control					
	TU	C	A	G	A+T	G+C
Vv_Indonesia_Basanti	32.9	19.1	25.9	22.1	58.8	41.2
Vv_Indonesia_Jubile	32.9	19.1	25.9	22.1	58.8	41.2
Vv_Indonesia_Ninel	32.9	19.1	25.9	22.1	58.8	41.2
Vv_Indonesia_GosV	32.9	19.1	25.9	22.1	58.8	41.2
Vv_Indonesia_Heliodor	32.9	19.1	25.9	22.1	58.8	41.2

Phylogenetic tree reconstruction

Phylogenetic tree reconstruction suggested 7 different clades across the sample using the *matK* sequences in **Figure 4**. However, *V. vinifera* L., *V. vinifera* subsp. *sylvestris*, and *V. vinifera* subsp. *causica* were not clustered together based on the *matK* sequences. The largest clade included *V. vinifera* L., *V. vinifera* subsp. *sylvestris*, and *V. vinifera* subsp. *causica*, while clades 2, 3, 4, and 5 consisted mostly of *V. vinifera* subsp. *sylvestris* and *V. vinifera* subsp. *causica*. Clade 6 was from *V. vinifera* L., while Clade 7 was more similar to the outgroup due to an SNP not shared with any other samples in clades 1–6. Meanwhile, the phylogenetic tree reconstruction that used the partial *matK* gene alongside the Indonesian samples yielded a single clade.

