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Short Communication: *rbc*L and *mat*K chloroplast DNA composition of green chireta (*Andrographis paniculata*) from Indonesia

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Abstract. *Arif MF, Aristya GR, Subositi D, Sai AN, Kasiamdari RS. 2019. Short Communication: rbc*L and *mat*K chloroplast DNA composition of green chireta (*Andrographis paniculata) from Indonesia. Biodiversitas 20: 3575-3583.* Green chireta (*Andrographis paniculata* (Burm.f.) Wall. ex Nees.) is often used by the traditional community as a medicinal herb in Indonesia. However, nucleotide study of green chireta from Indonesia has never been done. The objectives of this study were to analyze the *rbc*L and *mat*K chloroplast gene composition of green chireta from Indonesia and to analyze the relationship with other *Andrographis* species. The result proved that all the DNA composition of green chireta used in this study was similar to the *A. paniculata* from GenBank. The *rbc*L composition was similar to *A. paniculata* KF521878, *A. paniculata* JQ922118, and *A. paniculata* JQ230990 from GenBank while the *mat*K composition was similar to *A. paniculata* LC461762. Total 501 bp of *rbc*L and 639 bp of *mat*K can be aligned produced 167 and 213 amino acid from translation. The amino acid translation result showed no different expression from *rbc*L genes but there was one sample expressed different translations from the *mat*K gene. The phylogenetic tree was reconstructed using the Neighbor-Joining method with 1000 bootstrap values and the Kimura 2-Parameter (K2P) model in the MEGA7 software. Both *rbc*L and *mat*K genes grouped the samples into the same clade as *A. paniculata* from GenBank. No variation was detected from the *rbc*L gene but two haplotypes were detected from the *mat*K gene. This composition and sequence data serve as a database for *Andrographis* species from Indonesia which can be used for various studies.

Keywords: *Andrographis paniculata*, chloroplast DNA, medicinal plant, *rbc*L, *mat*K

INTRODUCTION

Indonesia has around 3.000 species used as a medicinal plant (Ministry of Trade Republic Indonesia, 2009). The RISTOJA (Research on Medicinal Plants and Herbs) is a study to produce the baseline data of the diversity of Indonesian plants carried out by the Indonesian Center for Medicinal and Traditional Medicine Research and Development in 2012, 2015, and 2017. Community-based exploratory research conducted in 405 ethnic groups throughout Indonesia includes 2.354 traditional healers as sources of information. The methods used were interview and observation of the selected traditional healer by purposive sampling and observation of medicinal plants and how to concoct traditional medicines carried out by traditional healers. The results of RISTOJA have identified 2.848 types of medicinal plants from 47.466 plant information used in traditional medicine. 32.014 concoction information was grouped into 74 categories of complaints/indications of disease (Wahyono 2017).

One of the target plants studied under the RISTOJA program is green chireta or *Andrographis paniculata* (Burm.f) Wall. ex Nees. This plant is being used by most traditional communities as an external medicine, consumption medicine, and a mixture with other medicinal plants (Mulyati and Setyowati 1996). The plant is distributed in most regions of the Asian continent such as South Asia, Southeast Asia, China and several parts of Europe (Kumar et al. 2014). The plant is usually used as an antidote, febrifuge, anti-bacterial, anti-cancer, and anti-inflammatory, (Joselin and Jeeva, 2014).

Many studies have revealed the biochemical contents and benefits of green chireta (Chao et al. 2010; Li et al. 2007; Shin et al. 2000), but the *rbc*L sequence, *mat*K sequence, and the nucleotide composition study of green chireta from Indonesia has never been done. DNA sequence data is important to be studied for completing the existing database (Neves and Forest 2011). The DNA sequence database is useful for analyzing variations of DNA sequences, DNA barcoding analysis, phylogenetic analysis, breeding and others (Daniell et al. 2016). The *rbc*L and *mat*K chloroplast genes are relatively often used for the analysis because both of them play an important role in plant photosynthesis and their mutation rates can be

measured (Kool et al. 2012). CBOL recommended using *rbc*L and *mat*K genes for DNA barcoding analysis (CBOL 2009). The *rbc*L sequence, *mat*K sequence, and the composition data are expected to provide and to preserve the database of *Andrographis* species from Indonesia which can be used for various studies.

MATERIALS AND METHODS

Sampling and DNA Isolation

Leaves were taken from each location of the accession representing the islands of Sumatra, Java, Sulawesi, Nusa Tenggara, and Papua (Table 1) during the RISTOJA 2017 project from Balai Besar Penelitian dan Pengembangan Tanaman Obat dan Obat Tradisional, Ministry of Health. Ten Leaves were taken from different individuals (Figure 1). The samples were collected and recorded. The leaves were stored at -20 ºC for long term storage.

