ANALYSIS OF SEVERAL DNA BARCODES FOR IDENTIFICATION OF *Desmodium styracifolium*

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SUMMARY

DNA barcoding is a short DNA fragment, is used to distinguish between species, especially morphologically similar species and processed specimens that are difficult to distinguish species. For plants, the regions of DNA used as barcodes in identification or taxonomy are usually sequences of the chloroplast genome and the nuclear genome, including the coding regions and the non-coding regions. In this study, three DNA barcode sequences, *mat*K, *rcb*L, and *ITS* were used to evaluate the ability to distinguish *Desmodium styracifolium* species. The results of analysis and comparison of DNA barcode sequences in *D. styracifolium* with species of the same genus *Desmodium* showed that *ITS* had the highest similarity with *Desmodium styracifolium* species in Genbank (100%), followed by *mat*K with 99.53%, and *rbc*L with 98.60%. The tree diagrams showing the phylogenetic relationship show that the studied samples are most similar to *Desmodium styracifolium*. Thereby, it shows that the use of DNA barcode sequences for species identification is very effective, in which the use of several DNA barcode sequences together will increase the reliability of the assessment results. The *mat*K, *rcb*L, and *ITS* sequences were deposited in Genebank with accession numbers MW789613, MW789614, and MW315931, respectively, to contribute to the construction of the barcode DNA database for plant species.

Keywords: *Desmodium styracifolium*, DNA barcode, *ITS*, *mat*K, *rbc*L.

1. INTRODUCTION

Desmodium styracifolium is a valuable medicinal plant belonging to the family Fabaceae. The genus *Desmodium* includes about 350 species, most of which are used as cereals and herbs. *D. styracifolium* is a herbaceous plant, crawling, cylindrical, yellowish-green, and covered with fine hair. The needle leaves are staggered, single or double with odd feathers, including 1 - 3 leaflets, the middle leaflet is larger than the two side leaves. Flowers Flowers are hermaphrodite and are gathered in clusters (Figure 1). This species is suitable for hot, humid, or cool temperature conditions, and less acidic soil, they can also tolerate poor soil and drought. The plant is usually harvested in the summer and autumn, and can be used fresh or dried (Vo, 1997; Do, 1999). All parts of *D. styracifolium* such as roots, stems, and leaves can be used. Modern pharmacological studies show that *D. styracifolium* has diuretic, choleretic, antibiotic, anti-inflammatory, vasodilating, lowering blood pressure, increasing bile secretion, reducing bile duct pain, increasing kidney blood flow, increase blood circulation to the brain and femoral arteries... But the main use of *D. styracifolium* is bile, urinary catheter, often used to treat kidney stones, gallstones, bladder stones, urinary tract stones, hepatitis jaundice, nephritis edema, hemorrhoids, cholecystitis (Do, 1999). In addition, *D. styracifolium* also has effects on the cardiovascular system, blood pressure, inhibits the formation of kidney stones (Doan, 2013). At present, most of the medicinal herbs are imported from abroad and the origin of *D. styracifolium* is not clear. Therefore, it is necessary to have an effective method to accurately identify this species to ensure a source of good quality medicinal herbs.

The DNA barcode method has become an effective tool for scientists in classifying and evaluating genetic diversity and genetic relationships of both animals and plants. This technique is based on a short DNA fragment (about 400 - 800 bp) as the standard for quickly and accurately identification of species. Each DNA barcode has its own distinctive characteristics and is capable of distinguishing organisms at different taxon: family, genus, species, or subspecies. The DNA barcodes in the plants can be those located in the nuclear genome (18S, 5.8S, 26S, and *ITS*...); (Baharum, 2012; Chen, 2010; Schoch et al., 2012) and the chloroplast genome of *mat*K, *rbc*L, *trn*H – *psb*A, *rpo*, *trn*L-*trn*F, and *ycf* (Yu et al., 2011; Hollingsworth, 2011). However, there is no single DNA barcode that is used as a common

barcode for all taxons, instead, it is necessary to select specific DNA barcode and combine DNA barcodes to bring higher efficiency (Schoch et al., 2012). Depending on the object of examination, the DNA barcode fragments will be used appropriately (Chase et al., 2007; Hollingsworth, 2011). Medicinal plants of the genus *Phyllanthus* (*P. amarus*, *P. urinaria*, *P. niruri*, and *P. tenellus*), Brazilian folk medicinal plants for the treatment of kidney stones and other diseases, were identified by DNA barcode markers such as *ITS*, *ITS*2, *mat*K, *rbc*L, *psb*A-*trn*H, *trn*L, and *trn*L-*trn*F. The results show that the markers *ITS*, *ITS*2, *mat*K, *psb*A-*trn*H, *trn*L and *trn*L-*trn*F are capable of identifying all 4 species, in which *ITS* and *mat*K have the best identification efficiency. The *rbc*L marker showed that the ability to identify all 4 species of the genus *Phyllanthus* was not good (Inglis et al., 2018).

