GENETIC CHARACTER OF *Tacca chantrieri* André AND THEIR PHYLOGENETIC INFERENCE IN THE GENUS *Tacca* BASED ON *ITS-rDNA* SEQUENCES ANALYSIS

Vu Dinh Giap[1*], Pham Mai Phuong[2], Bui Thi Tuyet Xuan[3], Bui Van Thang[4], Trinh Thi Thuy Linh[4], Vu Kim Dung[4], Vu Dinh Duy[2*]

[1]*HaUI Institute of Technology, Hanoi University of Industry*

[2]*Vietnam - Russia Tropical Centre*

[3]*Institute of Ecology and Biological Resource, Vietnam Academy of Science and Technology*

[4]*Vietnam National University of Forestry*

SUMMARY

Tacca J.R. & G. Forst is the only genus in Taccaceae includes 17 species distributed on the world, of which have found 7 species in Vietnam. DNA barcoding is a method of rapid species identification and discovery using short, standardized genes or DNA regions. In this study, we have sequences nuclear gene region (*ITS-rDNA*) from 9 individuals of *Tacca chantrieri* André belonging to three natural populations (Song Hinh Nature Reserve, Phu Yen Province; Cuc Phuong National Park, Ninh Binh province and Ba Vi National Park, Ha Noi city) to investigate genetic character and their phylogenetic inference in the genus *Tacca*. DNA was extracted from the tissue of leaves. The nucleotide sequence of *ITS-rDNA* was determined to be 674bp. All samples collected at three different geographic locations for the *ITS-rDNA* gene regions showed that their length and nucleotide sequence similarity were 100%. The analysis indicated the mean base compositions were nucleotide T(U) (16.9%), C (29.5%), A (20.3%) and G (33.2%). The TA content was found to be low (37.2%), compared to 62.8% of the CG content. Phylogenetic analyses using maximum likelihood (ML) indicated that all samples from Vietnam have a close relationship with *Tacca chantrieri* in GenBank with strong supporting values (99%). Genetic p-distances interspecific divergence within and among *Tacca* species were varied from 0% to 19%, mean genetic distance 10%. The gene (*ITS-rDNA*) is used as a barcode in the identification of the *Tacca* species in Vietnam.

Keywords: DNA barcodes, ITS-rDNA gene, *Tacca chantrieri*, Phylogenetic tree.

1. INTRODUCTION

Tacca J.R. & G. Forst is the only genus in Taccaceae, a new family derived from Dioscoreaceae (Caddick *et al.,* 2002; Ding and Larsen, 2000; Stevens, 2001; Mabberley, 2017). According to the World Checklist of Monocotyledons (http://apps.kew.org/wcsp/home.do), the genus *Tacca* includes 17 species (*T. ampliplacenta* L. Zhang & Q.J.Li, *T. ankaranensis* Bard. Vauc. *T. bibracteata* Drenth, *T. borneensis* Ridl, *T. celebica* Koord, *T. chantrieri* André, *T. ebeltajae* Drenth, *T. integrifolia* Ker Gawl., *T. lanceolata* Spruce, *T. leontopetaloides* (L.) Kuntze, *T. maculata* Seem., T. *palmata* Blume, *T. palmatifida* Baker, *T. parkeri* Seem, *T. plantaginea* (Hance) Drenth, *T. reducta* P.C. Boyce & S. Julia và *T. subflabellata* P.P. Ling & C.T. Ting) of which in Vietnam have found 7 species: *T. chantrieri*, *T. integrifolia*, *T. palmata*, *T. leontopetaloides*, *T. plantaginea*, *T. subflabellata* and *T. khanhhoaensis* (Nguyen Tien Ban *et al.,* 2005; Nguyen Tap *et al.,* 2006; MOST and VAST, 2007; Vo Van Chi, 2012; Dang *et al.,* 2018).