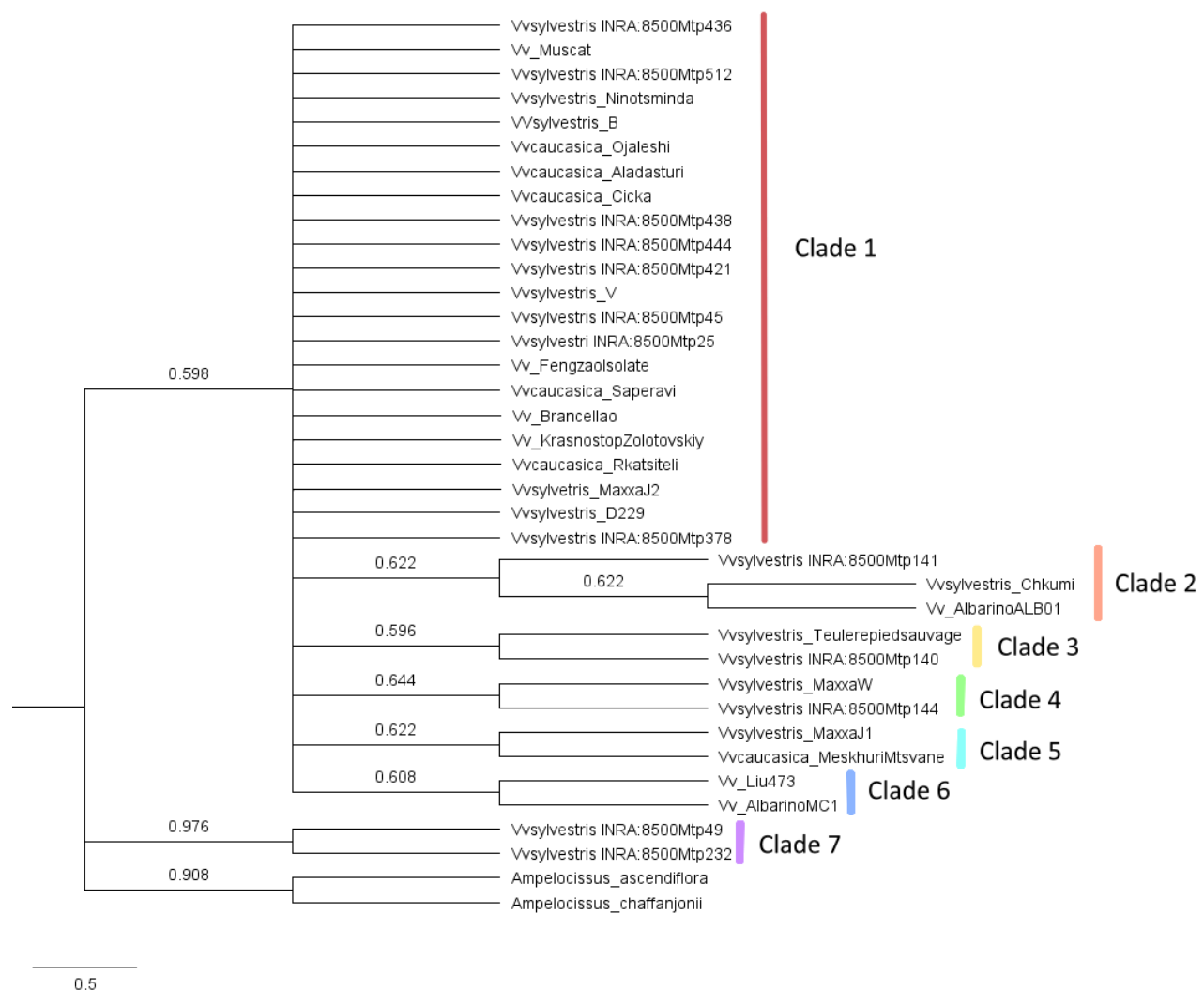


Figure 4. Phylogenetic tree reconstruction using 35 complete *matK* genes consisting of 7 of *V. vinifera* L., 22 of *V. vinifera* subsp. *sylvestris*, 6 of *V. vinifera* subsp. *causica*, and 2 outgroups



Figure 5. Phylogenetic tree reconstruction using 40 partial *matK* genes: 7 from *V. vinifera* L., 22 from *V. vinifera* subsp. *sylvestris*, 6 from *V. vinifera* subsp. *caucasica*, 5 Indonesian *V. vinifera* samples, and 2 outgroups

When comparing the *matK* sequences of 35 samples from NCBI, it can be observed that there are few to no mutations across the sequences. Notably, a single nucleotide polymorphism exists between Vvsylvestris_INRA:8500Mtp49 and Vvsylvestris_INRA:8500Mtp232 from Spain and Morocco, respectively. At position 262, a thymine was replaced with a guanine, converting tyrosine to aspartic acid. However, these differences were not enough to discriminate between cultivars.

Splitstree analysis

Splitstree analyses of the *matK* gene separated the gene into two clades, as shown in **Figure 6**. This result agrees with the prior alignment, in which only Vvsylvestris_INRA:8500Mtp49 and Vvsylvestris_INRA:8500Mtp232 differed by one nucleotide from other *V. vinifera* *matK* genes. However, as shown in **Figure 7**, the partial *matK* gene from the Indonesian samples could not be separated into clades.