The DNA extraction was executed using the GeneJET Plant Genomic DNA Purification Kit (Thermo Fisher Scientific) protocol. A total of 0,1 g leaf was ground with the help of liquid nitrogen until smooth. Ground leaf was transferred into a 1.5 ml microtube. A total of 350 µL of lysis buffer A was mixed with the ground leaf into the microtube. The mixture was homogenized using vortex for 20 s. A total of 50 µL lysis buffer B and 20 µL of RNAse were added to the mixture. The samples were then incubated for 15 min at 65ºC. A total of 130 µL of Precipitation Solution were added homogenized. The mixture was freeze into the -20ºC temperature for 5 min, then centrifuged for 5 min at 8.200 g. A total of 450 µL of the supernatant from the centrifugation was placed into a new microtube. 400 µL of Plant gDNA Binding Solution and ethanol absolute were added to the supernatant and then homogenized. The mixture then placed into a spin column and centrifuged for 1 min at 3.000 g. The liquid phase from the centrifugation in the collection tube was removed and the collection tube was set back in the spin column. 500 µL of wash buffer 1 was added to the spin column and centrifuged for 1 min at 5.000 g. The liquid phase from the centrifugation in the collection tube was discarded and the collection tube was set back in the spin column. 500 µL of wash buffer 2 was then added into a spin column and the mixture was centrifuged at 8.200 g for 3 min. The process was repeated twice. The collection of tubes was discarded and replaced with a 1.5 mL microtube. 150 µL of elution buffer was added to the spin column and centrifuged at 5.000 g for 1 min.

DNA was analyzed quantitatively using a nanodrop spectrophotometer in UV light (Multiskan Sky, Thermo Fisher Scientific. The DNA concentration and purity were calculated from absorbance of 260 nm (A260) and 280 nm (A280). Only appropriate samples were used for DNA amplification.

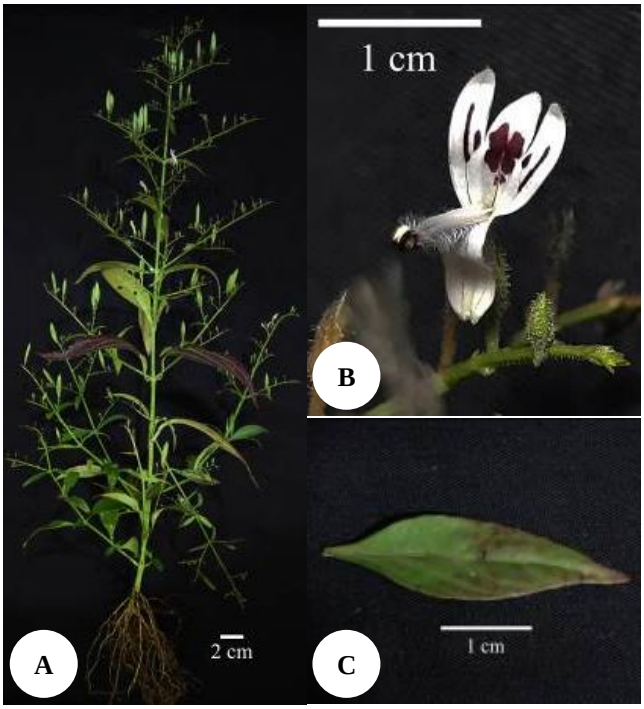

Figure 1. Morphology of green chireta from Indonesia (A) whole plant, (B) flower, and (C) leaf

Table 1. Sampling location and accession number of green chireta from Indonesia

Island	Origin of accessions	Sample code	Acc. no. from GenBank		Coordinate location	
			*rbc*L	*mat*K	Latitude	Longitude
Sumatra	South Sumatra	AP-SUM1	MN540094	MN544599	3°18' 12,3"	104° 39' 29,2"
		AP-SUM2	MN540095	MN544560		
Java	West Java	AP-JAV1	MN540086	MN544591	7° 20' 58,4"	108° 12' 29,6"
		AP-JAV2	MN540087	MN544592		
Sulawesi	Central Sulawesi	AP-SUL1	MN540092	MN544597	2° 13' 35"	121° 45' 52,4"
		AP-SUL2	MN540093	MN544598		
Nusa Tenggara	NTB	AP-NT1	MN540088	MN544593	8° 31' 11"	118° 50' 27"
		AP-NT2	MN540089	MN544594		
Papua	Papua	AP-PAP1	MN540090	MN544595	2° 34' 21,1"	140° 41' 27,1"
		AP-PAP2	MN540091	MN544596		

