Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2018 15(1): 192-198. **OPEN ACCESS**

Evaluation of *mat*K and *rbc*L genes as markers in DNA barcoding of *Codiaeum variegatum* (L.) Blume.

Song Ai Nio, Beivy Jonathan Kolondam, Trina Ekawati Tallei

Department of Biology, Faculty of Mathematics and Natural Sciences, University of Sam Ratulangi, Kampus Unsrat, Manado 95115, North Sulawesi, **Indonesia**

.

***Correspondence:** niosongai@unsrat.ac.id **Accepted:** 25 Dec. 2017 **Published online:** 04 Mar. 2018

Codiaeum variegatum (L.) Blume., commonly referred to garden croton, has variation in leaf shape and colour. The molecular identification using DNA barcode in this species has been scarcely carried out. In this study we focused on two cultivars with different leaf shape and color, i.e. gold star and royal. The aim of this study was to evaluate *mat*K and *rbc*L genes as markers in DNA barcoding of croton and to provide recommendation which marker was to be used in identifying croton more properly. The DNA extraction used innuPREP Plant DNA Kit and the Kit PCR 5x FirePol Master Mix was used to amplify *mat*K and *rbc*L genes fragments using available universal primers. Sequence alignment using *mat*K and *rbc*L showed that both varieties were 100% identical. Sequence identification of *mat*K revealed 98.96% similarity with *Philodendron radiatum*, *Monstera* sp. and *Homalomena speariae* using BOLD Systems and 100% similarity with *H. asperifolia* and *H. asmae* using BLAST. Sequence identification of *rbc*L gene using BOLD Systems and BLAST demonstrated that both varieties had 100% similarities with *C. variegatum*. In conclusion, *rbc*L gene was more reliable to be used as DNA barcode for identification of *C. variegatum* than *mat*K gene.

Keywords: barcode, *Codiaeum variegatum*, *mat*K, *rbc*L

INTRODUCTION

Codiaeum is the second largest genus of the family Euphorbiaceae and it is native plant in the Mollucan Islands of Indonesia as well as Philippines, Thailand, Malaysia, New Guinea, Australia, India, Sri Lanka and some other Pacific Islands. Garden croton (*Codiaeum variegatum* (L.) Blume.) is a group of small evergreen trees, perennial, tropical ornamental herbs and shrubs, with variation in leaf shape and colour. Leaf shapes vary from simple ovate to linear; some are slightly or deeply cut, and others are connected with the blade only by the midrib. The leaf color performed as shades, blends, combinations, or solid patches of red, pink, orange, yellow, lavender, black, and green (Deng et al., 2010). The variations of leaf color in crotons could be related to the combination of produced pigment in the plants, such as chlorophyll, carotene, phaeophytin, xanthophyll (Ogunwenmo et al. 2007) and anthocyanin (Papafotiou et al., 2007). In addition to its ornamental value, croton phytochemicals are known to have protective and preventive properties against diseases as well as antioxidants (Ogunwenmo et al., 2007; Deng et al., 2010).

Reliable methods to identify and distinguish ornamental plants specimen may help in solving the problem of doubt caused by counterfeited ornamental plants and many other illegal activities in the horticultural industry. Traditionally, the identification and characterization of cultivars and species was based on morphological and physiological properties, however, this identification is not effective and reliable (Elansary et al., 2017). In recent days the identification

method has been developed using molecular markers, such as DNA barcode (Hebert et al. 2003; Kadkhodaei et al., 2010) to analyze the diversity of plants and to determine plant specimens to their species although the morphological diagnostic characters are unavailable (Elansary et al., 2017).

As a technique for taxonomic identification, DNA barcoding can utilize one or several standardized DNA regions that are universally present in the target lineages and have sufficient sequence variation to recognize species and identify individuals correctly. DNA barcoding is a potential tool to detect error in identifying species because similarity-based approaches using DNA barcoding combined with morphology would solve the misidentification based on morphology (Liu et al. 2017). For the identification of plant species, two defined regions of the chloroplast DNA (maturase K or *mat*K and ribulose-1,5-biphosphate carboxylase oxygenase large subunit or *rbc*L) have been widely used for standard barcodes as endorsed by the Plant Working Group of the Consortium for the Barcode of Life (CBOL) in 2009 (CBOL, 2009). DNA barcode served fast and accurate identification of a plant species, and the sequences are available in the sequence library such as GenBank and BOLD (Kress and Erickson, 2007). Ogunwenmo et al., (2007) reported that cultivars of *C. variegatum* showed variability in content of pigments and chromosome numbers. Deng et al., (2010) studied genetic relationship of 44 cultivars of *C. variegatum* using amplified fragment length polymorphism (AFLP) markers. The molecular identification using DNA barcode in croton, however, has been scarcely carried out. There are so many cultivars of croton, but in this study we focused on two cultivars with different leaf shape and color, i.e. gold star and royal. The study aimed to evaluate *mat*K and *rbc*L genes as markers in DNA barcoding of croton and to provide recommendation which marker was to be used in identifying croton more properly.

