

Phylogenetic analysis of local Papuan (Indonesia) sweet potato genotypes using the maturase K (*matK*) molecular marker

NOUKE L. MAWIKERE¹, SARASWATI PRABAWARDANI^{1,✉}, ANTONIUS SUPARNO¹, SARTJI TABERIMA², NAHOR OLLUDIUM³, AGUSTINUS WARBAAL⁴, ZARIMA WIBAWATI¹, LULUT DWI SULISTYANINGSIH⁵

¹Program of Agrotechnology, Faculty of Agriculture, Universitas Papua. Jl. Gunung Salju, Manokwari 98314, West Papua, Indonesia.

Tel.: +62-986-211974, ✉email: s.prabawardani@unipa.ac.id

²Department of Soil Science, Faculty of Agriculture, Universitas Papua. Jl. Gunung Salju, Manokwari 98314, West Papua, Indonesia

³Graduate Program of Agriculture Science, Universitas Papua. Jl. Gunung Salju, Manokwari 98314, West Papua, Indonesia

⁴Graduate Program of Environmental Science, Universitas Papua. Jl. Gunung Salju, Manokwari 98314, West Papua, Indonesia

⁵Herbarium Bogoriense, Research Center for Biosystematic and Evolution, National Research and Innovation Agency. Jl. Raya Jakarta-Bogor Km 46, Cibinong 16911, West Java, Indonesia

Manuscript received: 24 December 2024. Revision accepted: 16 March 2025.

Abstract. Mawikere NL, Prabawardani S, Suparno A, Taberima S, Olludium N, Warbaal A, Wibawati Z, Sulistyaningsih LD. 2025. Phylogenetic analysis of local Papuan (Indonesia) sweet potato genotypes using the maturase K (*matK*) molecular marker. *Biodiversitas* 26: 1367-1375. This study aimed to investigate the genetic relationship among 12 local Papuan sweet potato genotypes using the maturase K (*matK*) molecular marker. DNA was extracted from fresh leaf samples and amplified using the *matK* primer, with sequencing conducted through the Sanger method. Sequence analysis was performed using MEGA11 software, employing BLAST for alignment with GenBank data. Clustering analysis was performed using the neighbor-joining method. While, the phylogenetic tree was constructed using Maximum Likelihood analysis with 1,000 bootstrap replications. The study showed extensive morphological diversity among the sweet potato genotypes, except for L-MKW and Biak-1 genotypes. Nucleotide variation ranges from 858 bp to 867 bp, with polymorphic sites identified at key nucleotide positions. Sweet potato genotype with the largest number of nucleotides was Numfor (867 bp). There were 12 polymorphic sites in nucleotide sequences 1-8, 837, 838, 864, and 865. The average nucleotide composition of twelve sweet potato genotypes was T/U (28.34%), C (15.76%), A (38.01%), and G (17.89%). The number of amino acids formed varied from 265 (Nabire) to 271 (Prafi-1, Mokwam), with an average number of 267.58. The highest amino acid composition was Lysine (Lys/K) 11.87%. Only four sites had polymorphic amino acids in the amino acid sequences 1, 2, 3, and 280. The grouping pattern of Papuan sweet potato genotypes based on nucleotide similarities amplified by the *matK* primer formed three groups based on their nucleotide similarities, namely i) Mokwam, Numfor, L-MKW; ii) Airani Serui, Prafi, Nabire; and iii) Ungu MKW, L-Sorong, Koya-4, Tinta-1, Biak-1, Amban Pantai-2. This cluster highlights the genetic diversity within Papuan sweet potato populations. Phylogenetic analysis revealed a close relationship between the studied genotypes and other species of the *Ipomoea* genus. These findings provide valuable insights into the genetic diversity of Papuan sweet potatoes, offering potential applications for conservation and local crop improvement efforts.

Keywords: Diversity, genetic, *Ipomoea batatas*, *matK*, Papua

INTRODUCTION

West Papua is recognized as a center of sweet potato (*Ipomoea batatas* (L.) Lam.) diversity, yet sweet potato production in this region remains inconsistent and below its potential. Sweet potato is a staple food for approximately 90% of the population in the central highlands of Papua, playing a crucial role in local food security (Indow et al. 2022). However, production has fluctuated significantly in recent years, decreasing by 5.46% from 13,101 tons in 2016 to 12,385 tons in 2017. It increased to 15,429 tons in 2018, then again reduced to 12,113 tons in 2019, a decline of 21.49% (BPS 2020), while the production data from 2020 to 2023 is not available.

Various factors contribute to the decrease in sweet potato production in Papua, including the reduction in the area of cultivated land due to the conversion of agricultural land, the use of low-yielding cultivars, and suboptimal cultivation techniques. Increasing sweet potato production

is critical, as the current supply is insufficient to meet growing consumer demand. This shortfall is reflected in the significantly higher price of sweet potatoes in Papua, which is 4-5 times greater than that of rice. At the local farmer level, average sweet potato yields in Papua remain low (10 t/ha) compared to the national average production (13-14 t/ha) (Saraswati et al. 2013), despite the crop's potential productivity reaching 20-40 t/ha under optimal condition (Saraswati et al. 2013; Martanto et al. 2016). Addressing this gap requires the adoption of high-yielding varieties, widely adaptable varieties alongside improved cultivation techniques to enhance productivity and meet the region's food security needs.

Genetic diversity was important in the plant breeding process, playing a crucial role in the successful selection of superior traits. Assessing genetic character diversity is an essential first step in efforts to develop sweet potato varieties with superior characteristics. This can be performed by the identification and characterization of sweet potato

characters using morphological, cytological, or molecular markers. Among these, molecular markers are now widely recognized as a standard tool for generating more accurate data, despite each molecular marker having its specific strengths and limitations (Raza et al. 2015; Scorsim et al. 2024). Probojati et al. (2021) emphasized that DNA sequence-based molecular approaches are more precise and efficient compared to traditional morphology-based and PCR-based methods.

Molecular markers can be classified into nuclear markers (from the cell nucleus) and cytoplasmic markers (from chloroplast or mitochondria). Among cytoplasmic markers, chloroplast genome markers, such as *rbcL* and *matK*, are commonly recommended markers for plant genetic analysis (Qiu et al. 2012; Immanissa et al. 2020; Sevindik et al. 2024). The *matK* is a gene in the plant chloroplast genome that shows little genetic recombination and is inherited maternally, making it useful for studying the evolutionary history of organisms (Wang et al. 2013; Jayaraj 2024). This gene encodes the maturaseK protein. *MatK* is approximately 1500 base pairs (bp) in size and is specifically found within the intron region of the *trnK* gene (Harnelly et al. 2018). The *matK* gene is generally used for plant identification due to its efficacy, slow mutation rate, and higher accuracy than other genes (Probojati et al. 2021).