DNA amplification of rbcL and matK

DNA was amplified by the PCR using pair primer of *rbc*L and *mat*K gene. The universal *mat*K primer was *mat*K Forward: 5'-GTCCATGTCGAAATCTTGGTTC-3' and *mat*K Reverse: 5'-ATGATAATGAGAAAGATTTC TGCC-3') (Yu et al. 2011). The universal *rbc*L primer was *rbc*L Forward: 5'-ATGTCACCACAAACAGAGACTAAA GC-3' and *rbc*L reverse: 5'-GTAAAATCAAGTCCACCG CG-3' (Bafeel et al. 2011). The final concentration of the DNA template was 5 ng/µL and the primers were 10 mM. The PCR reaction was performed referring to research from Arolla et al. (2015) with modification of annealing and extension temperature. Pre-denaturation was carried out at 94°C for 2 min, denaturation at 94°C for 30 s, annealing at 52°C for 3 s, extension at 72°C for 1 min, and a final extension at 72°C for 10 min. The reaction was repeated in 40 cycles. The reaction was stopped in the final hold at 4°C.

The amplified bands were separated and visualized on agarose stained with Florosafe DNA stain (1st BASE) gel with a 2% (w/v) concentration in the TBE buffer. Electrophoresis was executed with 50 volts for 60 min. The bands were visualized under UV light with the gel doc (Figure 2 and Figure 3). The *mat*K and *rbc*L gene which was successfully amplified then sequenced using the Sanger sequencing method.

Data analysis

The green chireta's *rbc*L and *mat*K sequence results were assembled using contig editor on GeneStudio software (GeneStudio, Inc). The forward and reverse of each sequence were observed carefully to ensure there was no mismatch on consensus sequence produced. The codon composition produced from the amino acid translation of the consensus sequence was checked to ensure there was no stop codon. The nucleotide composition of the *rbc*L and *mat*K gene were checked online using web-based software through The Sequence Manipulation Suite http: //www.bioinformatics.org/sms/). The G+C content was also validated using the DnaSP v.6.12.01 software (Librado and Rozas 2009). Samples were examined using BLAST nucleotide on GenBank (https: //blast.ncbi.nlm.nih.gov/ Blast.cgi). Data from GenBank were downloaded in FASTA format form. Both data from this research and from GenBank were processed and put together using MESQUITE software (Maddison and Maddison 2017). The FASTA format was also converted into FASTA for MEGA format using MESQUITE. The data obtained were aligned using ClustalW in MEGA7 (Kumar et al. 2016). The genetic distance estimation data was analyzed using Pairwise Distance with the Kimura 2-parameter model in MEGA7. The alignment result was converted into a MEGA format for variation analysis using DnaSP v.5.10.01 software. The phylogenetic tree was reconstructed using the Neighbour-Joining method with 1000 bootstrap values with the Kimura 2-Parameter (K2P) model in the MEGA7.

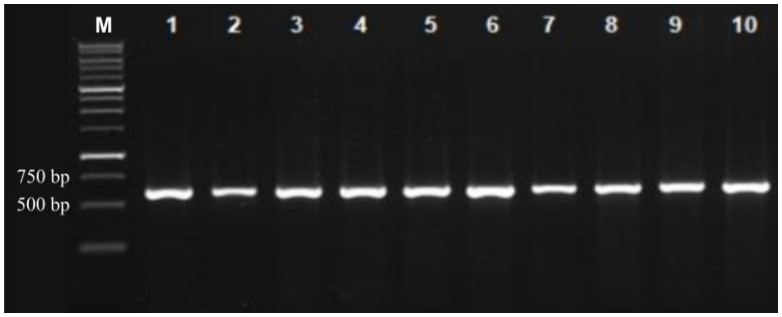

Figure 2. Amplicon of partial chloroplast *rbc*L gene (M: 1 kb DNA ladder, 1: AP-SUM1, 2: AP-SUM2, 3: AP-JAV1, 4: AP-JAV2, 5: AP-SUL1, 6: AP-SUL2, 7: AP-NT1, 8: AP-NT2, 9: AP-PAP1, 10: AP-PAP2.)