MATERIALS AND METHODS

DNA Extraction, Amplification and Sequencing

Total DNA was extracted from approximately 50 mg of plant materials (gold star and royal) using innuPREP Plant DNA Kit (Analytik Jena, Germany) according to manual, with a slight modification to increase the chloroplast DNA yield (Kolondam, 2015). Genes of *mat*K and *rbc*L were amplified using 5x Firepol PCR Master Mix Ready-to-load (Solis Biodyne). Primer pairs used for gene *mat*K were MatK-1RKIM-f 5'-ACC CAG TCC ATT CTG GAA ATC TTG GTT C-3' and MatK-3FKIM-r 5'-CGT ACA GTA CTT TTG TGT TTA CGA G-3' (Kuzmina et al., 2012). Primer pairs used for gene *rbc*L were *rbc*LaF 5'-ATG TCA CCA CAA ACA GAG ACT AAA GC-3' and *rbc*LaR 5'-GTA AAA TCA AGT CCA CCR CG-3' (Kress and Erickson, 2007). Amplification was performed as follows: predenaturation at 95^{o}C for 30 sec, annealing at 50^{o}C for 30 sec, and polymerization at 72^{o}C for 50 sec. Amplicons were separated using 1% agarose gel. Clear cut bands indicated the success of the amplification. The PCR products together with their primer pairs were sent to 1Base Malaysia for sequencing.

Data Analysis

The chromatograms were corrected using Geneious v5.6 (Kearse et al., 2012), and then processed using other available online programs, as suggested by Tallei and Kolondam (2015). The sequences were pairwise aligned using global alignment with free end gaps to identify regions of 95% similarity. Consensus sequences were generated by pairwise alignment of forward and reverse sequences using MUSCLE (Multiple Sequence Comparison by Log-Expectation) which is integrated in Geneious v5.6.

The sequences generated using each marker were aligned respectiveley, using multiple sequence alignment (multalin) with hierarchical clustering (Corpet, 1988; http://multalin.toulouse.inra.fr/multalin), and trimmed accordingly to get the core area of *mat*K and *rbc*L. The croton plants were identified using BOLD (Barcode of Life Database) Systems (www.boldsystems.org) (Ratnasingham and Hebert, 2007). The identification was correct if the highest identity percentage of searched sequences was derived from expected species or genus. On the other hand, the identification was ambiguous when the highest identity percentage of searched sequences was not derived from expected species or genus, or family. A homology search for matK and rbcL genes was performed using Basic Local Alignment Search Tool (BLAST) (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

RESULTS AND DISCUSSION

Sequence alignment of *mat*K gene using multalin showed that *Codiaeum variegatum* cv. Gold star (SPG2) and Royal (JM2) were 100% identical (Figure 1).