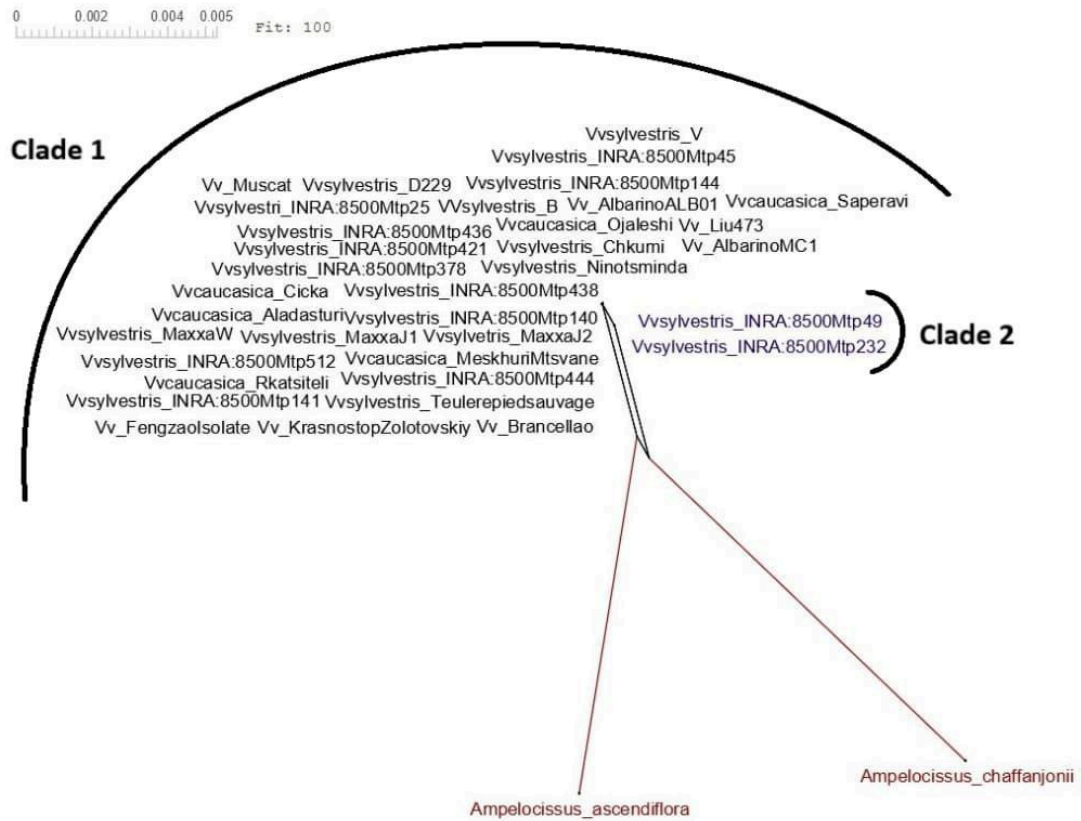


Figure 6. Tree generated using SplitsTree network based on genetic distance using 35 complete *matK* genes consisting of 7 of *V. vinifera* L., 22 of *V. vinifera* subsp. sylvestris, 6 of *V. vinifera* subsp. caucasica, and 2 outgroups. The result showed 2 differing clades.

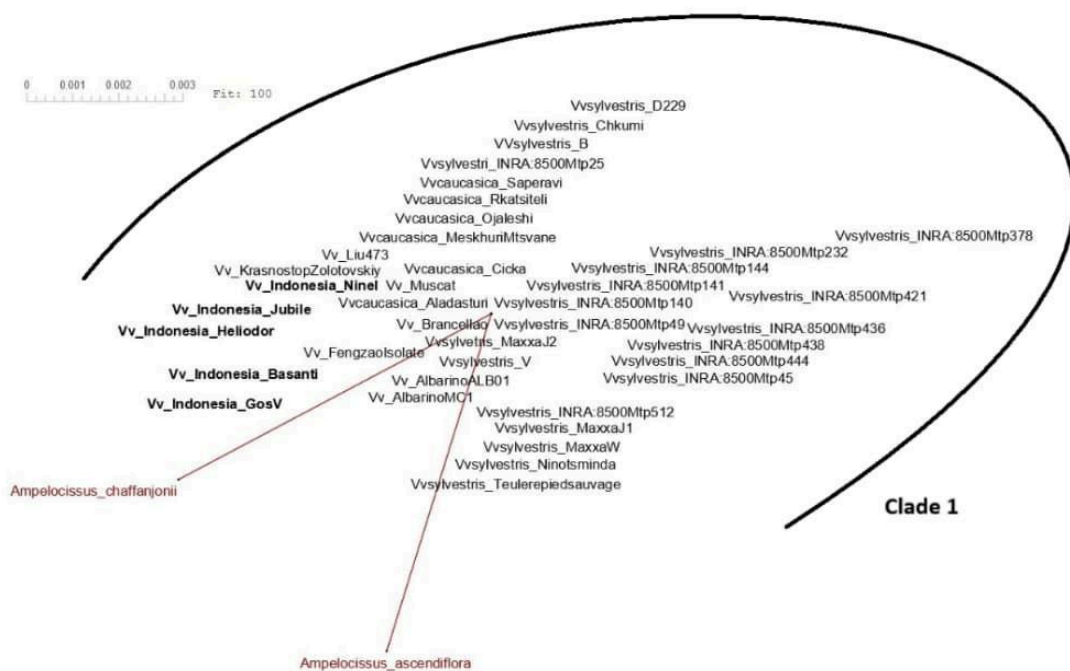


Figure 7. Tree generated using SplitsTree network based on genetic distance using 40 partial *matK* genes consisting of 7 of *V. vinifera* L., 22 of *V. vinifera* subsp. sylvestris, 6 of *V. vinifera* subsp. caucasica, 5 Indonesian *V. vinifera* samples, and 2 outgroups. The result showed a singular clade.