Genetic relationships between individuals or plant populations based on molecular markers can be identified using phylogenetic analysis. Phylogenetic methods are among the most commonly used in systematics. They allow us to understand the diversity of living organisms by reconstructing phylogenetic relationships (Wanke and Wicke 2023). Phylogenetic analysis can also identify the evolutionary relationships of groups of organisms. This study focuses on analyzing the genetic relationships of 12 local Papuan sweet potato genotypes and comparing them with other sweet potato species using the *matK* marker. These findings will support taxonomic studies, inform genetic conservation strategies, and guide local Papuan sweet potato breeding programs in Papua.

MATERIALS AND METHODS

Plant material and study area

The 12 sweet potato genotypes used in this study came from several regions in Papua, Indonesia, namely Manokwari, Nabire, Jayapura, Biak, Biak Numfor, Serui, Sorong, and Mokwam. The names of the 12 sweet potato genotypes that were molecularly analyzed were: Prafi-1, Nabire, Tinta-1, Koya-4, Numfor, Airani-Serui, Biak-1, Ungu Manokwari-1, Amban Pantai-2, Lokal Manokwari, Lokal Sorong, and Mokwam. Twelve sweet potato genotypes were collected at the Experimental Garden of the Faculty of Agriculture, University of Papua, Amban Manokwari. Fresh and young leaf samples from each genotype of two-month-old sweet potato plants were collected for DNA extraction. Molecular analysis was conducted between August and September 2024 at the Preparation Laboratory, KST Soekarno BRIN, Cibirong.

DNA extraction, amplification, and sequencing

DNA extraction of 12 local Papuan sweet potato genotypes using the Quick-DNA Magbead Plus Kit (Zymo Research, D4082) to obtain pure DNA. DNA from 12 local Papuan sweet potato genotypes that had been isolated and purified was then used as template DNA for the PCR amplification stage with the *matK* primer. PCR amplification using (2×) My Taq HS Red Mix (Bioline, BIO-25048). The PCR reaction mixture used was 25 µL, including: ddH₂O (adjust to a total mixture of 25 µL), My Taq HS Red Mix (12.5 µL), *matK*-F primer (0.4 µM), *matK*-R Primer (0.4 µM), and template DNA (50 ng). The stages of the PCR cycle are: 1) Pre-PCR 95°C (1 minute), 2) DNA Denaturation 95°C (10 seconds), 3) Annealing (primer attachment) 52°C (15 seconds), 4) Extension (lengthening of DNA) 72°C (15 seconds), and Post PCR 4°C. Stages 2 to 4 are carried out for 35 cycles. The *matK* primer sequences used are: Forward: 5'-CGTACAGTACTTTTGTGTTTACGAG-3' and Reverse: 5'-ACCCAGTCCATCTGGAAATCTTGGTTC-3'. The DNA resulting from the PCR was then electrophoresed in a 1% agarose gel using a UV transilluminator.

To be able to know with certainty the size and number of nucleotides from the DNA of 12 local Papuan sweet potato genotypes resulting from PCR amplification using the *matK* primer, an alignment or sequencing or alignment of the nucleotide sequences was carried out. The DNA sequencing stages of 12 local Papuan sweet potato genotypes were carried out at PT Genetica Science Indonesia Jakarta using the Sanger 1st base deoxy termination method (Bi-Directional Sequencing).

Data analysis

Sequencing results were analyzed using MEGA11 software. The Forward and Reverse sequences of each sample were aligned to obtain contig sequences. The *matK* gene sequences of 12 sweet potato genotypes were aligned with data in NCBI GenBank using Basic Local Alignment System Tools (BLAST). Taxonomic relationships were analyzed between 12 local Papuan sweet potato *matK* gene sequences and GenBank data. The research results of the sweet potato *matK* gene sequence and GenBank data were aligned using clustalW. The phylogenetic tree was carried out using Maximum Likelihood (ML) analysis with a number of bootstrap applications of 1,000. Clustering analysis of 12 local Papuan sweet potato genotypes was carried out using the neighbor-joining method.

RESULTS AND DISCUSSION

Visualization of Papua local sweet potato DNA resulting from PCR amplification with *matK*

Figure 1 shows the DNA electrophoresis visualization of 12 local Papuan sweet potato genotypes resulting from PCR amplification with the *matK* primer.

Visualization of the PCR results on a 1% agarose gel showed a single DNA band, indicating that the *matK* primer successfully amplified the *matK* fragment from 12 Papuan sweet potato genotypes. The single DNA band resulting from amplification appears very clear and intact, without any smearing when the DNA is electrophoresed, which

means the DNA is not fragmented. Good quality DNA is characterized by thick, clean DNA bands when visualized in gel electrophoresis photos (Singhal et al. 2010; Motohashi 2019). Intact DNA is crucial in the sequencing process to achieve more accurate results. The DNA bands of the local Papuan sweet potato genotypes range between 800-900 bp (base pairs). Thus, the DNA size of the 12 sweet potato genotypes is approximately 800 bp. The exact size of the DNA base pairs based on electrophoresis results cannot be determined precisely; it can only be estimated based on the size of the marker used, specifically the 1 kb DNA Ladder. This is in line with Karudapuram et al. (2007) and Gomes-Pereira and Monckton (2017) that size markers are crucial for accurate DNA length determination because they provide a reference for comparing the migration of DNA fragments.

Sequence and variation of nucleotides *matK* of local Papuan sweet potato

To determine with certainty the size and number of nucleotides of the DNA from 12 local Papuan sweet potato genotypes resulting from PCR amplification using the *matK* primer, alignment or sequencing of the nucleotide sequence was carried out. The DNA sequencing stages of the 12 local Papuan sweet potato genotypes were conducted at PT Genetika Science Indonesia, Jakarta, using the Sanger first base dideoxy termination method (BiDirectional Sequencing). The number or size of nucleotides of the 12 local Papuan sweet potato genotypes resulting from amplification using the *matK* primer are shown in Table 1.