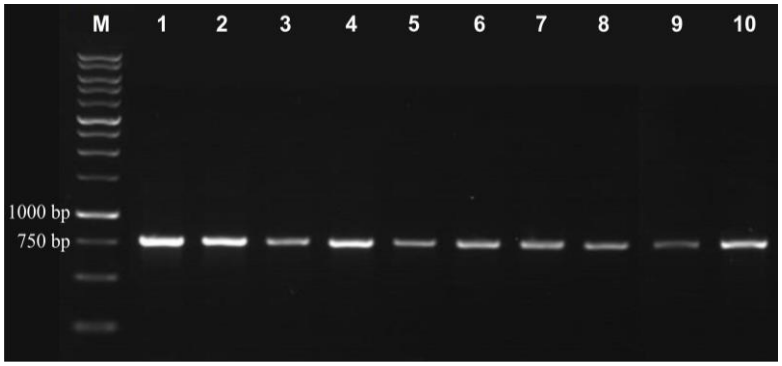

Figure 3. Amplicon of partial chloroplast *mat*K gene (M: 1 kb DNA ladder, 1: AP-SUM1, 2: AP-SUM2, 3: AP-JAV1, 4: AP-JAV2, 5: AP-SUL1, 6: AP-SUL2, 7: AP-NT1, 8: AP-NT2, 9: AP-PAP1, 10: AP-PAP2.)

RESULTS AND DISCUSSION

DNA Amplification and Nucleotide Composition

The *rbc*L chloroplast gene was ~600 bp (Figure 2) and the size of the *mat*K chloroplast gene was ~700 bp (Figure 3). The sequences were read using the Sanger Sequencing method. The green chireta samples studied have 99% to 100% similarity with the *Andrographis paniculata* species from the GenBank database. Alignment results with the data from GenBank showed that there were a total of 501 bp *rbc*L chloroplast gene compared without gaps. A total of 167 amino acids were translated from the partial *rbc*L gene in this study. While the alignment of the *mat*K chloroplast gene showed that 639 nucleotides can be compared with GenBank data. A total of 213 amino acids can be translated from the *mat*K gene sequence studied in this study.

The result indicated that all of the green chireta's *rbc*L gene used in the study have similar nucleotide compositions (Table 2). Data stated that the *rbc*L gene of green chireta from Indonesia consisted from 27,54% thymine (T), 22,55% cytosine (C), 28,54% adenine (A), and 21,36% guanine (G). The composition of nucleotides was similar to the composition of nucleotides of *A. paniculata* KF521878, *A. paniculata* JQ922118, and *A. paniculata* JQ230990 from the Genbank database. The *rbc*L gene from the study was also compared with the *rbc*L gene from other *Andrographis* species in GenBank. The samples also compared with *A. alata* KP455301, *A. echioides* KP455302, *A. glandulosa* KP455303, *A. lineata* KP455304, and *A. nallamalayana* KP455305 from GenBank (Table 2). All of the samples produced the same amount of thymine (T) content. The cytosine (C) content of *A. alata* KP455301, *A. echioides* KP455302, *A. glandulosa* KP455303, and *A. nallamalayana* KP455305 was lower than green chireta from Indonesia. The adenine (A) content of all samples compared were 28,54% except for *A. alata* KP455301 which was lower at 28,34%. The guanine (G) content of *A. alata* KP455301, *A. echioides* KP455302, *A. glandulosa* KP455303, and *A. nallamalayana* KP455305 was higher than green chireta from Indonesia (Table 2).

All samples showed the same *mat*K nucleotide composition except for the AP-NT1 sample originating from Nusa Tenggara (Table 3). The data indicated that almost all of the green chireta's *mat*K gene from Indonesia consisted of 35.84% thymine (T), 17,37% cytosine (C), 30,20% adenine (A), and 16,59% guanine (G). This composition was similar to the composition of *A. paniculata* LC461762 from the GenBank database. AP-NT1 sample had different nucleotide content, which was 35,68% thymine (T) and 30,36% adenine (A) nucleotides (Table 2). The *mat*K gene composition from this study was also compared with the *mat*K gene from other *Andrographis* species from GenBank such as *A. alata* KP455307, *A. echioides* KP455308, *A. glandulosa* KP455309, *A. lineata* KP455310, and *A. nallamalayana* KP455311 (Table 3). *A. glandulosa* KP455309 and *A. lineata* KP455310 indicated a higher content of thymine (T) nucleotides which were 35,99%. The content of cytosine (C) of *A. glandulosa* KP455309, *A. lineata* KP455310, and *A. nallamalayana* KP455311 was higher than green chireta from Indonesia, which was 17,68%, 17,53%, and 17,68%. The adenine (A) content of all compared samples was lower than the Samboloto from this study, which ranged from 28,95% to 29,42%. The guanine content (G) of the other *Andrographis* species were higher and ranging from 17,21% to 18,15% (Table 3).