Figure 1. Morphology of *Desmodium styracifolium*

2. RESEARCH METHODOLOGY

2.1. Sample selection and collection

D. styracifolium leaves were selected from 03 plants at the Medicine Research and Production Center of the Traditional Medicine Hospital of the Ministry of Public Security in Suoi Hai – Ba Vi - Hanoi. 01 sample of *D. styracifolium* tissue cultured at the Laboratory of Plant Cell Technology – College of Forest Biotechnology, Vietnam National University of Forestry. Requirements for leaf samples and restoration: cut the intact leaves, green and free from pests and diseases. After that, the leaf samples were stored in a plastic bag containing silica gel desiccant, transported to the laboratory, and carried out DNA extraction within the same day.

Sample label: three samples of *D. styracifolium* collected in the wild were labeled as KTT1, KTT2, and KTT3, and one sample of *D. styracifolium in vitro* tissue culture was labeled as KTTi.

2.2. Genomic DNA extraction

Total genomic DNA was extracted using the CTAB (Cetyl trimethyl ammonium bromide) method of Saghai Maroof et al. (1984) with little modification. Approximately 100 mg of leaf tissue was ground with a mortar and pestle in 600 µl of CTAB buffer (2% CTAB, 20 mM EDTA, 1.4 M NaCl, 2% beta-mercaptoethanol, 100 mM Tris-HCl pH 8.0). The sample was transferred to a 1.5 ml centrifuge tube and incubated at 65^0C in a thermostatic bath for 30 min, then allowed to cool at room temperature and extracted with one volume of chloroform:isoamyl alcohol (v/v = 24:1). Samples were centrifuged at 10,000 rpm for 15 min at room temperature. The upper phase of the solution was transferred to a new 1.5 ml centrifuge tube. DNA was precipitated by adding 500 µl of cold isopropanol and centrifuging at 10,000 rpm at 4°C for 15 min. The precipitated DNA was then washed with 70% alcohol and then dried and dissolved in 100 µl distilled H_2O. DNA samples were stored at -20°C.

2.3. PCR and DNA sequencing

PCR reaction was performed on PCR machine GX100 (Biologix) with ingredients including 10 µl PCR master mix 2X, 10 µM each primer, 50 ng template DNA, the addition of distilled H_2O to 20µl. The temperature program of the PCR reaction was as follows: denaturation at 95°C for 5 min; 35 repetitions of three steps 95°C – 30s, 51°C - 60°C (depending on primer pair) – 30s, 72°C – 1 min; finish synthesis at 72°C for 5 min; PCR products were stored at 4°C. Primer sequences of three DNA barcoded primer pairs, and the annealing temperatures of the primers used in the study are shown in Table 1. PCR products were electrophoresed on 1% agarose gel with nucleic acid dye addition (Redsafe). After electrophoresis, the agarose gel was examined under UV light and photographed.

Table 1. Primer sequences

Target gene	Primer	Primer sequence (5'- 3')	T_m (°C)	Amplified fragment size (bp)	Reference
*mat*K	*mat*K_F	ACCCAGTCCATCTGGAAATCTGGTTC	52	850	Kress and Erickson, 2007
	*mat*K_R	CGTACAGTACTTTTGTGTTTACGAG			
*rbc*L	*rbc*L_F	ATGTCACCACAAACAGAGACTAAAGC	52	600	Kress and Erickson, 2007
	*rbc*L_R	GTAAAATCAAGTCCACCTCG			
ITS	*ITS*_F	ACGAATTCATGGTCCGGTGAAGTGTTCG	60	800	Wen and Zimmer, 1996
	*ITS*_R	TAGAATTCCCCGGTTCGCTCGCCGTTAC			

After successful amplification, PCR products were purified using the "DNA Purification Kit" of InTRON - Korea. After purification, the PCR products were sent to 1st Base laboratory in Malaysia for sequencing. The nucleotide sequence of the DNA fragment was determined in the forward and reverse directions on an automated Sanger sequencer using the BigDye® Terminator v3.1 Cycle Sequencing Kit.