The number of nucleotides of each sweet potato genotype varies from 858 bp to 867 bp. The sweet potato genotype with the largest number of nucleotides is Numfor (867 bp), while the smallest is Nabire (858 bp). The difference in nucleotide bases of the 12 sweet potato genotypes ranged from 1-9 bp. Still, there are several genotypes with the same number of nucleotides, namely: i) Koya-4, Ungu MKW-1, L-Sorong (864 bp), and ii) Tinta-1, Biak-1, Mokwam (863 bp). The nucleotides amplified by the *matK* primer in each plant can vary in number and size. Fajri et al. (2024) obtained results of *matK* primer amplification with wild banana DNA of 540-561 bp, while in the *Nepenthes spathulate* plant it was 800-900 bp (Utama et al. 2024). The leilem plant (*Clerodendrum minahassae*) produced an amplification of nucleotide fragments measuring 843 bp

(Kalangi et al. 2014). In the Solanaceae and Fabaceae families, it produced an amplification of nucleotide fragments measuring 800-782 bp (Herman et al. 2023). In Papua sago plants, the amplification of nucleotide fragments measuring 578 bp was produced (Wibawati et al. 2024). Rumere (2021) obtained amplification results of sweet potato nucleotide fragments from Biak Numfor with *matK* measuring 830 bp, which is shorter compared to the results obtained in this study.

The sequence of nucleotide bases from the sequencing results was adjusted to the electropherogram that appeared. Each nucleotide has a different electropherogram peak color. The DNA nucleotide sequence of sweet potato genotypes amplified by the *matK* primer consists of the bases Adenine (A), Guanine (G), Thymine (T), and Cytosine (C). Based on the electropherogram (data not shown), it appears that the nucleotide sequence of the 12 sweet potato genotypes is highly monomorphic (conserved) or contains the same bases.

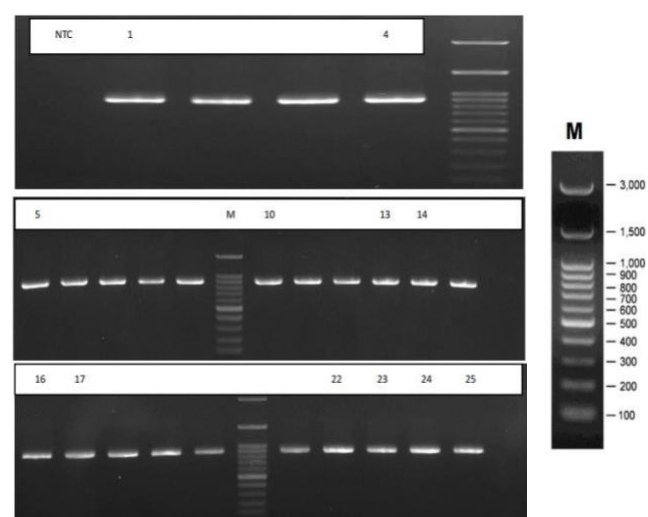


Figure 1. Electrophoresis visualization of local Papuan sweet potato DNA resulting from amplification with *matK*. Note: NTC: Control without DNA sample; 1-25: 12 genotypes of sweet potato DNA samples; M: Marker 1 kb ladder

Table 1. Nucleotide composition of 12 local Papuan sweet potato genotypes

Genotype	Acc. No. Genebank	T(U) (%)	C (%)	A (%)	G (%)	A+T (%)	G+C (%)	Total
Prafi-1	PV067023	28.34	15.80	38.10	17.77	66.32	33.68	861
Nabire	PV067024	27.97	15.85	38.34	17.83	66.32	33.68	858
Tinta-1	PV067025	28.39	15.76	37.89	17.96	66.28	33.72	863
Koya-4	PV067026	28.36	15.74	37.96	17.94	66.32	33.68	864
Numfor	PV067027	28.60	15.69	37.83	17.88	66.43	33.57	867
Airani-Serui	PV067028	28.26	15.81	38.14	17.79	66.40	33.60	860
Biak-1	PV067029	28.27	15.76	38.01	17.96	66.28	33.72	863
Ungu MKW-1	PV067030	28.36	15.74	37.96	17.94	66.32	33.68	864
Amban Pantai-2	PV067031	28.44	15.72	37.92	17.92	66.36	33.64	865
L-MKW	PV067032	28.31	15.78	38.05	17.87	66.35	33.65	862
L-Sorong	PV067033	28.36	15.74	37.96	17.94	66.32	33.68	864
Mokwam	PV067034	28.39	15.76	38.01	17.84	66.40	33.60	863
Average		28.34	15.76	38.01	17.89	66.35	33.65	

There are only 12 polymorphic sites, namely in the nucleotide sequences 1-8, 837, 838, 864, and 865. In the nucleotide sequence 1-8, the polymorphism is quite high, as several sweet potato genotypes do not have nucleotides like other genotypes. This is because the initial attachment site of the *matK* primer has different positions in each sweet potato genotype.

Polymorphic nucleotides at the other four sites are: i) At the 837th nucleotide sequence, 11 genotypes have a T base, and only Nabire has a C base, ii) At the 838th nucleotide sequence, 11 genotypes have a C base, only Nabire has an A base, iii) At the 864th nucleotide sequence, 11 genotypes have an A base, only Tinta-1 has a G base, and 4) At the 865th nucleotide sequence, 11 genotypes have a G base, only Tinta-1 has an A base. Variations in the *matK* nucleotide sequence in the sweet potato genotypes studied are likely caused by point mutations, either substitutions, transversions, or transitions that occur naturally at their growing location, resulting in genetic diversity among sweet potato genotypes within one species (intraspecies). *matK* gene has a higher level of evolution and sequence variability, providing more accurate and better results in identifying the genetic diversity of a plant species (Barthet and Hilu 2007; Thi et al. 2023; Jayaraj 2024).

Nucleotide composition of Papua local sweet potato based on *matK* marker

Based on the number of nucleotides amplified by *matK* primer (Table 2), the nucleotide composition of T(U), C, A, and G can be calculated in each Papua local sweet potato genotype. The average nucleotide composition of 12 sweet potato genotypes is T/U (28.34%), C (15.76%), A (38.01%), and G (17.89%). The nucleotide composition of T(U) of each genotype varies from 27.97% (Nabire) to 28.60% (Numfor). Nucleotide C varies from 15.69% (Numfor) to 15.85% (Nabire). Nucleotide A varies from 37.83% (Numfor) to 38.34% (Nabire). G nucleotides varied from 17.77% (Prafi-1) to 17.96% (Tinta-1 and Biak-1). The number of A+T nucleotides was greater than G+C, with an average of 66.35% and 33.65%, respectively (Table 1). This condition indicates that the DNA sequences of all sweet potato genotypes are easily denatured. The stability of double-stranded DNA is determined by the arrangement of bases and hydrogen bonds formed along the chain; 2 hydrogen bonds bind A and T, while three hydrogen bonds bind G and C. The C $\equiv$ G bond is more difficult to separate than A=T. Vlijm et al. (2015) stated that DNA sequences that have a lot of A+T content are more easily denatured than DNA sequences that have a lot of G+C content. The number of nucleotides A tends to be more similar to T, and the same goes for G and C. This condition occurs because in the double structure of DNA, nucleotide A will pair with T (and vice versa), and G will pair with C (and vice versa).