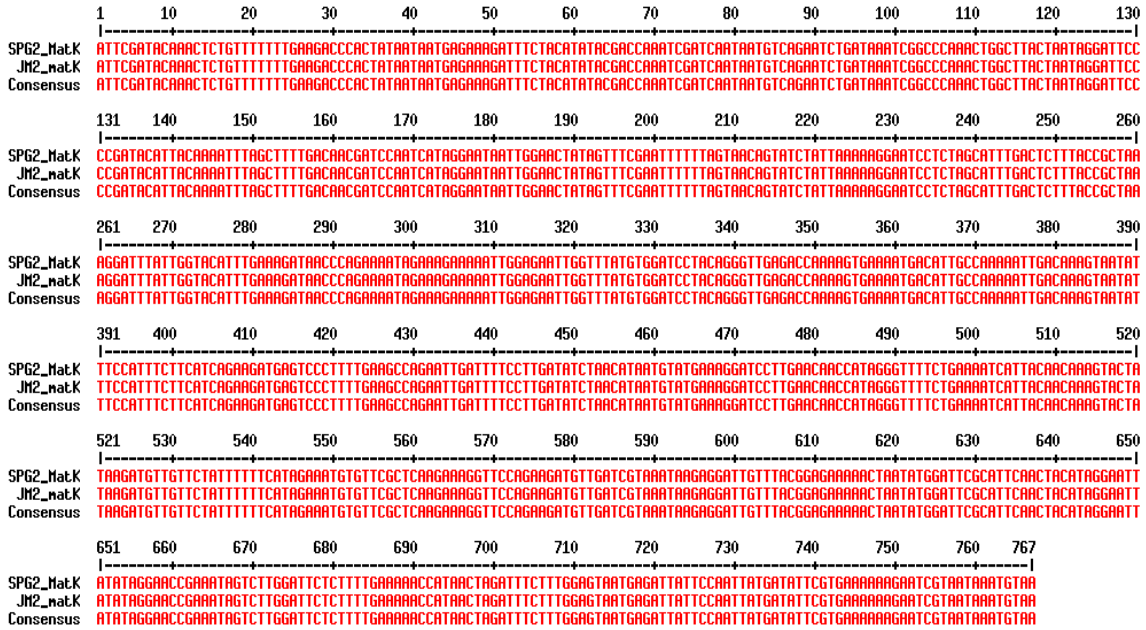


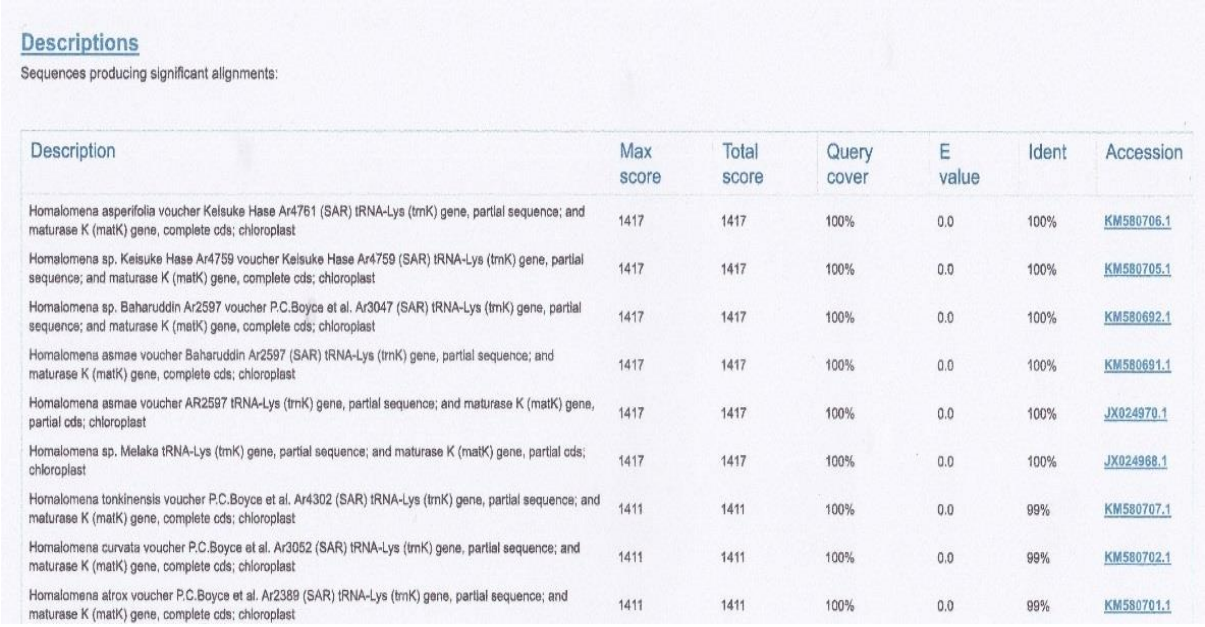
Figure 1. Sequence alignment of *mat*K gene of *Codiaeum variegatum* cv. Gold star (SPG2) and royal (JM2) using Multalin (http://multalin.toulouse.inra.fr/multalin).

Match Rank	Phylum	Class	Order	Family	Genus	Species	Subspecies	Score	Similarity	E-Value	Status
1	Magnoliophyta	Liliopsida	Alismatales	Araceae	*Philodendron*	*radiatum*		751	98.96	0	Early-Release
2	Magnoliophyta	Liliopsida	Alismatales	Araceae	*Monstera*			751	98.96	0	Early-Release
3	Magnoliophyta	Liliopsida	Alismatales	Araceae	*Monstera*			751	98.96	0	Early-Release
4	Magnoliophyta	Liliopsida	Alismatales	Araceae	*Homalomena*	*speariae*		750	98.96	0	Published
5	Magnoliophyta	Liliopsida	Alismatales	Araceae	*Philodendron*	*fragrantissimum*		749	98.83	0	Published
6	Magnoliophyta	Liliopsida	Alismatales	Araceae	*Philodendron*	*fragrantissimum*		749	98.83	0	Published
7	Magnoliophyta	Liliopsida	Alismatales	Araceae	*Philodendron*	*sulcatum*		747	98.7	0	Early-Release
8	Magnoliophyta	Liliopsida	Alismatales	Araceae	*Philodendron*	*jacquinii*		745	98.57	0	Early-Release
9	Magnoliophyta	Liliopsida	Alismatales	Araceae	*Furtadoa*	*mixta*		744	99.73	0	Published
10	Magnoliophyta	Liliopsida	Alismatales	Araceae		*Jorge170*		741	99.08	0	Published

Figure 2. Identification based on BOLD System of *mat*K gene of *Codiaeum variegatum* cv. Gold star (SPG2) and royal (JM2).

Although both varieties have different characteristics in leaf shape and color, this result revealed that they are the same species. The similar results were reported in green daluga, yellow daluga and mottled daluga from Sangihe Islands which included in the same species, i.e. *Cyrtosperma merkusii* (Julianti et al., 2015) and ornamental plant *Sansevieria trifasciata* var. Laurentii and Hahnii (Tallei et al., 2016a). Partial fragment of *matK* gene amplified using primer pairs of MatK-1RKIM and MatK-3FKIM-r did not indicate intraspecific variation between these croton cultivars.