2.4. Analysis of DNA barcode data

Nucleotide sequences were analyzed and processed using bioinformatics software such as BioEdit 7.2.5 (Hall, 1999) and NCBI tools (http://www.ncbi.nlm.nih.gov). The phylogenetic trees were built based on the Neighbor-Joining method.

3. RESULTS AND DISCUSSION

3.1. Genomic DNA extraction

DNA extraction is a very important initial step that determines the success of the PCR reaction later. To extract DNA with high efficiency and purity, it is necessary to choose an extraction method suitable for the studied object.

Total genomic DNA was extracted by the CTAB (Cetyl trimethyl ammonium bromide) method of Saghai Maroof et al. (1984) with little modification to suit the leaf samples of *D. styracifolium*. The result of agarose gel electrophoresis showed bright DNA bands, indicating that the total DNA extracted from 04 samples of *D. styracifolium* leaves was not broken and the DNA concentration was relatively high (04 KTT samples had concentrations from 189 ng/µl to 276 ng/µl). As shown in figure 2, the DNA bands in all 4 samples had same brightness, showing that the total DNA content extracted from the samples was relatively uniform, but the spectrophotometric results show a difference in concentration between the samples. The total DNA samples had relatively high purity (A260/A280 index ranged from 1.73 to 1.95) and showed that the DNA extraction method by CTAB was as described in Section 2.2 is effective for *D. styracifolium* leaf samples, avoiding the use of phenol as a toxic agent. On the other hand, the genomic DNA samples also

contained a small amount of RNA which was shown in the lower light bands on the agarose gel. This was because Rnase was not removed during DNA extraction, so this amount of RNA was still mixed with genomic DNA. However, this amount of RNA did not affect the specific cloning results. These four DNA samples were used as templates to perform PCR reactions in this study.

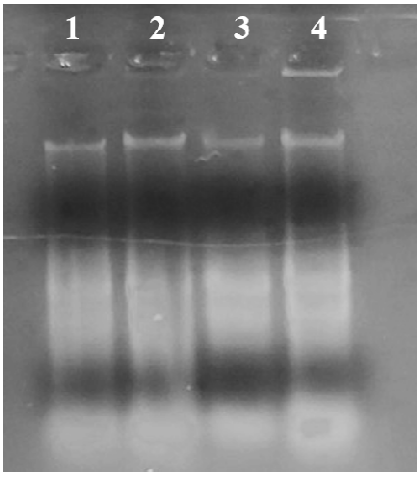

Figure 2. Genomic DNA isolated from *D. styracifolium* leaves
Lane 1: KTT1, 2: KTT2, 3: KTT3, 4: KTTi

3.2. Amplification of DNA barcode fragments by PCR technique

DNA barcode sequences have been widely used to identify species, including plants, animals, and microorganisms. Some DNA barcode sequences of *D. styracifolium* species have been published in GenBank, such as *mat*K, *trn*H-*psb*A, *rbc*L, *ITS*1, *ITS*2, *ITS* sequences.

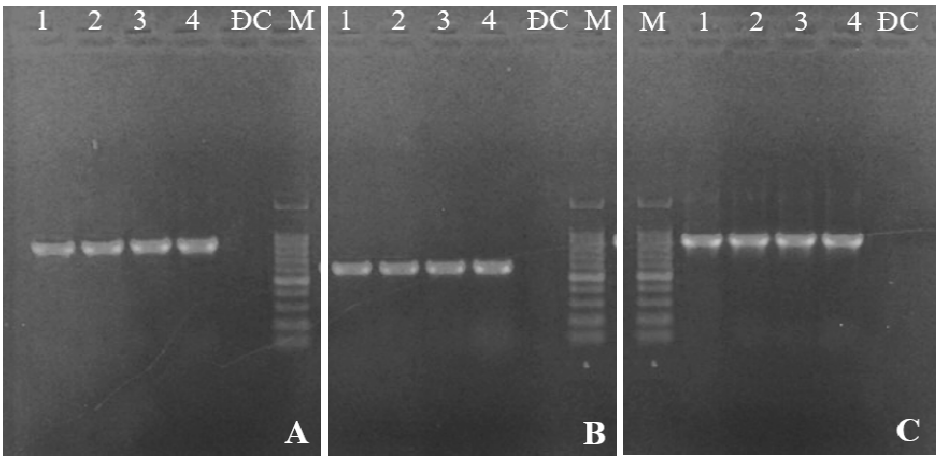

Figure 3. Amplification of DNA barcode sequences by PCR
*(PCR products of mat*K *(A); rbc*L *(B): Amplification of rbc*L *fragment; ITS (C; Lane 1-4: KTT1, KTT2, KTT3, and KTTi, respective; ĐC: no DNA template; M: DNA ladder 100 bp.)*