Amino acid sequence and composition of 12 local Papuan sweet potato genotypes

In the gene expression process, the DNA template fragment undergoes transcription into mRNA, assisted by

the RNA polymerase enzyme. Subsequently, the mRNA undergoes translation in the ribosome (rRNA), assisted by tRNA (transfer RNA), which forms an amino acid chain. Amino acids are the main components of proteins. The beauty of the genetic system is revealed as the amino acid sequence is translated from the sequenced DNA using the genetic code, where each amino acid is encoded by a codon consisting of three nucleotide bases (triplet).

In this study, we conducted amino acid sequencing of 12 local Papuan sweet potato genotypes performed using the Mega 11 program, according to Hall (2013) (Table 2). Our findings revealed a high degree of conservation in the amino acid sequences, similar to the nucleotide sequence in this sweet potato genotype. We observed monomorphism from the first to the last amino acid sequence, with only four locations showing polymorphic amino acids, specifically in the amino acid sequences 1, 2, 3, and 280. In amino acid sequences 1 to 3, several sweet potato genotypes lack amino acids compared to other genotypes. In amino acid sequence 280, the Nabire genotype contains the amino acid Asparagine (Asn/N), whereas the other genotypes contain Histidine (His/H).

The number of amino acids formed varies from 265 in Nabire to 271 in Prafi-1 and Mokwam, with an average number of 267.58 amino acids for the 12 local Papuan sweet potato genotypes (Table 2). This variation in the number of amino acids formed could potentially indicate differences in the protein structure and function among the sweet potato genotypes. The largest amino acid composition is Lysine (Lys/K) at 11.87%, while the smallest is Cysteine (Cys/C) at 0.81% of the total amino acids formed. The percentage of amino acid composition for each local Papuan sweet potato genotype is detailed in Table 2.

Clustering pattern of 12 local Papuan sweet potato genotypes, based on core genes controlling leaf shape, genotype based on nucleotides

Similarity clustering analysis was performed using the neighbor-joining method. This method employs a common data clustering technique for sequence analysis, using genetic distance as a clustering metric. The simple neighbor-joining method produces an unrooted tree and does not assume a constant rate of evolution across lineages. The principle of this method is to find pairs of Operational Taxonomic Units (OTUs, or "neighbors") that minimize the total branch length at each OTU clustering stage and initiate the tree branching. This method can quickly obtain the branch length and parsimonious tree topology.

The clustering pattern of 12 local Papuan sweet potato genotypes based on nucleotide similarity amplified by the *matK* primer is presented in Figure 2. It shows that three groups of genotypes are formed based on their nucleotide similarity, namely: i) Mokwam, Numfor, L-MKW with a bootstrap value of 51%; ii) Nabire, Prafi-1, Airani Serui with a bootstrap value of 90%; and iii) Ungu MKW, L-Sorong, Koya-4, Tinta-1, Biak-1, Amban Pantai-2 with a bootstrap value of 44%.

Table 2. Amino acid composition of 12 Papuan local sweet potato genotypes (%)

Genotype	Ala	Cys	Asp	Glu	Phe	Gly	His	Ile	Lys	Leu	Met	Asn	Pro	Gln	Arg	Ser	Thr	Val	Trp	Tyr	Total
Prafi-1	4.43	1.11	3.69	5.54	6.27	2.95	2.21	7.75	12.92	12.18	2.21	3.32	3.32	2.58	9.23	9.23	3.69	3.32	1.48	2.58	271.00
Nabire	2.64	1.13	3.40	6.42	5.66	4.15	3.02	8.30	10.57	9.81	0.38	8.68	4.53	4.91	5.28	5.66	3.40	4.15	2.26	5.66	265.00
Tinta-1	1.50	0.38	1.50	4.14	7.14	4.89	1.88	11.65	12.03	5.64	2.26	6.02	4.89	2.63	6.39	10.53	6.77	4.51	1.13	4.14	266.00
Koya-4	1.50	0.37	1.87	4.12	7.12	4.87	1.87	11.61	11.99	5.62	2.25	5.99	4.87	2.62	6.37	10.49	6.74	4.49	1.12	4.12	267.00
Numfor	1.49	0.37	1.87	4.10	7.46	4.85	1.87	11.57	11.94	5.60	2.24	5.97	4.85	2.61	6.34	10.45	6.72	4.48	1.12	4.10	268.00
Airani Serui	4.44	1.11	3.70	5.56	6.30	2.96	2.22	7.41	12.96	12.22	2.22	3.33	3.33	2.59	9.26	9.26	3.70	3.33	1.48	2.59	270.00
Biak-1	1.50	0.38	1.50	4.14	7.14	4.89	1.88	11.65	12.03	5.64	2.26	6.02	4.89	2.63	6.39	10.53	6.77	4.51	1.13	4.14	266.00
Ungu MKW	1.50	0.37	1.87	4.12	7.12	4.87	1.87	11.61	11.99	5.62	2.25	5.99	4.87	2.62	6.37	10.49	6.74	4.49	1.12	4.12	267.00
Amban Pantai2	2.62	1.50	3.37	6.37	5.62	4.12	3.00	8.24	10.49	9.74	0.37	8.61	4.49	4.49	5.24	5.99	3.37	4.49	2.25	5.62	267.00
L-MKW	2.63	1.13	3.38	6.39	5.64	4.14	3.01	8.27	10.53	9.77	0.38	8.65	4.51	4.51	5.26	6.02	3.38	4.51	2.26	5.64	266.00
L-Sorong	1.50	0.37	1.87	4.12	7.12	4.87	1.87	11.61	11.99	5.62	2.25	5.99	4.87	2.62	6.37	10.49	6.74	4.49	1.12	4.12	267.00
Mokwam	4.43	1.48	3.69	5.54	6.27	2.95	2.21	7.38	12.92	12.18	2.21	3.32	3.32	2.58	9.23	9.23	3.69	3.32	1.48	2.58	271.00
Average	2.52	0.81	2.65	5.05	6.57	4.20	2.24	9.75	11.87	8.32	1.78	5.98	4.39	3.11	6.82	9.03	5.14	4.17	1.49	4.11	267.58