Tacca chantrieri André (Taccaceae) is a widespread species in humid tropical regions of Southeast Asia (Zhang *et al.,* 2011). It's a herbal plant that grows in the humid tropical forests Vietnam (Nguyen Tap *et al.,* 2006). Its rhizomes have been used as folk medicine to treat gastric ulcers, enteritis and hepatitis (Vo Van Chi, 2012). Phytochemical investigations of this plant have resulted in the isolation of diarylheptanoids (Yokosuka *et al.,* 2002), steroidal saponins (Tinley *et al.,* 2003; Shwe *et al.,* 2010; Yokosuka *et al.,* 2004) and sterol saponins (Yokosuka *et al.,* 2005). In addition, these compounds show cytotoxic activities (Yokosuka *et al.,* 2002). *T. chantrieri* distribution is restricted by overexploitation, habitat destruction, and habitat fragmentation (Zhang *et al.,* 2011). Although morphological characteristics of *T. chantrieri* have been used to identify the species, these features are difficult to distinguish. On the other hand, the molecular characteristics of this species are still unknown in Vietnam.

**Correspondence authors: duydinhvu87@gmail.com; giapvudinh@gmail.com*

The use of DNA barcodes, which are short gene sequences taken from a standardized portion of the genome and used to identify species (Kress *et al.,* 2005). Short DNA barcode sequences can be used to identify patterns that aren't fully characterized by morphology (Vu Dinh Duy *et al.,* 2019, 2020, 2021). Genes coding for *45S* ribosomal mRNA are organized into tandem arrays of up to several thousand copies and contain *18S*, *5.8S*, and *26S rRNA* units separated by internal transcribed spacers *ITS1* and *ITS2*. While the rRNA units are evolutionary conserved, *ITS* shows a high level of interspecific divergence and has been frequently used in genetic diversity and phylogenetic studies of many plant groups (Hřibová *et al.,* 2011). Currently, different researchers have used some region genes such as *ITS, 18S, matK, psbA-trnH, rbcL, atpA, rbcL, trnL-F,* and *trnS-trnG* in buiding of DNA barcode for identification of *Tacca* species (Zhang *et al.,* 2006; Zhang *et al.,* 2011; Zhao and Zhang, 2015; Yeng and Shen, 2019). In this study, we sequenced nuclear genomic region nucleotides (*ITS-rDNA*) to identify genetic characteristics of *T. chantrieri* and their phylogenetic inference in the genus *Tacca*. This study contributes to the development of a DNA barcode database, as a foundation for conservation, evolution, and biological systems.

2. RESEARCH METHODOLOGY

2.1. Collection sampling

In this study, 9 samples (young leaves) of *T. chantrieri* were collected and placed in plastic bags with silica gel in the field, and transferred to the laboratory of Vietnam - Russia Tropical Centre, stored at -30^{o}C for DNA extraction (Table 1).

Table 1. Population descriptions of all sampled populations of *T. chantrieri* in Vietnam

Sample size	Pop. code	Locality	Latitude (N)	Longitude (E)	Altitude (m)	GenBank code
03	BV01-BV03	Ba Vi National Park, Ha Noi City	21^{o}36’48’’	105^{o}20’40’’	355	OL454071-OL454073
03	CP01-CP03	Cuc Phuong National Park, Ninh Binh province	20^{o}20’31’’	105^{o}35’51’’	356	OL454074-OL454076
03	SH01-SH03	Song Hinh, Phu Yen province	12°48'48"	109°00'33"	286	OL454077-OL454079

Figure 1. Adult plant of *T. chantrieri* species collected in Ba Vi National Park
(Photo: Dr. Vu Dinh Duy)

2.2. DNA isolation: Total genomic DNA was extracted using a plant/fungi DNA isolation kit (Norgenbiotek, Canada). The total DNA purity and integrity were tested by the Nanodrop ND-2000 spectrophotometer (NanoDrop Technologies, DE, USA) and then diluted to a concentration of 20ng/µl.

2.3. PCR amplification: The *ITS-rDNA* gene region was amplified through the following PCR cycling profile: an initial heating step at 94ºC for 3 min; followed by incubating for 40 cycles of 94ºC for 1 min, 55ºC for 1 min, respectively, and 72ºC for 1 min, and completed by incubating at 72ºC for 10 min. All PCR reactions were performed in 25 µl volumes using Gene Amp PCR Systems 9700. Double-stranded DNA was directly amplified by symmetric polymerase chain reaction (PCR) amplification using pairs of primers *ITS5* (5'-GGA AGT AAA AGT CGT AAC AAG G-3') and *ITS4* (5'-TCC TCC GCT TAT TGA TAT GC-3') (White *et al.,* 1990).