Table 2. Nucleotide composition of green chireta's *rbc*L partial chloroplast gene from Indonesia

Sample	Nucleotide percentage (%)					
	T (U)	C	A	G	A+T	G+C
AP-JAV1	27,54	22,55	28,54	21,36	56,09	43,91
AP-JAV2	27,54	22,55	28,54	21,36	56,09	43,91
AP-NT1	27,54	22,55	28,54	21,36	56,09	43,91
AP-NT2	27,54	22,55	28,54	21,36	56,09	43,91
AP-PAP1	27,54	22,55	28,54	21,36	56,09	43,91
AP-PAP2	27,54	22,55	28,54	21,36	56,09	43,91
AP-SUL1	27,54	22,55	28,54	21,36	56,09	43,91
AP-SUL2	27,54	22,55	28,54	21,36	56,09	43,91
AP-SUM1	27,54	22,55	28,54	21,36	56,09	43,91
AP-SUM2	27,54	22,55	28,54	21,36	56,09	43,91
A. alata KP455301*	27,54	22,36	28,34	21,76	55,89	44,11
A. echioides KP455302*	27,54	22,36	28,54	21,56	56,09	43,91
A. glandulosa KP455303*	27,54	22,36	28,54	21,56	56,09	43,91
A. lineata KP455304*	27,54	22,55	28,54	21,36	56,09	43,91
A. nallamalayana KP455305*	27,54	22,36	28,54	21,56	56,09	43,91
A. paniculata KF521878*	27,54	22,55	28,54	21,36	56,09	43,91
A. paniculata JQ922118*	27,54	22,55	28,54	21,36	56,09	43,91
A. paniculata JQ230990*	27,54	22,55	28,54	21,36	56,09	43,91
Avg.	27,54	22,51	28,53	21,41	56,08	43,92

Note: *data from GenBank

Table 3. Nucleotide composition of green chireta's *mat*K partial chloroplast gene from Indonesia

Sample	Nucleotide percentage (%)					
	T (U)	C	A	G	A+T	G+C
AP-JAV1	35,84	17,37	30,20	16,59	66,04	33,96
AP-JAV2	35,84	17,37	30,20	16,59	66,04	33,96
AP-NT1	35,68	17,37	30,36	16,59	66,04	33,96
AP-NT2	35,84	17,37	30,20	16,59	66,04	33,96
AP-PAP1	35,84	17,37	30,20	16,59	66,04	33,96
AP-PAP2	35,84	17,37	30,20	16,59	66,04	33,96
AP-SUL1	35,84	17,37	30,20	16,59	66,04	33,96
AP-SUL2	35,84	17,37	30,20	16,59	66,04	33,96
AP-SUM1	35,84	17,37	30,20	16,59	66,04	33,96
AP-SUM2	35,84	17,37	30,20	16,59	66,04	33,96
A. alata KP455307*	35,84	16,74	29,26	18,15	65,10	34,90
A. echioides KP455308*	35,84	16,74	29,42	18,00	65,26	34,74
A. glandulosa KP455309*	35,99	17,68	28,95	17,37	64,95	35,05
A. lineata KP455310*	35,99	17,53	29,26	17,21	65,26	34,74
A. nallamalayana KP455311*	35,84	17,68	29,11	17,37	64,95	35,05
A. paniculata LC461762*	35,84	17,37	30,20	16,59	66,04	33,96
A. paniculata KP455312*	35,84	17,53	29,89	16,74	65,73	34,27
A. paniculata MF349897*	35,84	17,53	29,89	16,74	65,73	34,27
Avg.	35,85	17,36	29,90	16,89	65,75	34,25

Note: *data from GenBank

Table 4. Nucleotide polymorphic sites of green chireta's *rbc*L partial chloroplast gene from Indonesia

Sample	Haplotype	Nucleotide number** 22224444 311390579 306445678
A. paniculata KF521878*	Hap-1	CTCTTCTAC
A. paniculata JQ922118*	Hap-1	. . . . .
A. paniculata JQ230990*	Hap-1	. . . . .
AP-JAV1	Hap-1	. . . . .
AP-JAV2	Hap-1	. . . . .
AP-NT1	Hap-1	. . . . .
AP-NT2	Hap-1	. . . . .
AP-SUL1	Hap-1	. . . . .
AP-SUL2	Hap-1	. . . . .
AP-SUM1	Hap-1	. . . . .
AP-SUM2	Hap-1	. . . . .
AP-PAP1	Hap-1	. . . . .
AP-PAP2	Hap-1	. . . . .
A. alata KP455301*	Hap-2	G..CG..TT
A. echioides KP455302*	Hap-3	..A...CGT
A. glandulosa KP455303*	Hap-3	..A...CGT
A. lineata KP455304*	Hap-4	.C...T...
A. nallamalayana KP455305*	Hap-3	..A...CGT