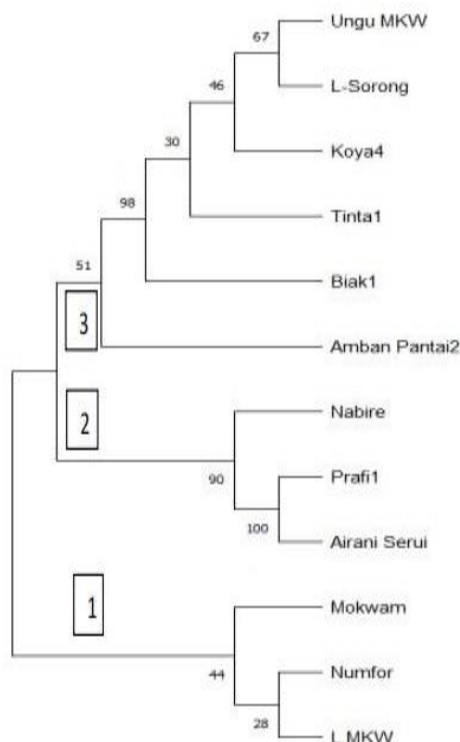


Figure 2. Neighbor-joining dendrogram based on *matK* profiles of 12 local Papuan sweet potato genotypes

The cluster analysis results showed that the Nabire, Prafi-1, and Airani Serui genotypes have a close relationship, as their bootstrap values reached 90%, higher than the other two groups. The number of times the branching pattern appears at a node in the original tree, indicated by the bootstrap value, shows repeats. A high degree of confidence in the node can be inferred if the bootstrap value is more than 95% (Dharmayanti 2011).

The grouping pattern of Papuan local sweet potato genotypes is not based on the proximity of their growing areas but is randomly grouped based on the nucleotide similarity of each sweet potato genotype. The 12 sweet potato genotypes come from different regions, namely West Papua Province (Prafi-1, L-MKW, Ungu MKW, Mokwam), Southwest Papua Province (L-Sorong), Papua Province (Koya-4, Serui, Numfor, Biak-1), and Central Papua Province (Nabire). Each region has different ecogeographic conditions, resulting in different characteristics between sweet potato genotypes.

The Prafi-1 and Airani Serui genotypes have the most similar *matK* nucleotide composition (bootstrap 100) compared to other genotypes. However, the phenotypic appearance of the leaf shape of these two genotypes is very different, with Prafi-1 having 5-fingered leaves while Airani Serui has 3-fingered oval leaves. This is because nuclear genes control leaf shape, while the *matK* gene is a chloroplast gene that encodes genes related to photosynthesis, which is in agreement with Qu et al. (2018).

Phylogenetic analysis

Phylogenetic analysis was conducted to compare the DNA sequences of local Papua sweet potato genotypes

with the GenBank database using the BLAST (Basic Local Alignment Search Tool) method in the MEGA 11 program. The study of phylogenetic relationships is fundamental to understanding biological evolution. Evolution is very slow, but it can accumulate changes in the characteristics of an organism over several generations, making simple species more complex. Phylogenetic analysis was carried out to determine the kinship of an organism with its ancestors based on character similarities. In phylogenetics, a group of organisms whose members share many similar characteristics or features are considered to be very closely related. They are thought to have descended from a single ancestor (Nater et al. 2015). The phylogenetic tree was statistically tested with 1,000 replications using the bootstrap method.

The phylogenetic tree is shown in Figure 3. Twelve local Papuan sweet potato genotypes, based on the *matK* marker, clustered in several different branches, but several genotypes clustered on the same branch. Sweet potato genotypes that are grouped on the same branch show a very close relationship, and vice versa. The sweet potato genotypes that have a close relationship are: i) L-MNK with L-SRG, Numfor, Aerani-Serui; ii) Ink-1 with MNK Purple; and iii) Prafi-1 with Nabire, Biak-1, Mokwam. The Amban Pantai-2 and Koya-4 genotypes are on different branches, indicating that they are quite closely related to other sweet potato genotypes. This could occur due to differences in nucleotides and amino acids based on the *matK* marker of each local Papuan sweet potato genotype.

Differences in environmental conditions in the area of origin of each sweet potato genotype were caused by genetic variation in several sweet potato characteristics. Different geographic origins led to the introduction and naturalization of cultivars from their original locations (Kiran et al. 2015; Dong et al. 2024). Isolation within the same geographic zone reduces the genetic distance between populations, leading to the emergence of traits with similar genetic features. In contrast, distinct geographical environments result in different adaptation patterns and genetic features (Mims et al. 2016; Karuwal et al. 2024).

The twelve sweet potato genotypes have the closest similarity to several species of the genus *Ipomoea*, namely, *Ipomoea cordatotriloba*, *Ipomoea × leucantha*, and *Ipomoea batatas*. The maximum scores or total values obtained from the alignment between the sequences of the 12 sweet potato genotypes and the database sequences range from 1574 to 1600, with total scores between 1574 and 1600 (Table 3). Query Coverage, which describes the percentage of the target DNA sequence that matches the sequences of the 12 sweet potato genotypes, is 99.77%-100%. This means that the DNA sequences of the 12 sweet potato genotypes cover the entire target DNA sequence in the NCBI database. The E Value of the 12 sweet potato genotypes is 0, indicating that there is no possibility that the alignment occurred by chance. The E Value, or expectation value, measures the likelihood that the alignment happened by chance; a good E Value is close to zero. The Percentage Identity of the 12 sweet potato genotypes is 99.77%, indicating how well the DNA sequences of the 12 sweet potato genotypes match the target DNA sequence from the database.