Each primer pair was used to amplify by PCR reaction on the basis of the products of total DNA. DNA barcode regions, including *mat*K, *rbc*L, and *ITS* were amplified with primer pairs: matK_F/R, rcbL_F/R and ITS_F/R, respectively. PCR products were checked by electrophoresis on 1.0% agarose gel.

*Mat*K is a sequence of genes located in the chloroplast that can be easily cloned in many plant species. The *mat*K gene sequence has been widely used to construct ecological relationships between closely related species or to identify plant species. In this study, the primer pair *mat*K_F and *mat*K_R were used to amplify the *mat*K region. The results of PCR

product testing on 1.0% agarose gel showed that the *mat*K gene segment was well amplified in all four samples of *D. styracifolium*. The obtained bands were bright with the same size >800 bp (compared with DNA ladder 100 bp), no extra band was present in all studied samples (Figure 3A). Therefore, it can be confirmed that the *mat*K gene segment has been successfully amplified in the species of *D. styracifolium* with a size of over 800 bp. The PCR product of the *mat*K gene fragment was purified and nucleotide sequenced. Compared to the *mat*K gene sequence amplified in *Camellia* species (*Camellia tamdaoensis*) in Tam Dao with 951 bp (Ha et al., 2015), the *mat*K gene fragment amplified in four samples of *D. styracifolium* had less number of nucleotides. Ha et al. (2015) used primer pair to amplify the *mat*K gene fragment with the forward primer sequence: 5'-TCCATGGG TTTATATGGATCCTTCCTGGTT-3' and reverse primer: 5'-CCCG CCATGGATG GAAGAATTCAAAAGATA-3', this primer pair sequence was different from the primer pair sequence used in this study. Thereby it can be seen that, although it is the same *mat*K gene, each study uses a pair of primers to amplify different sequences, thus producing different sized PCR products.

The *rbc*L gene is one of the genes encoding the elements of the photosynthetic system (rubisco), this gene is the first gene used to sequence in plants and is widely used in the identification of plant species. In the process of searching for some DNA barcodes of the *D. styracifolium* species, some sequences have been published in GenBank using *rbc*L gene sequences, so we had selected this sequence in the identification study of *D. styracifolium* species. Primer pairs *rbc*L_F and *rbc*L_R were used to amplify the *rbc*L gene fragment. The results of electrophoresis of the *rbc*L gene fragments on 1.0 % agarose gel showed that the *rbc*L gene fragment was well amplified in all four samples of *D. styracifolium*. DNA bands were obtained with the same size of about 600 bp (compared with DNA ladder 100 bp) (Figure 3B). From the obtained results, the *rbc*L gene segment was successfully amplified in *D. styracifolium* species. These PCR products were purified for nucleotide sequencing.

In the cell, rDNA is arranged as randomly repeat units consisting of DNA coding for 18S, 5.8S, and 28S ribosomes and interspersed with non-coding sequences *ITS*1 and *ITS*2 (internal transcribed spacers). They were located at two flanking regions of the 5.8S gene. The *ITS* region is both conservative and diverse enough to distinguish closely related species. The amplified results of the *ITS* gene fragment in four samples of *D. styracifolium* are shown in figure 3C. The *ITS* gene fragment was amplified successfully in all 04 *D. styracifolium* samples. The PCR products showed a clear bright DNA band with the size of about 850 bp. No extra DNA bands appeared. They were consistent with the theoretical size of the *ITS* gene fragment expected to be amplified. Similar results were also observed in the 2nd and 3rd PCR repeats. This result again confirmed that the PCR product cloning the *ITS* gene fragment was very specific and can be used directly for nucleotide sequencing. Le et al (2017) also used the primer pair ITS_F/R with the same sequence as the primer pair used in this study, but in some species of the *Ginseng* genus, the *ITS* gene fragment was found in the genus *Ginseng* is also 800bp in size. This result was similar to the amplified result of the *ITS* fragment for four *D. styracifolium* samples. This suggests that the *ITS* fragment size can be conservative in different plant species when the same primer pair is used for amplification. However, these results are only similar in size, and to confirm the diversity of nucleotide sequences, it is necessary to sequence the gene fragment and perform a comparative analysis.