Sequence identification of *mat*K gene using BOLD Systems (Figure 2) revealed 98.96% similarity with *Philodendron radiatum, Monstera* sp. and *Homalomena speariae*. The same sequence of *mat*K gene employed in BLAST demonstrated 100% similarity with *Homalomena asperifolia* and *Homalomena asmae* (Figure 3). The similar result was demonstrated by Tallei and Kolondam (2015) which showed that using *mat*K gene, Sangihe nutmeg (*Myristica fragrans*) had 100% similarity with *M. fatua*, *M. maingayi*, and *M. globosa*.

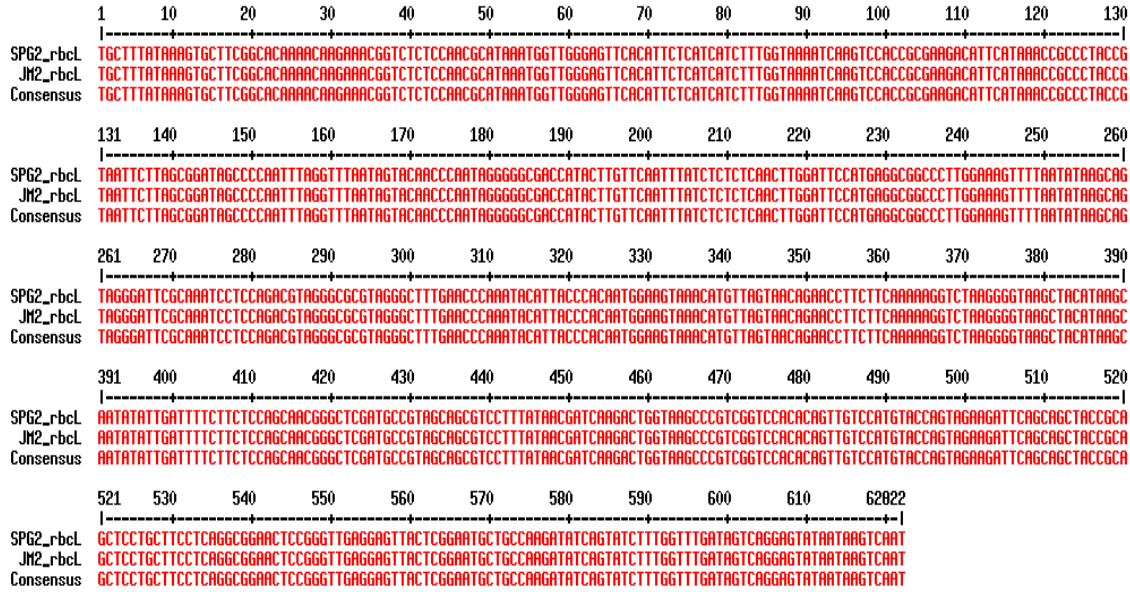


Descriptions

Sequences producing significant alignments:

Description	Max score	Total score	Query cover	E value	Ident	Accession
Homalomena asperifolia voucher Kelsuke Hase Ar4761 (SAR) tRNA-Lys (trnK) gene, partial sequence; and maturase K (matK) gene, complete cds; chloroplast	1417	1417	100%	0.0	100%	KM580706.1
Homalomena sp. Kelsuke Hase Ar4759 voucher Kelsuke Hase Ar4759 (SAR) tRNA-Lys (trnK) gene, partial sequence; and maturase K (matK) gene, complete cds; chloroplast	1417	1417	100%	0.0	100%	KM580705.1
Homalomena sp. Baharuddin Ar2597 voucher P.C.Boyce et al. Ar3047 (SAR) tRNA-Lys (trnK) gene, partial sequence; and maturase K (matK) gene, complete cds; chloroplast	1417	1417	100%	0.0	100%	KM580692.1
Homalomena asmae voucher Baharuddin Ar2597 (SAR) tRNA-Lys (trnK) gene, partial sequence; and maturase K (matK) gene, complete cds; chloroplast	1417	1417	100%	0.0	100%	KM580691.1
Homalomena asmae voucher AR2597 tRNA-Lys (trnK) gene, partial sequence; and maturase K (matK) gene, partial cds; chloroplast	1417	1417	100%	0.0	100%	JX024970.1
Homalomena sp. Melaka tRNA-Lys (trnK) gene, partial sequence; and maturase K (matK) gene, partial cds; chloroplast	1417	1417	100%	0.0	100%	JX024968.1
Homalomena tonkinensis voucher P.C.Boyce et al. Ar4302 (SAR) tRNA-Lys (trnK) gene, partial sequence; and maturase K (matK) gene, complete cds; chloroplast	1411	1411	100%	0.0	99%	KM580707.1
Homalomena curvata voucher P.C.Boyce et al. Ar3052 (SAR) tRNA-Lys (trnK) gene, partial sequence; and maturase K (matK) gene, complete cds; chloroplast	1411	1411	100%	0.0	99%	KM580702.1
Homalomena atrox voucher P.C.Boyce et al. Ar2389 (SAR) tRNA-Lys (trnK) gene, partial sequence; and maturase K (matK) gene, complete cds; chloroplast	1411	1411	100%	0.0	99%	KM580701.1

Figure 3. Identification based on BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) of *mat*K gene of *Codiaeum variegatum* cv. Gold star (SPG2) and royal (JM2).