Note: *data from GenBank, **vertical reading

Nucleotide variation of *rbc*L and *mat*K chloroplast gene

A total of 501 bp partial *rbc*L genes can be aligned for polymorphic site analysis. The results of the *rbc*L gene analysis indicated that all the samples were grouped as the same haplotype with *A. paniculata* KF521878, *A. paniculata* JQ922118, and *A. paniculata* JQ230990 from GenBank (Table 4). No polymorphic sites were detected from all the green chireta samples analyzed in this study generated from the *rbc*L gene (Table 4). When compared with the *A. alata* KP455301 from GenBank there were five polymorphic sites in nucleotides number 33, 234, 294, 477, and 498. The sample from this study had four nucleotide differences at numbers 216, 456, 477, and 498 with the *A. echioides* KP455302, *A. glandulosa* KP455303, dan *A. nallamalayana* from GenBank. The samples also had two nucleotide differences in the numbers 210 and 405 with the sample *A. lineata* KP455304 from GenBank. Overall, the *rbc*L sequence data analyzed in this study generated four haplotypes at the level of the genus *Andrographis* (Table 4).

A total of 639 bp partial *mat*K genes can be aligned for the polymorphic site analysis. The results of the *mat*K gene analysis emphasized that almost all the samples from Indonesia were grouped in the same haplotype as the *A. paniculata* LC461762 sequence data from GenBank (Table 5). A polymorphic site from AP-NT1 was detected. The AP-NT1 sample differs in its nucleotide in number 632. Based on these data, it is known that the samples used for this study divided into two haplotypes. The samples also had different nucleotides in numbers 386, 529, 627, and 639 compared to *A. paniculata* KP455312 and *A. paniculata* MF349897 from GenBank. The *mat*K gene indicated a higher variation. A total of 46 polymorphic sites were recorded in the analysis of the genus *Andrographis* level. This number was higher than the polymorphic sites of the *rbc*L gene which only amounts to 9 sites. Overall, *mat*K sequence data analyzed yielded seven types of haplotypes at the level of the genus *Andrographis* (Table 5).

Amino acid variation of *rbc*L and *mat*K chloroplast gene

The results of the amino acid translation of the *rbc*L and *mat*K genes from ten bitter samples from Indonesia were analyzed and compared with data from GenBank. The results of the *rbc*L gene analysis at the *Andrographis* genus showed no different amino acids translation throughout the observed site. While the results of the *mat*K gene translational analysis stated that almost all samples had the

same amino acid translation as the *A. paniculata* LC461762 sample from GenBank (Table 6). AP-NT1 sample expressed a difference in the translation of amino acids in codon number 211. AP-NT1 sample produced asparagine (N) amino acid while the other samples produced isoleucine (I) amino acid (Table 6). The translation results were also different when compared with *A. paniculata* KP455312 and *A. paniculata* MF349897 on codons number 129, 177, and 213 (Table 6). *A. paniculata* KP455312 and *A. paniculata* MF349897 produced serine (S) amino acids, lysine (K), and aspartic acid (D) while samples from this study produced tyrosine (Y) amino acids, glutamine (Q), and glutamic acid (E) (Table 6).

Phylogenetic tree reconstruction

The results of the phylogeny tree reconstruction showed that both using the *rbc*L and *mat*K genes, the samples were grouped into the same clade as *A. paniculata* from GenBank. Clade analysis results from *rbc*L chloroplast gene generated bootstrap 86% value and from the *mat*K chloroplast gene generated 87% of the bootstrap value. Analysis of the two genes also revealed that *A. lineata* species were the most closely related species to *A. paniculata*. While other *Andrographis* species are further away than *A. paniculata* and *A. lineata*. The limitations of existing data make only a small amount of data that can be analyzed.

Table 5. Nucleotide polymorphic sites of green chireta's *mat*K partial chloroplast gene from Indonesia