3.3. Nucleotide sequence analysis of DNA barcode fragments

3.3.1. *Mat*K sequence

The sequence of *mat*K gene fragments amplified in 04 *D. styracifolium* samples had

the same size of 864 bp. All four samples had 100% *mat*K sequence similarity. Therefore, sample KTT2 was selected as a representative sample for all 04 samples of *D. styracifolium*. The nucleotide sequence of the *mat*K fragment in the *D. styracifolium* species was deposited in GenBank with accession number MW789613. From the 100% similarity in *mat*K gene sequences, it was found that the studied samples of *D. styracifolium* had no variation in nucleotide sequence. This result may be because the samples of *D. styracifolium* were taken from the same origin as Ba Vi, even the *in vitro* cultured *D. styracifolium* (KTTi) samples were also sampled from the Suoi Hai Drug Research and Production Center – Ba Vi.

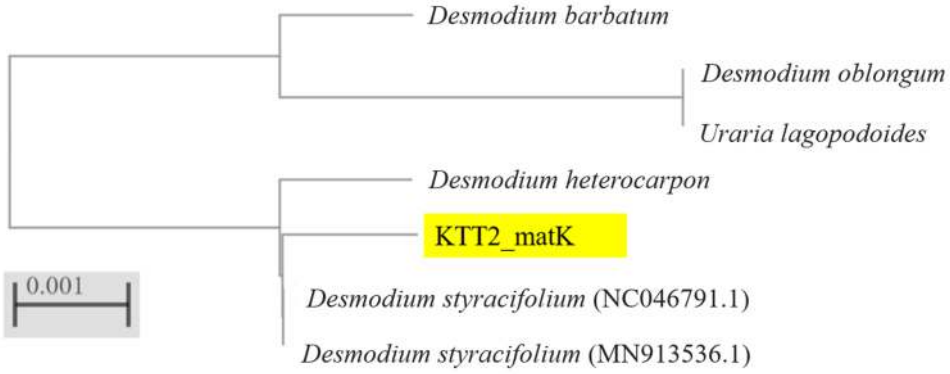

Figure 4. Phylogenetic tree of *D. styracifolium* and related species in GenBank based on *mat*K sequence

Using the *mat*K gene sequence of the samples to compare with the similar sequences in GenBank, the KKT2 sample was 99.53% similar to the species of *D. styracifolium* whose scientific name is *Desmodium styracifolium* (NC_046791.1, MN913536.1) and 98.47% to 99.18% similar to three species of the same genus *Desmodium* (MK933632.1, EU717420.1, LC080896.1), 98.47% similar to another species (*Uraria lagopodoides* (KF621107.1)) (Figure 4).

3.3.2. *Rbc*L sequence

The nucleotide sequences of the *rbc*L gene fragments from four *D. styracifolium* samples were obtained with the size of 575 bp. The nucleotide sequence the *rbc*L gene fragment in four *D. styracifolium* samples was deposited in GenBank with accession number MW789614. The nucleotide sequence of *rbc*L gene fragments was 100% similar to that of all four studied samples of *D. styracifolium*, and the KTT2 sample was selected as a representative sample for all four *D. styracifolium* samples. This result showed that the *D. styracifolium* samples have no *rbc*L gene sequence difference. This is a completely reliable result because the *rbc*L region is considered a fairly conservative region between species, so within a species, they are even more similar.

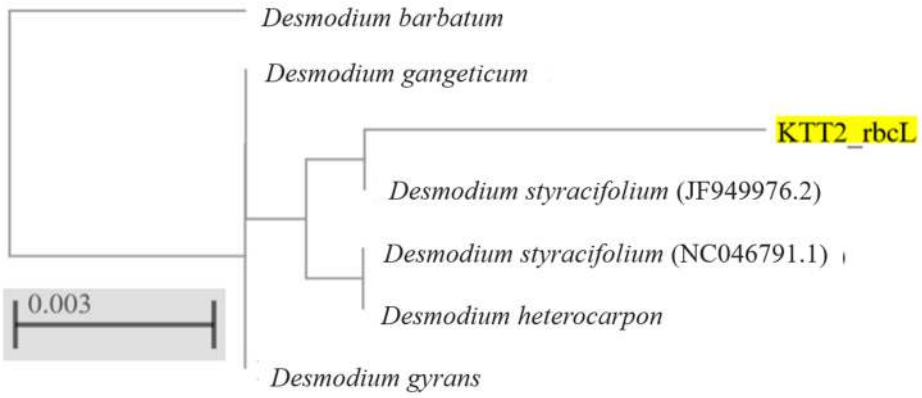

Figure 5. Phylogenetic tree of *D. styracifolium* and *Desmodium* species in GenBank based on *rbc*L sequence