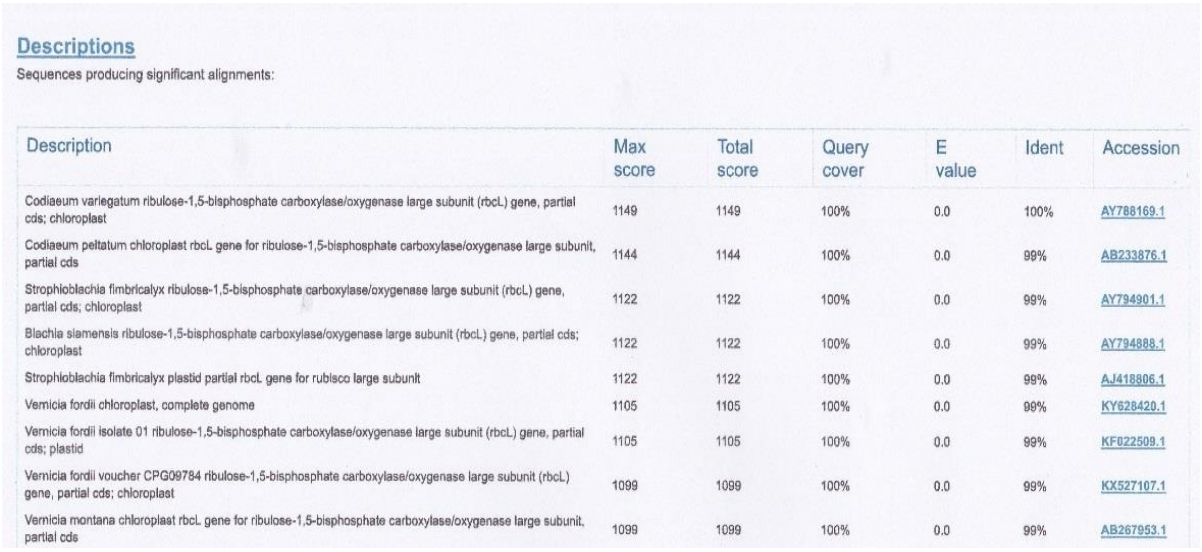


```
                1       10        20        30        40        50        60        70        80        90       100       110       120       130
                |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
SPG2_rbcL       TGCTTTATAAAGTGCTTCGGCACAAAACAAGAAACGGTCTCTCCAACGCATAAATGGTTGGGAGTTCACATTCTCATCATCTTTGGTAAAATCAAGTCCACCGCGAAGACATTCATAAACCGCCCTACCG
JM2_rbcL        TGCTTTATAAAGTGCTTCGGCACAAAACAAGAAACGGTCTCTCCAACGCATAAATGGTTGGGAGTTCACATTCTCATCATCTTTGGTAAAATCAAGTCCACCGCGAAGACATTCATAAACCGCCCTACCG
Consensus       TGCTTTATAAAGTGCTTCGGCACAAAACAAGAAACGGTCTCTCCAACGCATAAATGGTTGGGAGTTCACATTCTCATCATCTTTGGTAAAATCAAGTCCACCGCGAAGACATTCATAAACCGCCCTACCG

                131     140       150       160       170       180       190       200       210       220       230       240       250       260
                |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
SPG2_rbcL       TAATTCTTAGCGGATAGCCCCAATTTAGGTTTAATAGTACAACCCAATAGGGGGCGACCATACTTGTTCAATTTATCTCTCTCAACTTGGATTCCATGAGGCGGCCCTTGGAAAGTTTTAATATAAGCAG
JM2_rbcL        TAATTCTTAGCGGATAGCCCCAATTTAGGTTTAATAGTACAACCCAATAGGGGGCGACCATACTTGTTCAATTTATCTCTCTCAACTTGGATTCCATGAGGCGGCCCTTGGAAAGTTTTAATATAAGCAG
Consensus       TAATTCTTAGCGGATAGCCCCAATTTAGGTTTAATAGTACAACCCAATAGGGGGCGACCATACTTGTTCAATTTATCTCTCTCAACTTGGATTCCATGAGGCGGCCCTTGGAAAGTTTTAATATAAGCAG

                261     270       280       290       300       310       320       330       340       350       360       370       380       390
                |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
SPG2_rbcL       TAGGGATTCGCAAATCCTCCAGACGTAGGGCGCGTAGGGCTTTGAACCCAAATACATTACCCACAATGGAAGTAAACATGTTAGTAACAGAACCTTCTTCAAAAAGGTCTAAGGGGTAAGCTACATAAGC
JM2_rbcL        TAGGGATTCGCAAATCCTCCAGACGTAGGGCGCGTAGGGCTTTGAACCCAAATACATTACCCACAATGGAAGTAAACATGTTAGTAACAGAACCTTCTTCAAAAAGGTCTAAGGGGTAAGCTACATAAGC
Consensus       TAGGGATTCGCAAATCCTCCAGACGTAGGGCGCGTAGGGCTTTGAACCCAAATACATTACCCACAATGGAAGTAAACATGTTAGTAACAGAACCTTCTTCAAAAAGGTCTAAGGGGTAAGCTACATAAGC

                391     400       410       420       430       440       450       460       470       480       490       500       510       520
                |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
SPG2_rbcL       AATATATTGATTTTCTTCTCCAGCAACGGGCTCGATGCCGTAGCAGCGTCCTTTATAACGATCAAGACTGGTAAGCCCGTCGGTCCACACAGTTGTCCATGTACCAGTAGAAGATTCAGCAGCTACCGCA
JM2_rbcL        AATATATTGATTTTCTTCTCCAGCAACGGGCTCGATGCCGTAGCAGCGTCCTTTATAACGATCAAGACTGGTAAGCCCGTCGGTCCACACAGTTGTCCATGTACCAGTAGAAGATTCAGCAGCTACCGCA
Consensus       AATATATTGATTTTCTTCTCCAGCAACGGGCTCGATGCCGTAGCAGCGTCCTTTATAACGATCAAGACTGGTAAGCCCGTCGGTCCACACAGTTGTCCATGTACCAGTAGAAGATTCAGCAGCTACCGCA

                521     530       540       550       560       570       580       590       600       610       62822
                |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+-|
SPG2_rbcL       GCTCCTGCTTCCTCAGGCGGAACTCCGGGTTGAGGAGTTACTCGGAATGCTGCCAAGATATCAGTATCTTTGGTTTGATAGTCAGGAGTATAATAAGTCAAT
JM2_rbcL        GCTCCTGCTTCCTCAGGCGGAACTCCGGGTTGAGGAGTTACTCGGAATGCTGCCAAGATATCAGTATCTTTGGTTTGATAGTCAGGAGTATAATAAGTCAAT
Consensus       GCTCCTGCTTCCTCAGGCGGAACTCCGGGTTGAGGAGTTACTCGGAATGCTGCCAAGATATCAGTATCTTTGGTTTGATAGTCAGGAGTATAATAAGTCAAT
```