2.4. Sequencing of the ITS-rDNA region: Sequencing was performed on an Avant 3100 automated DNA sequencer using the Dye Terminator Cycle sequencing kit (PE Applied Biosystems). Sequencing of the 9 studied samples used the primers *ITS5* and *ITS4*.

2.5. Phylogenetic analysis: Chromas Pro 2.1.6 software (Technelysium Pty Ltd., Tewantin, Australia) was used to edit the sequences. Sequence alignments were made with Bioedit v7.0.5.2 (Hall, 1999). We used MEGA 7.0 (Kumar *et al.,* 2016) to analyse our data. Nucleotide sequence divergences were calculated using the Kimura two-parameter (K2P). Phylogenetic trees were performed using maximum likelihood (ML) on MEGA 7.0 software with 1000 replicates. Mega 7.0 was used to analyze p-distance between *Tacca* species.

3. RESULTS AND DISCUSSION

3.1. DNA Extraction and Polymerase Chain Reaction

In plants, secondary metabolites and polysaccharides interfere with genomic isolation procedures and downstream reactions such as restriction enzyme analysis and gene amplification (Amani *et al.,* 2011). DNA isolation represents the basic and probably the most important step in plant genetics and biotechnology. Despite the development of molecular protocols for DNA isolation of plant species, there are still many drawbacks depending on sample composition. To maximize DNA yields and minimize the co-extraction of PCR inhibitors, we used the Plant DNA Isolation Kit for DNA extraction from 9 leaf tissues of *T. chantrieri* in Vietnam. Results of DNA electrophoresis on 1% agarose gel showed that each sample had a single, sharp and bold bands indicating successful DNA extraction (Figure 2). The purity of extracted DNA was excellent, as evident DNA concentrations A260/A280 ratio ranging from 1.840 to 1.956, which also suggested that the preparations were sufficiently free of proteins and polyphenolic/polysaccharide compounds. The DNA concentration ranged from 700 to 920ng/µl. The extracted DNA was suitable for PCR amplification of plant barcode genes. For the PCR reaction, the DNA concentration was diluted to 20 ng/µl. The primer pair *ITS5*/ITS4 were successfully cloned for 09 samples at a primer temperature of 55ºC (Figure 3). The PCR product was approximately 700bp in length. Electrophoresis on a 1.5% agarose gel showed high quality PCR product, with only a single bright band that was qualified for nucleotide sequencing.

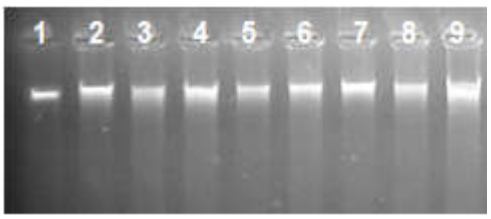

Figure 2. Electrophoresis of total DNA from 9 samples of *T. chantrieri* using 1% agarose gel

Table 2. The quantity and purity of extracted DNA from 9 leaf samples of *T. chantrieri*

No	Sample	Concentration (ng/µl)	$A260_{nm}$	$A280_{nm}$	$A260_{nm}/A280_{nm}$
1	BV01	700	0.070	0.038	1.842
2	BV02	890	0.089	0.049	1.816
3	BV03	860	0.086	0.046	1.869
4	CP01	840	0.084	0.045	1.866
5	CP02	900	0.090	0.046	1.956
6	CP03	920	0.092	0.050	1.840
7	SH01	900	0.090	0.048	1.875
8	SH02	890	0.089	0.046	1.935
9	SH03	860	0.086	0.047	1.830

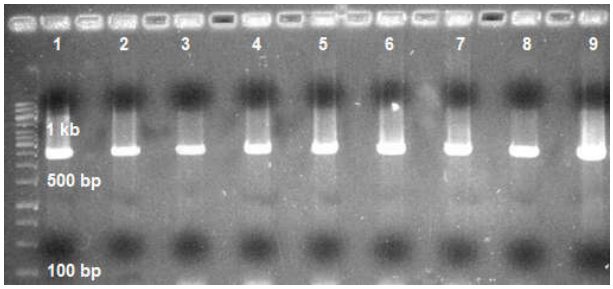

Figure 3. PCR products from 9 samples of *T. chantrieri* were electrophoresed on 1.5% agarose gel (M: DNA ladder 100bp; 1-9: No. samples)