Using the BLAST tool on NCBI to compare the *rbc*L gene sequence of the KTT2 sample with the species in GenBank, the KTT2 sample was 98.60% similar to *Desmodium styracifolium* and several *Desmodium* species. Six species with the highest sequence similarity of *rbc*L gene fragment with KTT2 sample were selected to build a phylogenetic tree (Figure 5). Results of analysis of genetic relationship tree between KTT2 sample and six *Desmodium* species showed that KTT2 sample was most closely related to *Desmodium styracifolium* in GenBank. Although the similarity between the *rbc*L fragment sequence of KTT2 sample with species was not 100%, it also showed a very close genetic relationship (a very high degree of similarity among species of the *Desmodium* genus). This result can confirm that the studied species has the scientific name of *Desmodium styracifolium* when using the DNA barcode marker as the *rbc*L sequence. Morphological examination of the *D. styracifolium* samples also initially confirmed that it was the species *Desmodium styracifolium*. However, the DNA barcode method could once again verify the identification results by this morphology. The use of molecular markers to identify species in general and plants, in particular, has proven to be very reliable and when combined with traditional identification methods, the accuracy can reach 100%.

3.3.3. *ITS* sequence

The *ITS* sequence fragment amplified for four studied *D. styracifolium* samples was 852 bp in size. Due to the 100% similarity of *ITS* fragments between 04 *D. styracifolium* samples, so *ITS* fragment sequence of the KTT2 sample was used as a representative sample and the nucleotide sequence of *ITS* fragment was deposited on GenBank with accession number MW315931. Using the BLAST tool on NCBI to compare the *ITS* gene sequence of the KTT2 sample with sequences in GenBank, the KTT2 sample showed the highest similarity (100%) with the species whose scientific name is *Desmodium styracifolium*.

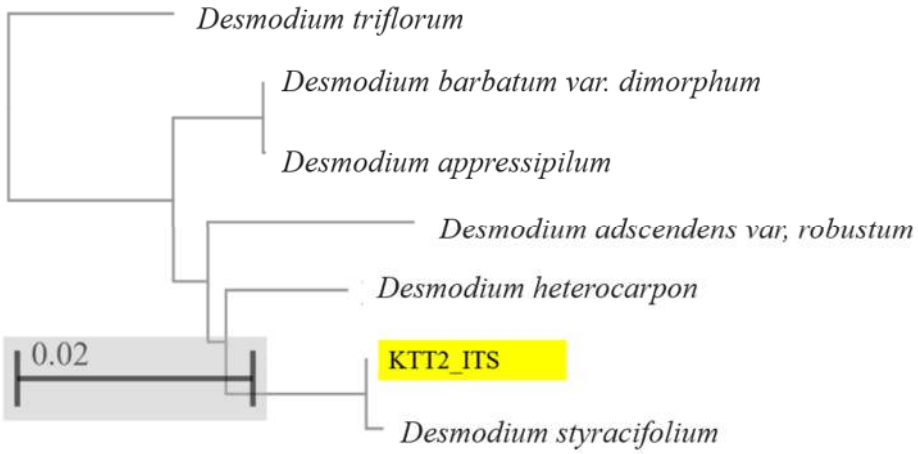

Figure 6. Phylogenetic tree of *D. styracifolium* and *Desmodium* species in GenBank based on *ITS* sequence

This result showed that the efficiency of identification of the *ITS* sequence was very high. In addition to the species *D. styracifolium*, the KTT2 sample was also similar to five other species of the same genus *Desmodium*. The phylogenetic tree of the KTT2 sample with 06 similar species on NCBI is shown in Figure 6. The analyzed result of the phylogenetic tree based on the *ITS* sequence showed that the KTT2 sample was *Desmodium styracifolium* with the degree of 100% similarity. Compared with 05 other species belonging to the same genus *Desmodium*, the similarity with the KTT2 sample reached from 94.74% to 97.40%. This confirms that the studied species had the exact scientific name of *Desmodium styracifolium* when using the DNA barcode marker as the *ITS* sequence.