Figure 4. Sequence alignment of *rbc*L gene of *Codiaeum variegatum* cv. Gold star (SPG2) and royal (JM2) (http://multalin.toulouse.inra.fr/multalin).

Match Rank	Phylum	Class	Order	Family	Genus	Species	Subspecies	Score	Similarity	E-Value	Status
1	Magnoliophyta	Magnoliopsida	Malpighiales	Euphorbiaceae	*Codiaeum*	*variegatum*		622	100	0	Published
2	Magnoliophyta	Magnoliopsida	Malpighiales	Euphorbiaceae	*Codiaeum*	*peltatum*		620	99.84	0	Published
3	Magnoliophyta	Magnoliopsida	Malpighiales	Euphorbiaceae	*Strophioblachia*	*fimbricalyx*		612	99.2	0	Published
4	Magnoliophyta	Magnoliopsida	Malpighiales	Euphorbiaceae	*Blachia*	*siamensis*		612	99.2	0	Published
5	Magnoliophyta	Magnoliopsida	Malpighiales	Euphorbiaceae	*Strophioblachia*	*fimbricalyx*		612	99.2	0	Published
6	Magnoliophyta	Magnoliopsida	Malpighiales	Euphorbiaceae	*Vernicia*	*montana*		604	98.55	0	Published
7	Magnoliophyta	Magnoliopsida	Malpighiales	Euphorbiaceae	*Hylandia*	*dockrillii*		604	98.55	0	Published
8	Magnoliophyta	Magnoliopsida	Malpighiales	Euphorbiaceae	*Ostodes*	*paniculata*		604	98.55	0	Published
9	Magnoliophyta	Magnoliopsida	Malpighiales	Euphorbiaceae	*Ostodes*	*paniculata*		604	98.55	0	Published
10	Magnoliophyta	Magnoliopsida	Malpighiales	Euphorbiaceae	*Vernicia*	*montana*		604	98.55	0	Published

Figure 5. Identification based on BOLD System of *rbc*L gene of *Codiaeum variegatum* cv. Gold star (SPG2) and royal (JM2).