3.2. Characteristic analysis of *ITS-rDNA* sequences

All the studied samples were successfully amplified for *ITS-rDNA* gene regions with a high sequencing rate of 100%. Chromaspro2.1.6 software was used to display the results and edit the sequences. After removing the two ends, we identified that the remaining size of each sample was 674 nucleotides. These sequences have been compared with similar sequences on GenBank using the BLAST tool. Results showed that the studied species was 100% similar to *T. chantrieri* (JN850567). The sequences for the *ITS-rDNA* gene region from *T. chantrieri* in Vietnam have been deposited in GenBank (Table 1). Moreover, the results of comparing nucleotide sequences with each other using Bioedit software between nine samples collected at three different locations in the *ITS–rDNA* gene region showed that their length and nucleotide sequence similarity were 100%. Therefore, the following study took only one representative sample of this species in Vietnam.

For this study, *ITS-rDNA* sequences of *T. chantrieri* consisted of 674 nucleotide positions. The mean base compositions were 16.9, 29.5, 20.3 and 33.2% for T (U), C, A and G, respectively. The TA content was low (37.2%), compared to 62.8% of the CG content. This difference showed a low TA content at all three codon positions. These values were 27.1%, 47.9% and 36.8% for the 1^{st}, 2^{nd} and 3^{rd} codons, respectively. The average R rate at the second position was 1.8 times, higher than the rate at the two remaining codon positions (Table 3).

Table 3. Nucleotide base compositions (%) for the *ITS-rDNA* sequences of *T. chantrieri*

	Codon position	Base				Length (bp)	R=Si/Sv
		T(U)	C	A	G		
Tacca chantrieri	All positions	16.9	29.5	20.3	33.2	674	1.5
	1st positions	16.1	28.1	21.0	34.4	224	1.8
	2nd positions	17.2	29.1	20.7	33.0	227	1.7
	3rd positions	17.5	30.9	19.3	32.3	223	1.1

Note: Transitionsal Pairs (Si); Transversional Pairs (Sv)

3.3. The genetic distance and phylogenetic inference in the genus *Tacca*

The genetic distances and the maximum likelihood (ML) tree were used to determine genetic relationships between samples and 14 species of the genus *Tacca* (Table 4 and Figure 4). The mean genetic distance was 10% ranging from *T. leontopetaloides*/*T. maculate* (0%) to *T. chantrieri/T. maculata* (19%). The species pairs: *T. chantrieri/T. maculata* (19%), *T. cristata*/*T. maculata* (18%) showed highest genetic distances, whereas lowest genetic distances were observed between species pairs: *BV*/*T. chantrieri* (0%), *T. leontopetaloides*/*T. maculate* (0%), *T. havilandii*/*T. cristata* (1%), *T. reducta*/*T. cristata* (2%). Our results showed the highest genetic distances among species in genus *Tacca*.

Table 4. Different genetic distance among species in genus *Tacca* based on *ITS-rDNA* analysis

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. BV	-														
2. *T. leontopetaloides* MK144476	0.19	-													
3. *T. havilandii* MK144500	0.07	0.18	-												
4. *T. cristata* MK144506	0.06	0.17	0.01	-											
5. *T. borneensis* MK144485	0.06	0.17	0.04	0.02	-										
6. *T. integrifolia* MK144478	0.04	0.18	0.06	0.05	0.05	-									
7. *T. maculata* MK144480	0.19	0.00	0.19	0.18	0.17	0.18	-								
8. *T. reducta* MK144487	0.06	0.18	0.03	0.02	0.03	0.06	0.18	-							
9. *T. ampliplacenta* JF978853	0.05	0.18	0.06	0.05	0.05	0.01	0.18	0.06	-						
10. *T. bibracteata* MK144508	0.12	0.19	0.13	0.12	0.12	0.11	0.20	0.13	0.12	-					
11. *T. parkeri* JN850573	0.13	0.22	0.16	0.15	0.15	0.13	0.22	0.15	0.13	0.15	-				
12. *T. palmata* MK144499	0.11	0.17	0.10	0.09	0.09	0.10	0.17	0.10	0.10	0.12	0.15	-			
13. *T. palmatifida* JN850572	0.10	0.16	0.10	0.09	0.09	0.08	0.16	0.09	0.08	0.09	0.13	0.06	-		
14. *T. subflabellata* JF978885	0.03	0.18	0.07	0.06	0.05	0.03	0.18	0.06	0.03	0.12	0.14	0.11	0.10	-	
15. *Tacca chantrieri* JN850567	0.00	0.19	0.07	0.06	0.06	0.05	0.19	0.07	0.05	0.12	0.13	0.11	0.10	0.03	-