Sample	Haplotype	Nucleotide number**
		111111222223333333334444444555555556666666
		157678999015692267888990044679112334781122233
		111307735941437470126923264988203968873672 7929
A. paniculata LC461762*	Hap-1	CCATTAACTGGAAGAACGTGAGCCCCAACCACGCGTACGTCGAATA
A. paniculata KP455312*	Hap-2	.....C.....A.....G..C
A. paniculata MF349897*	Hap-2	.....C.....A.....G..C
AP-JAV1	Hap-1	.....
AP-JAV2	Hap-1	.....
AP-NT1	Hap-3	.....A.
AP-NT2	Hap-1	.....
AP-SUL1	Hap-1	.....
AP-SUL2	Hap-1	.....
AP-SUM1	Hap-1	.....
AP-SUM2	Hap-1	.....
AP-PAP1	Hap-1	.....
AP-PAP2	Hap-1	.....
A. alata KP455307*	Hap-4	....GCGGC..G..G.T.AA.....GG.TG.G...CTG..T.G..C
A. echioides KP455308*	Hap-5	TA....GG...G..GC..A...A.G.G....G...CCG..T.G..C
A. glandulosa KP455309*	Hap-6	...G.GGGGATGGAGC.CA..A..T.GG..GGC.TCCGTATTGC.C
A. lineata KP455310*	Hap-7	..C.....G..G.....G.....C...T.G..C
A. nallamalayana KP455311*	Hap-7	..C...G....G..G.....CCG..T.G..C

Note: *data from GenBank, **vertical reading

Table 6. Amino acid polymorphic sites of green chireta's *mat*K partial chloroplast gene from Indonesia

Sample	Amino acid site**
	1111111 1111111111 222222
	12556666 7880222333 5677778899 000111
	1475493578 1588489016 0001790335 678013
A. paniculata LC461762*	PALWYIRLVK NHRKLVYRTH KQNQQRSELG LPDNIE
A. paniculata KP455312*	....S... ....K..... .....D
A. paniculata MF349897*	....S... ....K..... .....D
AP-JAV1	....
AP-JAV2	....
AP-NT1	....N.
AP-NT2	....
AP-SUL1	....
AP-SUL2	....
AP-SUM1	....
AP-SUM2	....
AP-PAP1	....
AP-PAP2	....
A. alata KP455307*	....D.GV.. D...HI...D .EK...PVV. .S...D
A. echioides KP455308*	SD....GV.. D..NH...N. ..K...PAV. .S...D
A. glandulosa KP455309*	...G.MGVIN DRKNH..K.. R.KH.MPAVW ISYT.D
A. lineata KP455310*	..F..... D..... .....A.. .S...D
A. nallamalayana KP455311*	..F...G... D..... ......PAV. .S...D