Descriptions

Sequences producing significant alignments:

Description	Max score	Total score	Query cover	E value	Ident	Accession
Codiaeum variegatum ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	1149	1149	100%	0.0	100%	AY788169.1
Codiaeum peltatum chloroplast rbcL gene for ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, partial cds	1144	1144	100%	0.0	99%	AB233876.1
Strophioblachia fimbricalyx ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	1122	1122	100%	0.0	99%	AY794901.1
Blachia siamensis ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	1122	1122	100%	0.0	99%	AY794888.1
Strophioblachia fimbricalyx plastid partial rbcL gene for rubisco large subunit	1122	1122	100%	0.0	99%	AJ418806.1
Vernicia fordii chloroplast, complete genome	1105	1105	100%	0.0	99%	KY628420.1
Vernicia fordii isolate 01 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; plastid	1105	1105	100%	0.0	99%	KF022509.1
Vernicia fordii voucher CPG09784 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	1099	1099	100%	0.0	99%	KX527107.1
Vernicia montana chloroplast rbcL gene for ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, partial cds	1099	1099	100%	0.0	99%	AB267953.1

Figure 6. Identification based on BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) of *rbc*L gene of *Codiaeum variegatum* cv. Gold star (SPG2) and royal (JM2).

Sequence alignment using *rbc*L gene showed that both varieties were 100% identical (Figure 4). Based on the identification of *rbc*L barcode using BOLD System (Figure 5) and BLAST, these two croton varieties had 100% similarities with *Codiaeum variegatum* (Figure 6).

The results of this study indicated that *rbc*L gene was more potential than *mat*K gene as DNA barcode for identifying *Codiaeum variegatum* because these two croton varieties had 100% similarities with *Codiaeum variegatum* based on the identification of *rbc*L barcode using BOLD System and BLAST. It was reported that ITS (internal transcribed spacer) and ITS 2 (internal transcribed spacer 2) were more potential than *rbc*L and *mat*K as barcodes for identifying the Euphorbiaceae species. At the interspecific level, the highest divergence was provided by ITS 2 followed by ITS. The marker *rbc*L indicated the intraspecific divergence and there were no significant differences of divergences among *mat*K, ITS and ITS 2 (Pang et al., 2010). In addition, the combination of ITS+*mat*K was recommended as the optimal DNA barcode for large flowering plant genera based on their

evaluation of DNA barcodes in Dendrobium (Orchidaceae) from Mainland Asia (Xu et al., 2015) as well as in Codonopsis (Campanulaceae) and in some large angiosperm plant genera (Wang et al., 2017). Mishra et al., (2017) also showed that multilocus regions had a higher distriminatory power than single barcodes, and the combination of matK+ITS showed the highest resolution rate (94.44%). Furthermore, the most efficient DNA barcode for identifying species and genera was ITS2 in Apocynaceae (Selvaraj et al., 2015) and ITS in Lauraceae from China (Liu et al., 2017). Tallei et al., (2016b) suggested that DNA barcodes must produce a significant barcoding gap between interspecific divergence and intraspecific distance. Elansary et al., (2017) reported that although the core DNA barcodes cannot always discriminate species, but at least it is potential to control the market place of horticultural crops and protect copyrights of new species and cultivars.

The size and completeness of barcode databases affected the success rate of DNA barcode to differentiate closely related species such as reported in the identification of African rainforest tress (Parmentier et al. 2017). Several combinations of two or three barcodes have been proposed as core barcodes to increase the species identification success rate (Xu et al. 2015). It was generally considered that combining DNA barcodes could improve species identification. For example, the discrimination rates of the combinations varied from 10.6% to 32.6% with *rbc*L+*mat*K < *rbc*L+*mat*K+*trn*H-*psb*A < *rbc*L+*mat*K+*trn*H-*psb*A+ITS2 < *rbc*L+*mat*K+*trn*H-*psb*A+ITS at the species level in Lauraceae from China. The rate of sequence recovery as well as the discrimination power of DNA barcodes should be considered to use them as markers (Liu et al. 2017). It will be valuable to evaluate ITS, ITS 2, *trn*H-*psb*A as single barcode or combination of these DNA barcode with rbcL and *mat*K to obtain higher identification success rate in some varieties of *Codiaeum variegatum*.

CONCLUSION

*rbc*L gene was more reliable to be used as DNA barcode for identification of *C. variegatum* than *mat*K gene.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

We are grateful to Dr. Agus Darwanto for his suggestions on drafts of this manuscript.

AUTHOR CONTRIBUTIONS

SAN collected the plant samples and also wrote the manuscript. BJK carried out the laboratory work and TET analyzed the data and also reviewed the manuscript. All authors read and approved the final version.

REFERENCES

CBOL, 2009. A DNA barcode for land plants. Proc. Natl Acad Sci 106 (31):12794-12797.

Corpet F, 1988. Multiple sequence alignment with hierarchical clustering. Nucleate Acids Res 16 (22):10881-10890.

Deng M, Chen J, Henny RJ, Li Q, 2010. Genetic relationship of *Codiaeum variegatum* cultivars analyzed by amplified fragment length polymorphism markers. Hortsience 45 (6):868-874.