Automatic barcoding gap discovery

Based on the ABGD analysis, the analysis could not detect a meaningful barcoding gap within the *matK* sequences collected from NCBI. ABGD analysis of the partial *matK* gene with Indonesian samples produced no results due to 100% similarity. It can be observed in **Figure 8** that the distribution of pairwise differences showed very small intraspecific divergence but no interspecific divergence. Commonly, pairwise differences would yield two different bars separated by a gap, which implies the percentage by which a species could be differentiated from another. The X-axis shows the class of genetic distance, while the Y-axis shows the number of genetic distances across the samples. The larger the genetic difference between two different sequences, the further apart they will be along the X-axis, thus creating a visualization of the barcoding gap.

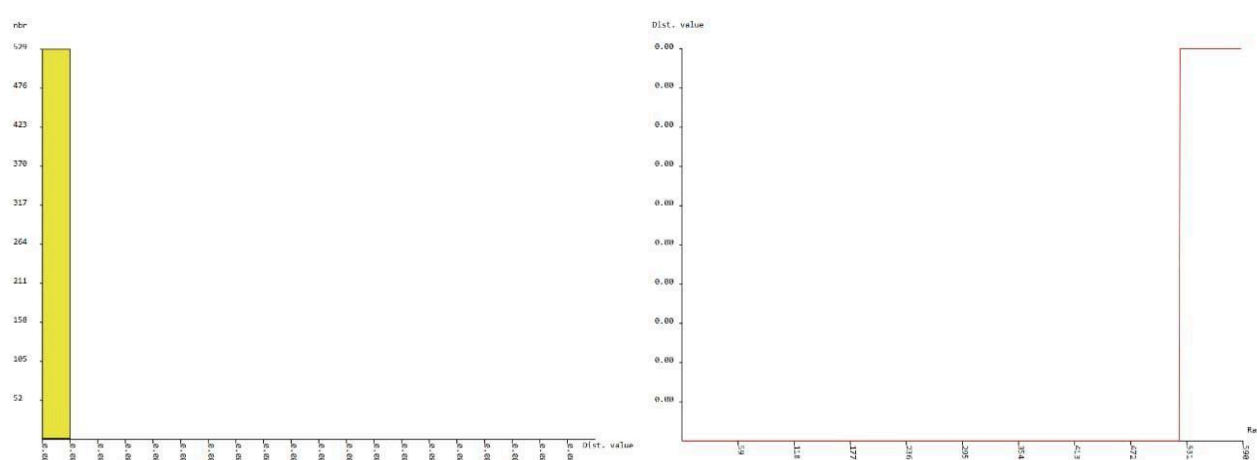


Figure 8. Automatic Barcoding Gap Detection. Left: Distribution of pairwise differences of the full 1500 bp *matK* sequence. Right: ranked pairwise differences of the full 1500 bp *matK* sequence

The distribution of pairwise differences typically represents the interspecific and intraspecific differences across multiple species, highlighted by a gap in which the barcoding gap can be inferred. Ranked pairwise differences would also locate the barcoding gap where the value of pairwise differences jumps from a low value to a high value, indicated by a sharp slope. However, results showed that there was no interspecific variation and no observable barcoding gap, while the slope showed the homogeneity of the sample size instead of indicating the barcoding gap.

DISCUSSION

rbcl and *matK* are multi-loci markers that have multiple annealing sites, including within the nuclear genome. Loci in the nuclear genome accumulated more mutations because they evolve faster than chloroplast loci. There was a slight peculiarity in which the Basanti PCR product band disappeared when the annealing temperature rose, as can be observed in **Figure 1**. However, the Ninel PCR product band seemed to intensify with the increase in temperature. This could be attributed to the purity of the extracted DNA, particularly the phenolic compounds that would disturb the amplification process in Basanti. Additionally, a similar conserved *rbcl* primer annealing site could appear in a higher quantity within the Ninel sample within its genome, causing intensified bands. However, this could not be further confirmed. Further optimization, such as optimization of annealing temperature and adjustment of primer concentration, could improve the PCR product yield (Zhang et al., 2024).