3.4. Comparison of identification efficiency of three DNA barcode sequences for *D. styracifolium* species

To compare the identification efficiency of *D. styracifolium* species based on the 03 DNA barcode sequences analyzed above, we use the parameter that was the similarity ratio of each sequence between the studied *D. styracifolium* species and the species *Desmodium styracifolium* in Genbank (NCBI). Summary of all three DNA barcode sequences including *mat*K, *rbc*L, and *ITS* showed that the similarity of studied species with *Desmodium styracifolium* species in GenBank was 99.53%, 98.6%, and 100, respectively. From the results of this similarity comparison, it can be seen that the identification efficiency of the three DNA barcodes is arranged in the following order: *ITS* > *mat*K > *rbc*L.

The results of the assessment of the identification efficiency of *D. styracifolium* species showed that the use of the ITS marker, which is a barcoded DNA sequence in the nuclear genome, gave the highest identification efficiency. This result also completely coincides with the study of author Le et al. (2016) on some species of the *Ginseng* genus. Inglis et al. (2018) also announced the best able to identify 4 species of *P. amarus*, *P. urinaria*, *P. niruri*, and *P. tenellus* belonging to *Phyllanthus* genus by the *ITS* and *mat*K sequences, while the sequences *rbc*L had the worst detection efficiency.

4. CONCLUSION

- Three DNA barcode sequences, namely *mat*K, *rbc*L, and *ITS* have been identified, serving the effective identification of D. *styracifolium* species. All three sequences *mat*K, *rbc*L, *ITS* were successfully deposited in GenBank with the following accession numbers: MW789613, MW789614, and MW315931, respectively.

- Evaluate the effectiveness of identification of D. *styracifolium* species based on three DNA barcode sequences in the following order: *ITS* > *mat*K > *rbc*L.

REFERENCES

1. Vo V. C. (1997). Dictionary of Vietnamese medicinal plants. *Medical Publisher*, Ho Chi Minh City.
2. Do T. L. (1999). Medicinal plants and herbs of Vietnam. *Medical Publisher*, Hanoi.
3. Doan T. N. (2013). Treatment of urolithiasis, gallstones, hepatitis, nephritis using *Desmodium styracifolium* (Osb.) Merr. Pioneers' newspaper, Ho Chi Minh City.
4. Phan K. L., Vu D. D., Nguyen G. S., Nguyen T. P. T., Le T. M. L., Le T. S. (2014). Genetic relationships of ginseng samples collected in Lai Chau based on genetic analysis of nucleotide sequences of *mat*K and *ITS*-rDNA regions. *Saigon Technology Journal*, 2: 327-337.
5. Ha V. H., Nguyen V. P. (2015). Identification of DNA barcodes for Tamdao yellow tea (*Camellia tamdaoensis*): An endemic plant species of Vietnam. *Journal of agriculture and rural development*, 5:123-130.
6. Le T. H., Nguyen N. L., Bui M. N., Ha H. H., Huynh T. T. H., Nong V. H., Ha V. H., Le T. T. H. (2017). The application of DNA barcodes support species identification of some Ginseng samples of the Ginseng genus (*Panax* L.). *Journal of Biotechnology*, 15 (1), 63-72.
7. Baharum S.N. (2012). Application of 16s rDNA DNA cytochrome b ribosomal markers in studies of lineage ADN fish populations structure of aquatic species. *Molecular biology reports*, 39 (5): 5225-5232.
8. Chase M.W., Cowan R.S., Hollingsworth P.M., Berg C.V.D., Madriñán S., Petersen G., Seberg O., Jørgsensen T., Cameron K.M., Carine M., Pedersen N., Hedderson T.A.J., Conrad F., Salazar G.A., Richardson J.E., Hollingsworth M.L., Barraclough T.G., Kelly L., Wilkinson M. (2007). A proposal for a st ADN ardised protocol to barcode all ADN plants. *Taxon* 56 (2): 295-299.
9. Chen S., Yao H., Han J., Liu C., Song J., Shi L., Zhu Y., Ma X., Gao T., Pang X., Luo K., Li Y., Li X., Jia X., Lin Y., Leon C. (2010). Validation of the ITS2 region as a novel DNA barcode for identifying medicinal plant species. *PloS One*, 5 (1): e8613.
10. Chiou S.J., Yen J.H., Fang C-L., Chen H.L., Lin T.Y. (2007). Authentication of medicinal herbs using PCR- amplified ITS2 with specefic primers. *Planta medica*, 73 (13): 1421 – 1426.
11. Doyle J.J., Doyle J.L. (1990). Isolation of plant DNA from fresh tissue. *Focus.* 12: 13-15.
12. Hall T.A. (1999). BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95-98.
13. Hollingsworth, M.L., Clark A. (2009). Selecting barcoding loci for plants: evaluation of seven cDNAidate loci with species-level sampling in three divergent groups of lDNA plants. *Molecular Ecology Resources*, 9 (2): 439-457.