Note: *data from GenBank, **vertical reading

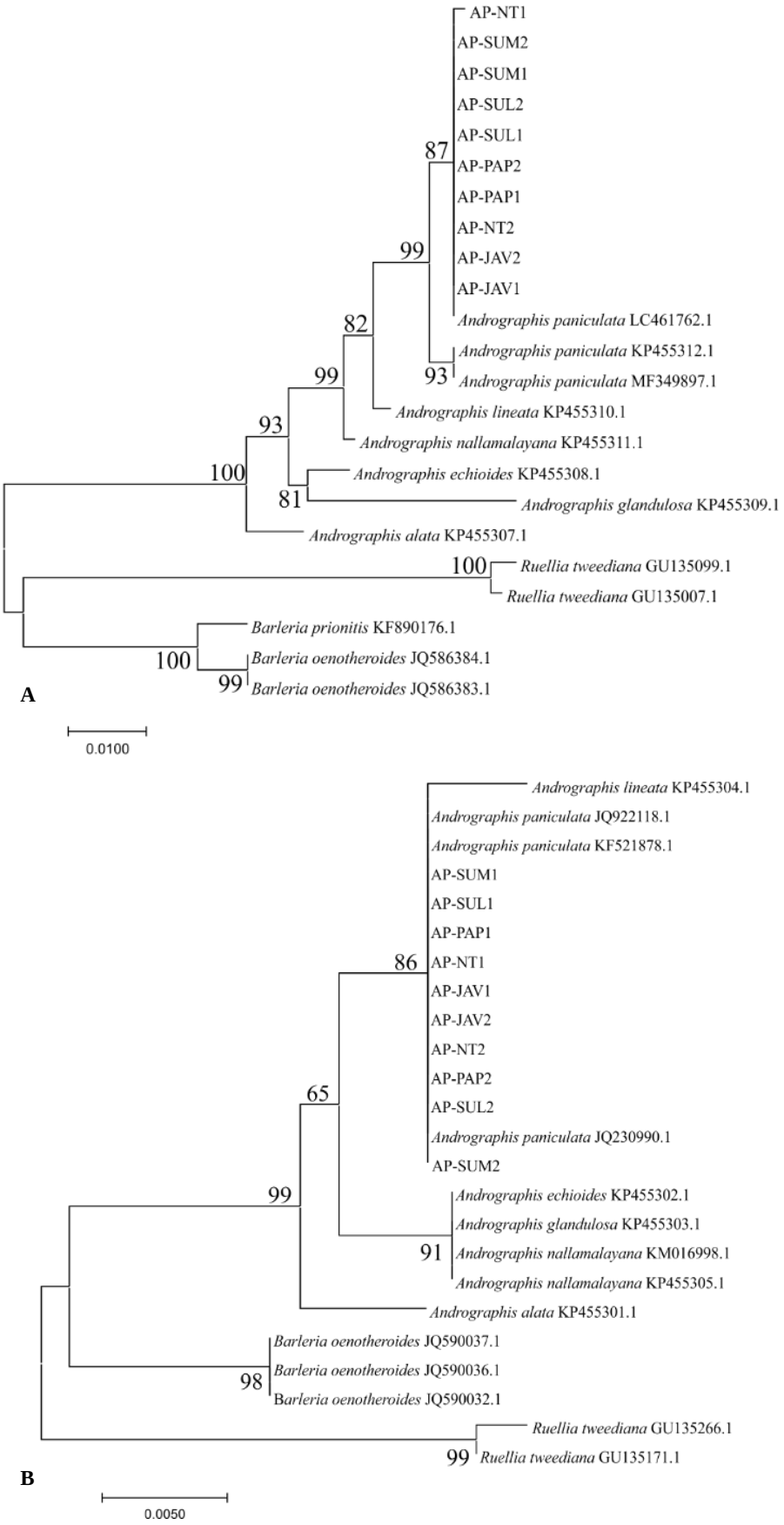

Figure 4. Phylogenetic tree reconstruction from (A) *mat*K gene and (B) *rbc*L gene around Acanthaceae. Each node shows a bootstrap value and the scale number indicates substitution rates

Discussion

The chloroplast gene used for DNA barcoding analysis, composition analysis, and haplotyping in this study were the *rbc*L and *mat*K genes. The *rbc*L gene is considered to have fewer variations than other chloroplast genes (Li et al. 2011). The *rbc*L gene has more primer universality than the *mat*K gene (Kool et al. 2012; Sass et al. 2007). Primer universality is one important point when identifying unknown species such as biodiversity samples or market products. Primers are more effective if they can be used to amplify different kinds of plants (Ghorbani et al. 2017). There is still no stable and universal primers in plants such as the *COI* gene in animals (Kress et al. 2005).

The analysis provided that the *mat*K gene generated more polymorphic sites than the *rbc*L gene. The *rbc*L gene evolves slowly and is most often used as material for phylogenetic analysis (Smith and Donoghue 2008). When compared to other chloroplast genes which are also often used as phylogenetic analysis materials such as the *mat*K chloroplast gene and *trn*H-*psb*A, the *rbc*L gene is more universal. The *rbc*L gene is weaker in the separation of the very closely related taxon (Hollingsworth et al. 2011). However, research conducted by Newmaster et al. (2006), revealed that the *rbc*L gene had enough nucleotide variations to distinguish species in angiosperms and fulfilled the criteria for material for DNA barcoding with some exceptions. The *mat*K gene is the chloroplast gene which is considered the fastest evolving (Hilu and Liang 1997). The *mat*K gene is also deliberated as the plant gene which is the closest analog to the animal *COI* gene. The entire results of DNA barcoding, the *rbc*L and *mat*K nucleotide analysis, and amino acid translation analysis of the samples revealed that the nucleotide composition was similar to the *A. paniculata* chloroplast gene from GenBank.

The phylogenetic tree reconstruction was carried out to determine the relationship between the samples studied and *Andrographis* species from GenBank also with other Acanthaceae. The reconstruction results revealed that the sample identified as *A. paniculata* and it was the closest relative to *A. lineata* from GenBank. The phylogenetic tree reconstruction in this study was done using the Neighbor-Joining method with 1000 times bootstrap value and Kimura 2-Parameter (K2P) model. The Neighbor-Joining method uses the calculation of the genetic distance as the basis for its reconstruction. The reconstruction measure the shortest branches produced from the genetic distance of Operational Taxonomic Units (OTUs) called neighbors (Saitou and Nei 1987). Kimura 2-Parameter Model calculates the evolutionary for the reconstruction of phylogeny trees by measuring the same rates of transition and transversion (Nei and Kumar 2000).

From this research, it is known that the nucleotide composition and haplotype of the chloroplast green chireta's *rbc*L and *mat*K genes Indonesia similar to the *A. paniculata* species from GenBank. Both *mat*K and *rbc*L genes contain higher adenine + thymine (A + T) compositions than guanine + cytosine (G + C). The *mat*K gene is able to separate *A. paniculata* from Indonesia into two types of haplotypes while the *rbc*L gene cannot separate the haplotypes of the samples analyzed. This composition and sequence data serve as a database for *Andrographis* species from Indonesia which can be used for various studies.

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