Grapevine leaves are particularly rich in polysaccharides, polyphenolic compounds, and secondary metabolites, making the process of DNA extraction particularly difficult (Öncü Öner et al., 2023). Polyphenols have similar size and charge; thus, conventional methods of extraction would result in high levels of contamination when extracting grapevine leaves. Polyphenols are also prone to oxidation, which would covalently bind to the backbone of nucleic acids during the extraction process, and the DNA precipitate would become brown due to its contamination, further affecting its purity (Rajaei et al., 2017). Meanwhile, leftover polysaccharides increase the viscosity of the solution and inhibit polymerase activity, which heavily affects the downstream processing of the DNA extraction (Ali et al., 2019). Plants with high polysaccharides and phenolic compounds commonly use the Cetyltrimethylammonium bromide (CTAB) DNA extraction method by Doyle & Doyle (1987), which is a highly successful method that would be modified according to species. The extraction method used in this study does not account for high levels of secondary metabolites in the sample; thus, essential reagents such as Polyvinylpyrrolidone (PVP) and CTAB were not used.

CTAB can bind to polysaccharides at high ionic concentrations while acting as a surfactant for cell lysis. It is a cationic detergent with a long hydrophobic hydrocarbon chain and forms micelles that would disturb membranes of the plant tissues (Heikrujam et al., 2020). Meanwhile, PVP is a polymer able to bind to phenolic compounds through adsorption by hydrogen bonding and other weak forces (Ranatunge et al., 2017). In a study by Öncü Öner et al. (2023), the use of PVP-40 reduced RNA contamination and protein concentration, improving the quality of the extracted DNA. Therefore, the effects of high polysaccharide and phenolic content during the extraction resulted in failure of amplification in samples Tamaki, Early Adora, and Baikonur. This further reduced amplification efficiency in Vetseedless, Ninel, and Gos V.

A search within the Google Scholar database proved that Tamaki, Basanti, Early Adora, Vetseedless, Jubile, Baikonur, Ninel, Gos V, and Heliodor were local names for grape cultivars. The term “Baikonur” appears once in a study by Tatarynova et al. (2022), where it was translated into its Russian localization, Байконур. The study also found Baikonur to be resistant to mildew, making it suitable for cultivation in Russia. Therefore, it is highly likely that common names of grapes were translated into local names when they were domesticated in Indonesia. Future studies into local grape cultivars should strive to also analyze the morphological characteristics to further investigate the origins and current identity of the cultivars. As previously mentioned, *matK* was used for species delimitation, even then its performance is accompanied by *rbcl* analysis as well. Cultivar identification for *matK* has been done previously by Probojati et al. (2021), in which the PCR fragments were able to show high variability and were enough to plot the ancestry of banana cultivars. This meant that *matK* could accumulate enough mutations for construction of a phylogenetic tree and estimate genetic divergence time, which was not the case in this study due to the high similarity of the sequence.

V. vinifera is a perennial crop that undergoes clonal propagation to preserve current traits; it also retains 95% of the neutral variation compared to annual crops like banana, which only retained 60% (Probojati et al., 2021). Its heterozygosity would not heavily affect domesticated progeny, as no sexual reproduction occurs, but its main source of mutation comes from deleterious mutations that occurred during clonal propagation. The domestication process also altered sexual reproduction, as previously mentioned, as *V. vinifera* L. is monoecious while *V. vinifera* sylvestris is dioecious. Monoecious plants can self-pollinate as their flowers possess female and male characteristics, while dioecious plants are unable to self-pollinate. In a study done by Zhou et al. (2017), domestication produced *V. vinifera* L. with larger berries, larger bunch sizes, higher sugar content, and altered seed morphology. However, it also caused a reduction in biodiversity.

Its identity as a perennial and domesticated crop could explain the stagnant results of this study, alongside *matK* as a conserved region, especially when collected from the chloroplast instead of the nuclear genome. *matK* enzyme is highly important for the splicing of around 20 Group II introns from precursor RNAs, which are needed for photosynthesis and plant viability. Its loss of function would lead to defective splicing and impair chloroplast development; therefore, slight changes within *matK* would compromise the health of the plant (Liang *et al.*, 2026). Furthermore, environmental stresses also involve the upregulation of *matK*, which cemented its importance within the well-being of a plant (Liu *et al.*, 2025). This study found that the *matK* gene was too conserved for the identification of *V. vinifera* cultivars, and no genetic relationship or ancestry can be determined from the results of this analysis, whether it used a complete *matK* gene or a partial *matK* gene.