The maximum likelihood tree of sequence divergences (K2P) in the *ITS-rDNA* region reflects the above findings. It shows that all 14 species in the genus *Tacca* were distinctly separated and characterized by a high bootstrap value and a branch length of 0.05 (Figure 4). The ML tree showed a clear separation of samples (BV) into one clade together with

Tacca chantrieri (JN850567) with a bootstrap value of 99%. BV/*T. chantrieri* had highly identical *ITS* sequences. The results showed that this species pair was determined as one species.

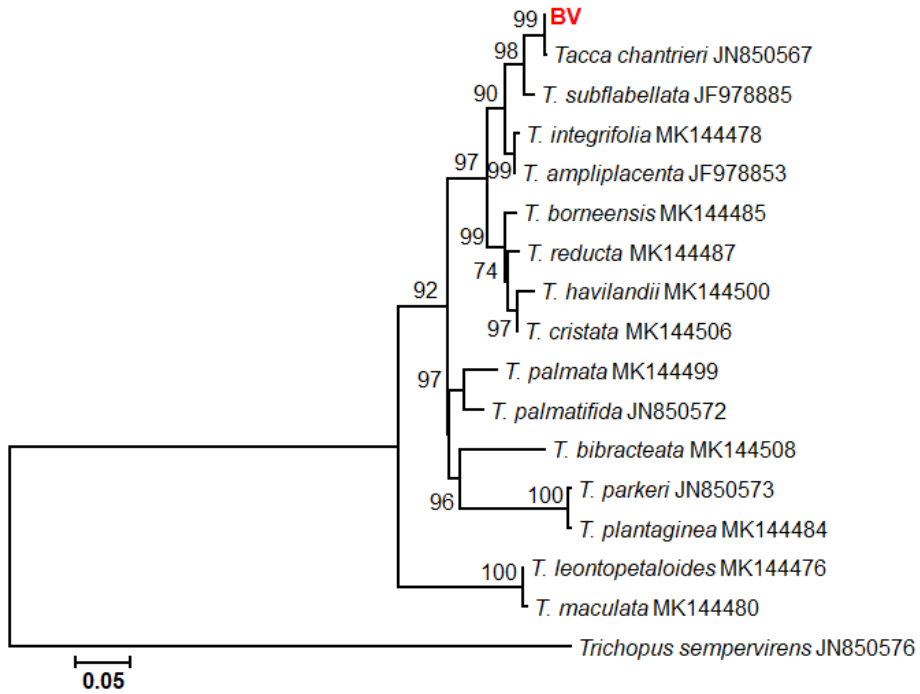

Figure 4. Phylogenetic relationships among *Tacca* species based on sequence of nuclear genes (*ITS-rDNA*) using Maximum likelihood (ML) tree. Numbers above branches represent bootstrap

Accurate species indentification is essential for the management and conservation of species (Trias-Blasi and Vorontsova, 2015). Species identification based on morphology is mostly laborious and less accurate. However, molecular-based methods such as DNA barcoding have been shown to be rapid and accurate for specific identification (Kress *et al.,* 2015; Tahir *et al.,* 2018; Kang *et al.,* 2021). A previous study by Zhao and Zhang (2011) recommended four candidate DNA barcoding regions, three (*rbcL, matK*, and *trnH-psbA*) from the chloroplast genome and one (*ITS*) from the nuclear genome, which were evaluated among 36 accessions representing 6 species of *Tacca*. The results indicated that both *ITS* and the core barcode *rbcL+matK* proposed by the Consortium for the Barcode of Life (CBOL) exhibited the highest resolution as single and combined data, respectively. Based on overall performance, *matK+rbcL* can be considered a potential barcode for identifying the species of *Tacca*, *ITS* can be used as a supplementary barcode. DNA barcoding revealed two distinct lineages of *T. integrifolia* distributed allopatrically in Tibet and Malaysia. And these two lineages with morphological variations may potentially represent new species. Zhang *et al.* (2011) used DNA sequences from one nuclear, one mitochondrial and three plastid loci (*ITS, atpA, rbcL, trnL-F,* and *trnH-psbA*) to reconstruct molecular phylogeny in the genus *Tacca*. Phylogenetic analysis of 16 *Tacca* species utilizing nuclear *ITS* and plastid *matK* gene areas (Yeng and Shen, 2019). Our results agree with the previous finding, confirm the core barcode's effectiveness, and suggest using *ITS-rDNA* gene region as DNA barcode sequences in the genus *Tacca* in Vietnam.