Elansary HO, Ashfaq M, Ali HM, Yessoufou K, 2017. The first initiative of DNA barcoding of ornamental plants from Egypt and potential applications in horticulture industry. PloS ONE 12(2):e0172170.

Hebert PDN, Cywinska NA, Ball SL, de Waard JR, 2003. Biological identifications through DNA barcodes. Proc Roy Soc B-Biol Sci 270: 313–321.

Julianti E, Pinaria A, Lengkong EF, Kolondam BJ, 2015. DNA Barcoding tanaman daluga (Cyrtosperma spp) dari Kepulauan Sangihe berdasarkan gen *mat*K. Jurnal Bioslogos 5 (2):46-54.

Kadkhodaei S, Elahy M, Nekouei MK, Imani A, Shahnazari M, Mardi M, Javanmard A, Ariff AB, 2010. A panel of cultivate specific marker based on polymorphisms at microsatellite markers for Iranian cultivated Almonds (*Prunus dulcis*). Aust J Crop Sci

4:730-736.

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Mentjies P, Drummond A, 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28(12):1647-1649.

Kolondam BJ, 2015. Applying matK gene for identification of Liliopsida plant species from North Sulawesi through Bold System. Int J Appl Biol and Pharm Technol 6 (2):242-245.

Kress WJ, Erickson DL, 2007. A two-locus global DNA barcode for land plants: the coding *rbc*L gene complements the non-coding trnH-psbA spacer region. PLoSONE 2 (6): e508.

Kuzwina ML, Johnson KL, Barron HR, Herbert PDN, 2012. Identification of vascular plants of Churchill, Manitoba, using a DNA barcode library. BMC Ecology 12:1-11.

Liu ZF, Ci XQ, Li L, Li HW, Conran JG, Li J, 2017. DNA barcoding evaluation and implications for phylogenetic relationships in Lauraceae from China. PLoS ONE 12(4):e0175788.

Mishra P, Kumar A, Nagireddy A, Shukla AK, Sundaresan V, 2017. Evaluation of single and multilocus DNA barcodes towards species delineation in complex tree genus Terminalia. PLoS One 12(8):e0182836.

Ogunwenmo KO, Idowu OA, Innocent C, Esan EB, Oyelana OA, 2007. Cultivars of *Codiaeum variegatum* (L.) Blume (Euphorbiaceae) show variability in phytochemical and cytological characteristics. African J Biotech 6 (20):2400-2405.

Pang X, Song J, Zhu Y, Xie C, Chen S, 2010. Using DNA barcoding to identify species within Euphorbiaceae. Planta Med 76:1784-1786.

Papafotiou M, Avajianneli B, Michos Costas. 2007. Coloration, anthocyanin concentration, and growth of croton (*Codiaeum variegatum* L.) as affected by cotton gin trash compost use in the potting medium. Hortscience 42 (1):83-87.

Parmentier I, Duminil J, Kuzmina M, Philippe M, Thomas DW, Kenfack D, Chuyong GB, Cruaud C, Hardy OJ. 2013. How effective are DNA barcodes in the identification of African rainforest trees? PloS One 8(4):e54921. Doi:10.1371/journal.pone.0054921.

Ratnasingham S, Hebert PDN, 2007. BOLD: The barcode of life data system. Molecular Ecology Notes 7: 355-364.

Selvaraj D, Sarma RK, Shanmughanandhan D, Srinivasan R, Ramalingam S, 2015. Evaluation of DNA barcode candidates for the discrimination of the large plant family Apocynaceae. Plant Systematics and Evolution 301 (4):1263-1273.

Tallei TE, Kolondam BJ, 2015. DNA barcoding of Sangihe nutmeg (*Myristica fragrans*)

using matK gene. Hayati J Biosci 22 (1):41-47. DOI: 10.4308/hjb.22.1.41.

Tallei TE, Rembet RE, Pelealu JJ, Kolondam BJ, 2016a. Sequence variation and phylogenetic analysis of *Sansevieria trifasciata* (Asparagaceae). Biosci Res 13(1): 01-07.

Tallei TE, Irawan PD, Kolondam BJ, 2016b. DNA Barcoding analysis of *mat*K gene of some Syzygium species. In: Bioinformatics Workshop 2016: Developing knowledge and skill in bioinformatics for Young Indonesian Scientists in improving research quality in life science and sustainable exploration of biodiversity in Indonesia, 13 – 15 September 2016, Al Azhar University Jakarta. DOI: 10.13140/RG.2.2.10710.24641.

Wang DY, Wang Q, Wang YL, Xiang XG, Huang LQ, Jin XH, 2017. Evaluation of DNA barcodes in Codonopsis (Campanulaceae) and in some large angiosperm plant genera. PLoS ONE 12 (2): e0170286.doi:10.1371/journal.

Xu S, Li D, Li J, Xiang X, Jin W, Huang W, Jin X, Huang L, 2015. Evaluation of the DNA barcodes in Dendrobium (Orchidaceae) from Mainland Asia. PLoS ONE 10 (1): e0115168.doi:10.1371/journal.