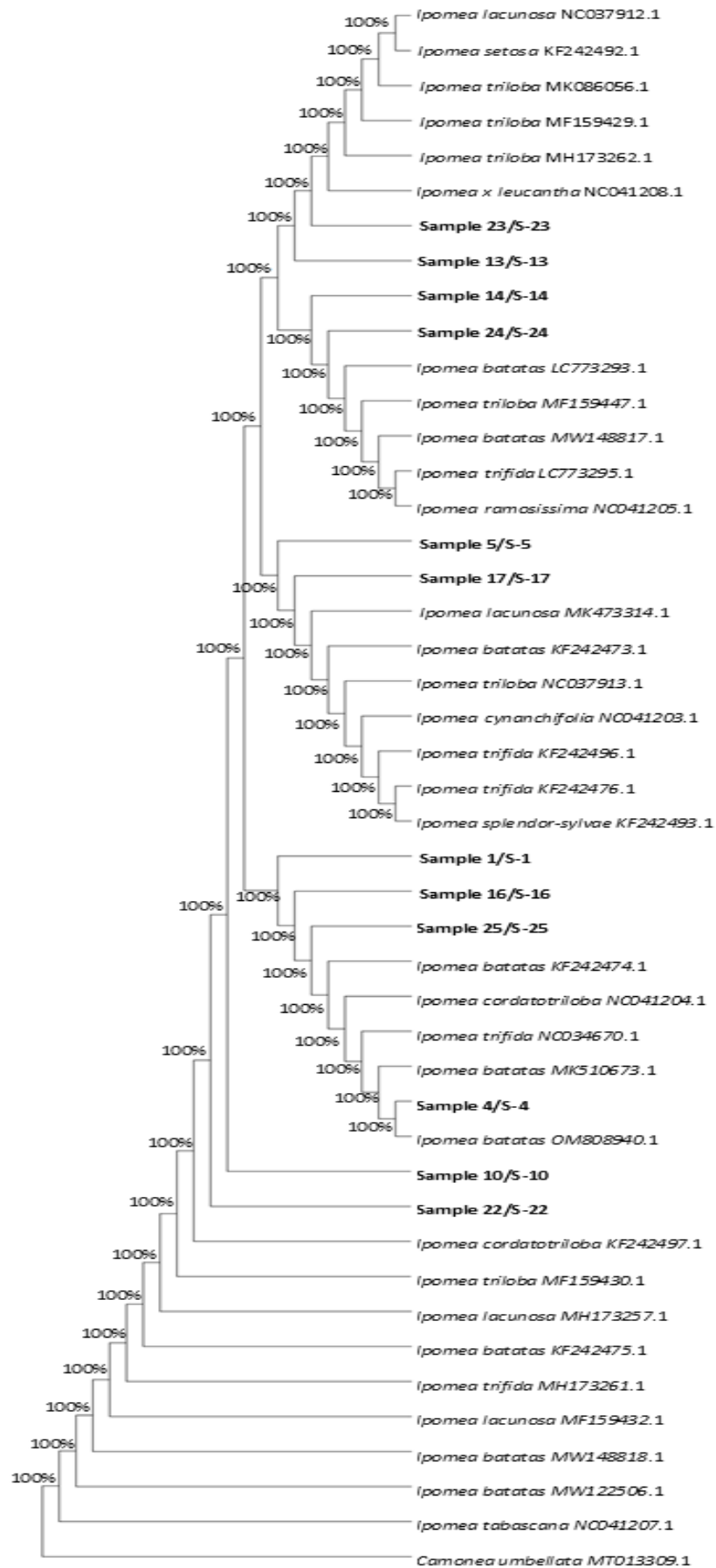


Figure 3. Phylogenetic tree of 12 local Papuan sweet potato genotypes based on *matK* markers from GenBank

Table 3. Sweet potato accessions sequence similarity analysis with NCBI using BLAST

Accession name	Nearest BLAST sequence	Max Score	Total score	%Query cover	E-value	% Identity	Accession number
Prafi-1	<i>Ipomoea cordatotriloba</i> chloroplast, partial genome	1591	1591	100	0.0	100	KF242497.1
	<i>Ipomoea</i> × <i>leucantha</i> chloroplast, complete genome	1591	1591	100	0.0	100	NC_041208.1
	<i>Ipomoea batatas</i> chloroplast, partial genome	1591	1591	100	0.0	100	KF242474.1
Nabire	<i>Ipomoea cordatotriloba</i> chloroplast, partial genome	1574	1574	100	0.0	99.77	KF242497.1
	<i>Ipomoea</i> × <i>leucantha</i> chloroplast, complete genome	1574	1574	100	0.0	99.77	NC_041208.1
	<i>Ipomoea batatas</i> chloroplast, partial genome	1574	1574	100	0.0	99.77	KF242474.1
Tinta-1	<i>Ipomoea lacunose</i> isolate CBT-208-03 <i>matK</i> .gene. partial cds: chloroplast	1589	1589	100	0.0	99.88	MK473314.1
	<i>Ipomoea cordatotriloba</i> chloroplast, partial genome	1587	1587	99	0.0	99.88	KF242497.1
	<i>Ipomoea lacunose</i> isolate CBT-208-03 <i>matK</i> .gene. partial cds: chloroplast	1596	1596	100	0.0	100	MK473314.1
Koya-4	<i>Ipomoea cordatotriloba</i> chloroplast, partial genome	1594	1594	99	0.0	100	KF242497.1
	<i>Ipomoea lacunose</i> isolate CBT-208-03 <i>matK</i> .gene. partial cds: chloroplast	1600	1600	99	0.0	100	MK473314.1
	<i>Ipomoea cordatotriloba</i> chloroplast, partial genome	1596	1596	100	0.0	99.88	KF242497.1
Numfor	<i>Ipomoea cordatotriloba</i> chloroplast, partial genome	1589	1589	100	0.0	100	KF242497.1
	<i>Ipomoea</i> × <i>leucantha</i> chloroplast, complete genome	1589	1589	100	0.0	100	NC_041208.1
	<i>Ipomoea lacunose</i> isolate CBT-208-03 <i>matK</i> .gene. partial cds: chloroplast	1594	1594	100	0.0	100	MK473314.1
Airani-Serui	<i>Ipomoea cordatotriloba</i> chloroplast, partial genome	1592	1592	99	0.0	100	KF242497.1
	<i>Ipomoea lacunose</i> isolate CBT-208-03 <i>matK</i> .gene. partial cds: chloroplast	1596	1596	100	0.0	100	MK473314.1
	<i>Ipomoea cordatotriloba</i> chloroplast, partial genome	1594	1594	99	0.0	100	KF242497.1
Biak-1	<i>Ipomoea cordatotriloba</i> chloroplast, partial genome	1592	1592	99	0.0	100	KF242497.1
	<i>Ipomoea</i> × <i>leucantha</i> chloroplast, complete genome	1592	1592	100	0.0	100	NC_041208.1
	<i>Ipomoea lacunose</i> isolate CBT-208-03 <i>matK</i> .gene. partial cds: chloroplast	1596	1596	100	0.0	100	MK473314.1
Ungu MKW-1	<i>Ipomoea cordatotriloba</i> chloroplast, partial genome	1594	1594	99	0.0	100	KF242497.1
	<i>Ipomoea lacunose</i> isolate CBT-208-03 <i>matK</i> .gene. partial cds: chloroplast	1598	1598	100	0.0	100	MK473314.1
	<i>Ipomoea cordatotriloba</i> chloroplast, partial genome	1594	1594	99	0.0	100	KF242497.1
Amban Pantai-2	<i>Ipomoea cordatotriloba</i> chloroplast, partial genome	1592	1592	100	0.0	100	KF242497.1
	<i>Ipomoea</i> × <i>leucantha</i> chloroplast, complete genome	1592	1592	100	0.0	100	NC_041208.1
	<i>Ipomoea lacunose</i> isolate CBT-208-03 <i>matK</i> .gene. partial cds: chloroplast	1596	1596	100	0.0	100	MK473314.1
L-MKW	<i>Ipomoea cordatotriloba</i> chloroplast, partial genome	1594	1594	99	0.0	100	KF242497.1
	<i>Ipomoea cordatotriloba</i> chloroplast, partial genome	1592	1592	100	0.0	100	KF242497.1
	<i>Ipomoea</i> × <i>leucantha</i> chloroplast, complete genome	1592	1592	100	0.0	100	NC_041208.1
L-Sorong	<i>Ipomoea lacunose</i> isolate CBT-208-03 <i>matK</i> .gene. partial cds: chloroplast	1596	1596	100	0.0	100	MK473314.1
	<i>Ipomoea cordatotriloba</i> chloroplast, partial genome	1594	1594	99	0.0	100	KF242497.1
	<i>Ipomoea cordatotriloba</i> chloroplast, partial genome	1594	1594	100	0.0	100	KF242497.1
Mokwam	<i>Ipomoea</i> × <i>leucantha</i> chloroplast, complete genome	1594	1594	100	0.0	100	NC_041208.1