ABGD program detects the first barcode gap larger than the intraspecific variance based on the population mutation rate that was set beforehand, and it will create the primary partition where it sorts the initial samples into different candidate species. It would then split the primary partition by overlapping intraspecific and interspecific diversity for mutation rate variability until no splitting occurs (Pentinsaari *et al.*, 2016). This method would be used as a secondary analysis alongside a phylogenetic tree to organize species into clades to confirm the number of species within the dataset (Yeo *et al.*, 2020). Commonly, large datasets with known origins and multiple sequences belonging to one species would be used for this analysis (Puillandre *et al.*, 2017). The intended usage of this analysis was unable to be fulfilled as there is a low number of *matK* sequences for *V. vinifera* of the same cultivar, and so far, barcoding gap has been primarily used for species delimitation, not differentiation of cultivars (Tnah *et al.*, 2023). Therefore, the results were inconclusive because the 131-bp *matK* sequence was too short and highly conserved to provide sufficient genetic variation for ABGD to distinguish the grapevine samples. Consequently, no meaningful Operational Taxonomic Units (OTUs) or barcode gap could be identified. This study concludes that a 131 bp PCR product was not sufficient for ABGD analyses or phylogenetic analysis; ABGD analysis was unable to distinguish between cultivars, and it suggests that larger PCR products should be used for future studies.

Within this study, there was a lack of full genome sequencing of grapevine samples across regions closely related to Indonesia, resulting in inconclusive ancestry of Indonesian grapevines. Sequences found within NCBI for the *matK* analysis, and the majority of them, are from subspecies *sylvestris*—which was previously mentioned as a *V. vinifera* wild type—and subspecies *causica*. Some cultivar names were also not stated within these accessions, and differing local names of grapevines further obfuscate its origins and identification.

Studies into Indonesian grapevines should consider the use of the CTAB method to reduce polyphenols and polysaccharides. It should move forward using another method of recognizing cultivars, such as utilizing Short Sequence Reads (SSR), Amplified Fragment Length Polymorphism (AFLP), Sequence Characterized Amplified Region (SCAR), Random Amplified Polymorphic DNA (RAPD), Sequence-Related Amplified Polymorphism (SRAP), or focusing on certain loci with a less conserved region, or through whole-genome sequencing (Öncü Öner *et al.*, 2023). An analysis of its haplotype diversity could also be conducted to further complement the diversity analysis, which was unable to be done due to the high degree of similarity within the study. Or, to find a locally advantageous cultivar, studies could also focus on the detection of genes connected with agronomic traits such as certain SSRs or biomarkers of QTLs. For ABGD analysis, it is recommended to use multiple species with multiple sequence reads to correctly determine the barcoding gap for species delimitation, as well as a much more certain phylogenetic tree reconstructed from its distance matrix. Furthermore, future studies utilizing the *matK* gene should also use

the *rbcL* gene for species delimitation and use universal primers that produce more than 600–800 bp PCR fragments. There is a lack of studies pursuing the barcoding gap within *V. vinifera*, which would ease the species delimitation of future grapevine studies as well.

CONCLUSION

This study showed that the 131 bp partial chloroplast *matK* fragment was highly conserved among the examined Indonesian grapevine (*Vitis vinifera* L.) cultivars from Magelang and showed no nucleotide variation. Consequently, this short *matK* fragment could not discriminate among grapevine cultivars or provide reliable phylogenetic resolution. Similarly, the phylogenetic and ABGD analyses did not clearly separate the examined grapevine taxa. These findings indicate the limited utility of this short partial *matK* region for grapevine cultivar discrimination. Future studies should use longer, more variable barcode regions, multiple molecular markers, or higher-resolution approaches such as SSR markers to improve cultivar delimitation and genetic characterization.

AUTHOR CONTRIBUTIONS

AH: Data Curation, Formal Analysis, Investigation, Project Administration, Visualization, Writing - Review and Editing. **WP:** Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing - Original Draft, Writing - Review and Editing. **AIS:** Investigation, Validation.

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COMPETING INTERESTS

The authors declare no conflict of interest.

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ADDITIONAL INFORMATION

None.

SUPPLEMENTARY MATERIALS

Grapevine dataset used for phylogenetic analysis

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