14. Inglis W. P., Mata M. Lorena, Silva J. M., Vieira R., Alves B. N. R., Silva D., Azevedo V. (2018). DNA Barcoding for the Identification of *Phyllanthus* Taxa Used Medicinally in Brazil. *Planta Medica*. 84 (17). 10.1055/a-0644-2688.

15. Kress WJ., Erickson DL. (2007). A Two-Locus Global DNA Barcode for Land Plants: The Coding rbcL Gene Complements the Non-Coding trnHpsbA Spacer Region. *PLoS ONE* 2(6): e508.

16. Kumar S., Stecher G., Tamura K. (2015). MEGA7: Molecular Evolutionary Genetics Analysis version 7.0. *Molecular Biology and Evolution* 30: 2725-2729.

17. Saghai-Maroof M. A., Soliman K. M., Jorgensen R. A., Allard R. W. (1984). Ribosomal DNAsepacer-length polymorphism in barley: mendelian inheritance, chromosomal location, and population dynamics. *Proc Natl Acad Sci*, 81, pp 8014–8019.

18. Schoch, C.L., Seifert K.A. (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. *Proceedings of the National Academy of Sciences*, 109 (16): 6241- 6246.

19. Wen J., Zimmer E.A. (1996). Phylogeny and biogeography of *Panax L.* (the ginseng genus, Araliaceae): inferences from ITS sequences of nuclear ribosomal DNA. *Mol Phylogenet Evol* 6(2): 167-177

20. Yu, J., Xue J.H., Zhou S.L. (2011). New universal matK primers for DNA barcoding angiosperms. *Journal of Systematics DNA Evolution*, 49 (3): 176-181.

XÁC ĐỊNH MỘT SỐ TRÌNH TỰ ADN MÃ VẠCH PHỤC VỤ CÔNG TÁC ĐỊNH DANH LOÀI KIM TIỀN THẢO (*Desmodium styracifolium*)

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TÓM TẮT

ADN mã vạch là một phương pháp định loại phân tử thường được sử dụng để hỗ trợ định danh hay giám định các loài khó nhận dạng về hình thái hay các mẫu vật đã qua chế biến. Đối với thực vật, các vùng ADN được sử dụng làm mã vạch trong giám định hay phân loại thường là các trình tự thuộc hệ gen lục lạp và hệ gen nhân, bao gồm cả vùng mã hóa và vùng không mã hóa. Trong nghiên cứu này, 03 trình tự ADN mã vạch là *mat*K, *rcb*L, và *ITS* được sử dụng để đánh giá khả năng phân biệt loài Kim tiền thảo (*Desmodium styracifolium*). Kết quả phân tích, so sánh các trình tự ADN mã vạch ở loài Kim tiền thảo với các loài cùng thuộc chi *Desmodium* cho thấy *ITS* có mức độ tương đồng cao nhất với loài *Desmodium styracifolium* trên ngân hàng gen quốc tế (100%). Tiếp đến là trình tự *mat*K với 99,53%, *ycf*1b với 98,90%, và cuối cùng là *rbcL* với 98,60%. Các biểu đồ hình cây thể hiện mối quan hệ phát sinh loài cho thấy mẫu Kim tiền thảo nghiên cứu đề tương đồng cao nhất với loài *Desmodium styracifolium*. Qua đó cho thấy việc sử dụng các trình tự ADN mã vạch để giám định loài là rất hiệu quả, trong đó việc sử dụng kết hợp một số trình tự ADN mã vạch với nhau sẽ làm tăng độ tin cậy của kết quả giám định. Các trình tự *mat*K, *rcb*L, và *ITS* được đăng ký lên ngân hàng gen Quốc tế với các mã số lần lượt là MW789613, MW789614, và MW315931 nhằm góp phần xây dựng cơ sở dữ liệu ADN mã vạch cho các loài thực vật.

Từ khóa: ADN mã vạch, *Desmodium styracifolium*, *ITS*, Kim tiền thảo, *mat*K, *rbc*L.

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