From the results of this study, it can be concluded that the number of nucleotides of each sweet potato genotype varies from 858 bp to 867 bp. The sweet potato genotype with the largest number of nucleotides is Numfor (867 bp), while the smallest is Nabire (858 bp). There are only 12 polymorphic sites, namely in the nucleotide sequences 1-8, 837, 838, 864, and 865. The average nucleotide composition of the 12 sweet potato genotypes is: T/U (28.34%), C (15.76%), A (38.01%), and G (17.89%). The number of amino acids formed varies from 265 (Nabire) to 271 (Prafi-1, Mokwam), with the average number of amino acids for the 12 local Papuan sweet potato genotypes being 267.58. The highest amino acid composition is Lysine (Lys/K) at 11.87%, and the lowest is Cysteine (Cys/C) at 0.81% of the total amino acids formed. Only four sites have polymorphic amino acids, namely in the amino acid sequences 1, 2, 3, and 280. The grouping pattern of the 12 local Papuan sweet potato genotypes based on nucleotide similarities amplified by the *matK* primer forms three groups of genotypes based on their nucleotide similarities: i) Mokwam, Numfor, L-MKW; ii) Nabire, Prafi-1, Biak-1, Mokwam; and iii) Ungu MKW, L-Sorong, Koya-4, Tinta-1, Biak-1, Amban Pantai-2. The 12 sweet potato genotypes have the closest similarity to several species of the genus *Ipomoea*, namely

I. batatas, *I. cordatotriloba*, *I. × leucantha*, *I. triloba*, and *I. lacunosa*.

ACKNOWLEDGEMENTS

Thanks to the Ministry of Education, Culture, Research and Technology of Indonesian Republic for funding this research in accordance with the grant competition scheme of Fundamental Research, with the contract number: 076/E5/PG.02.00.PL/2024/ and contract date of June 11, 2024.

REFERENCES

- Barthet MM, Hilu KW. 2007. Expression of *matK*: Functional and evolutionary implications. *Am J Bot* 94 (8): 1402-1412. DOI: 10.3732/ajb.94.8.1402.
- BPS [Bapan Pusat Statistik]. 2020. Central Bureau of Statistics of West Papua Province. West Papua Province in Figures. <https://www.papuabarat.bps.go.id>. [Indonesian]
- Dharmayanti NLPI. 2011. Molecular phylogenetic: Organism taxonomy methods based on evolutionary history. *Wartazoa* 21 (1): 1-10.
- Dong B-C, Yang Q, Kinlock NL, Pouteau R, Pyšek P, Weigelt P, Yu F-H, van Kleunen M. 2024. Naturalization of introduced plants is driven

- by life-form-dependent cultivation biases. *Divers Distrib* 30 (1): 55-70. DOI: 10.1111/ddi.13788.
- Fajri H, Sunandar A, Qurbaniah M. 2024. Phylogenetic analysis of wild bananas (*Musa* spp.) in West Kalimantan, Indonesia, based on maturase K (*matK*) genes. *Biodiversitas* 25 (8): 2612-2618. DOI: 10.13057/biodiv/d250833.
- Gomes-Pereira M, Monckton DG. 2017. Ethidium bromide modifies the agarose electrophoretic mobility of CAG•CTG alternative DNA structures generated by PCR. *Front Cell Neurosci* 11: 153. DOI: 10.3389/fncel.2017.00153.
- Hall BG. 2013. Building phylogenetic trees from molecular data with MEGA. *Mol Biol Evol* 30: 1229-1235. DOI: 10.1093/molbev/mst012.
- Harnelly E, Thomy Z, Fathiya N. 2018. Phylogenetic analysis of Dipterocarpaceae in Ketambe Research Station, Gunung Leuser National Park (Sumatra, Indonesia) based on *rbcL* and *matK* genes. *Biodiversitas* 19 (3): 1074-1080. DOI: 10.13057/biodiv/d190340.
- Herman, Al-Khairi H, Wansyah AR, Asmania, Utami RI, Anzaelina DN, Oktaviano Z, Lestari W, Adiwiirman, Roslim DI. 2023. The ability of *matK* and *trnL-trnL-trnF* intergenic spacer to discern certain species accessions of the families Solanaceae and Fabaceae. *Sabao J Breed Genet* 55 (1): 97-106. DOI: 10.54910/sabao2023.55.1.9.
- Immanissa S, Faizal I, Salamah A, Sari IA, Susilo AW. 2020. Genetic variation in Maturase K (*matK*) from cacao (*Theobroma cacao* L.) varieties in Indonesia. *IOP Conf Ser: Earth Environ Sci* 481: 012026. DOI: 10.1088/1755-1315/481/1/012026.
- Indow L, Maturbongs RA, Prabawardani S, Hendri, Lyons G. 2021. Implementation of the remote indigenous community empowerment program on the sustainability of the local food crops in West Papua, Indonesia. *Biodiversitas* 22: 5247-5254. DOI: 10.13057/biodiv/d221202.
- Jayaraj G. 2024. The role of *matK* gene in *Eucalyptus* species identification and its importance in phylogenetics. *BioMed Res J* 8 (2): 753-762.
- Kalangi C, Kamu VS, Kumaunang M. 2024. Barcode DNA tanaman Leilem (*Clerodendrum minahassae* L.) berdasarkan gen *matK*. *Jurnal Mipa Unsrat Online* 3: 108-112. DOI: 10.35799/jm.3.2.2014.5861. [Indonesian]
- Karudapuram S, Padilla R, Chen S, Koepf S, Hauser J, Wang Y, Jacobson K, White M, Bordoni R, Wenz M, Shah A, Joe L. 2007. P48-T a new high-density size standard for sizing large fragments across multiple fragment analysis capillary electrophoresis applications. *J Biomol Tech* 18 (1): 17.
- Karuwal RL, Kasiandari R, Daryono BS. 2024. Genetic variation of Fei banana (*Musa troglodytarum* L.) in Maluku Islands using RAPD markers. *Sabao J Breed Genet* 56 (1): 101-111. DOI: 10.54910/sabao2024.56.1.9.
- Kiran U, Moahnty SK, Roy PS, Behera L, Chand PK. 2015. Genetic diversity among banana cultivars from Odisha using RAPD markers. *Sci Res Rep* 5 (2): 118-124.
- Mims MC, Hauser L, Goldberg CS, Olden JD. 2016. Genetic differentiation, isolation-by-distance, and metapopulation dynamics of the Arizona Treefrog (*Hyla wrightorum*) in an isolated portion of its range. *PLoS One* 11: e0160655. DOI: 10.1371/journal.pone.0160655.
- Motohashi K. 2019. Development of highly sensitive and low-cost DNA agarose gel electrophoresis detection systems, and evaluation of non-mutagenic and loading dye-type DNA-staining reagents. *PLoS One* 14 (9): e0222209. DOI: 10.1371/journal.pone.0222209.
- Nater A, Burri R, Kawakami T, Smeds L, Ellegren H. 2015. Resolving evolutionary relationships in closely related species with whole-genome sequencing data. *Syst Biol* 64 (6): 1000-1017. DOI: 10.1093/sysbio/syv045.
- Probojati RT, Listyorini D, Sulisetijono S, Wahyudi D. 2021. Phylogeny and estimated genetic divergence times of banana cultivars (*Musa* spp.) from Java Island by maturase K (*matK*) genes. *Bull Natl Res Cent* 45: 33. DOI: 10.1186/s42269-021-00492-3.
- Qiu X, Zhang H, Wang Q, Jian H, Yan H, Zhang T, Wang J, Tang K. 2012. Phylogenetic relationships of wild roses in China based on nrDNA and *matK* data. *Sci Hortic* 140: 45-51. DOI: 10.1016/j.scienta.2012.03.014.
- Qu Y, Legen J, Arndt J, Henkel S, Hoppe G, Thieme C, Ranzini G, Muino JM, Weihe A, Ohler U, Weber G, Ostersetzer O, Schmitz-Linneweber C. 2018. Ectopic transplastomic expression of a synthetic *MatK* gene leads to cotyledon-specific leaf variegation. *Front Plant Sci* 9: 1453. DOI: 10.3389/fpls.2018.01453.
- Raza S, Farooqi S, Mubeen H. 2015. Role of molecular markers and their significance. *Am J Pharm Health Res* 3 (12): 1-8.
- Rumere J. 2021. Karakter morfo-agronomi dan molekuler beberapa aksesori ubijalar (*Ipomoea batatas* (L.) Lam.) asal Biak Numfor. [Thesis]. Universitas Papua, Manokwari. [Indonesian]
- Saraswati P, Soplanit A, Syahputra AT, Kossay L, Muid N, Ginting E, Lyons G. 2013. Yield trial and sensory evaluation of sweet potato cultivars in Highland Papua and West Papua Indonesia. *J Trop Agric* 51 (1): 74-83.
- Scorsim B, da Silva AC, Ramos LI, Passere MD, Thomaz SM, de Oliveira AV. 2024. Molecular markers in genetic studies of aquatic macrophytes: A systematic review. *Hydrobiologia* 851: 3809-3820. DOI: 10.1007/s10750-024-05556-9.
- Sevindik E, Korkom Y, Murathan ZT. 2024. Evaluating DNA barcoding using cpDNA *matK* and *rbcL* for species identification and phylogenetic analysis of *Prunus armeniaca* L. (Rosaceae) genotypes. *Genet Resour Crop Evol* 71: 1825-1835. DOI: 10.1007/s10722-023-01748-9.
- Singhal H, Ren YR, Kern SE. 2010. Improved DNA electrophoresis in conditions favoring polyborates and lewis acid complexation. *PLoS One* 5 (6): e11318. DOI: 10.1371/journal.pone.0011318.
- Thi NPA, Tam TT, Khang DT. 2023. Genetic diversity in the *matK* gene of *Dimocarpus longan* varieties in the Mekong Delta. *Asian J Plant Sci* 22 (3): 444-451. DOI: 10.3923/ajps.2023.444.451.
- Utama MN, Etikawati N, Sugiyarto, Susilowati A. 2024. New specific primer *matK* and *rbcL* region for DNA barcode pitcher plant *Nepenthes spathulate*. *Biodiversitas* 25 (6): 2515-2523. DOI: 10.13057/biodiv/d250621.
- Vlijm R, Torre JVD, Dekker C. 2015. Counterintuitive DNA sequence dependence in supercoiling-induced DNA melting. *PLoS One* 10 (10): e0141576. DOI: 10.1371/journal.pone.0141576.
- Wang J-F, Gong X, Chiang Y-C, Kuroda C. 2013. Phylogenetic patterns and disjunct distribution in *Ligularia hodgsonii* Hook. (Asteraceae). *J Biogeogr* 40 (9): 1741-1754. DOI: 10.1111/jbi.12114.
- Wanke S, Wicke S. 2023. Editorial: Phylogenomic discordance in plant systematics. *Front Plant Sci* 14: 1308126. DOI: 10.3389/fpls.2023.1308126.
- Wibawati Z, Abbas B, Mustamu YA, Mawikere NL, Noya AI. 2024. Filogenetik 5 kultivar sagu dan spesies palmae lainnya berdasarkan penanda molekuler *matK*. *Cassowary* 7 (1): 47-55. DOI: 10.30862/cassowary.cs.v7.i1.202. [Indonesian]