4. CONCLUSIONS

In the current study, we sequenced nuclear genomic region nucleotides (*ITS-rDNA*) to identify *T. chantrieri* in Vietnam, constructed phylogenetic trees of the genus *Tacca,* and suggest using *ITS-rDNA* gene region to identify *Tacca* species in Vietnam. The findings there will be significant in the study of

evolution, systematics, and conservation of the species.

REFERENCES

1. Amani J., Kazemi R., Abbasi AR., Salmanian AH. 2011. A simple and rapid leaf genomic DNA extraction method for polymerase chain reaction analysis. *Iranian Journal of Biotechnology* 9(1): 69-71.

2. Caddick LR., Rudall PJ., Wilkin P., Hedderson TAJ., Chase MW. 2002. Phylogenetics of Dioscoreales based on combined analyses of morphological and molecular data. *Bot J Linn Soc* 138: 123-144.

3. Chromas Pro1.2.1.6 (Technelysium Pty Ltd, Helensvale, Queensland, Australia).

4. Dang VS., Truong BV., Nguyen TPT., Hoang NS. 2018. *Tacca khanhhoaensis* V.S. Dang & Vuong (Taccaceae), a new species from southern Vietnam. *PhytoKeys* 114: 115–122.

5. Ding ZZ., Larsen K. 2000. Taccaceae. Flora China 24: 274-275.

6. Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/2007/NT. *Nucl Acids Symp Ser* 41: 95–98

7. Hřibová E., Čížková J., Christelová P., Taudien S., Langhe E., Doležel J. 2011. The ITS1-5.8S-ITS2 Sequence Region in the Musaceae: Structure, Diversity and Use in Molecular Phylogeny. *PLoS One* 6(3): e17863.

8. http://apps.kew.org/wcsp/home.do

9. Kang. 2021. Molecular identification of *Aquilaria* species with distribution records in China using DNA barcode technology. *Mitochondrial DNA B Resour* 6(4): 1525–1535.

10. Kress WJ., García-Robledo C., Uriarte M., Erickson DL. 2015. DNA barcodes for ecology, evolution, and conservation. *Trends in Ecology & Evolution* 30: 25–35.

11. Kress WJ., Wurdack KJ., Zimmer EA., Weigt LA., Janzen DH. 2005. Use of DNA barcodes to identify flowering plants. *Proceding of National Academy of Sciences* 102: 8369-8374.

12. Kumar S., Stecher G., Tamura K. 2016. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33: 1870-1874.

13. Mabberley D. 2017. Mabberley's Plant-book: A Portable Dictionary of Plants, their Classification and Uses. Cambridge University Press, Cambridge.

14. MOST., VAST. 2007. *Vietnam red data book, Part II. Plants*. Natural Science and Technology Publishing House.

15. Nguyen Tap, Ngo Van Trai, Nguyen Trieu, Do Huy Bich, Pham Thanh Huyen, Nguyen Quynh Nga, Minh Minh Khoi, Nguyen Duy Thuan, Bui Xuan Chuong, Nguyen Quang Hao, Nguyen Ba Hoat, Pham Duy Hung, Ngo Duc Phuong, Le Thanh Son, Hoang Van Toan, Phan Van Truong. 2006. *Checklist of medicinal plants in Vietnam*. Hanoi Science and Technology Publishing House.

16. Nguyen Tien Ban, Nguyen Khac Khoi, Vu Xuan Phuong. 2005. *List of plant species in Vietnam - Volume 3*. Agricultural publishing house.

17. Shwe HH., Aye M., Sein MM., Htay KT., Kreitmeier P., Gertsch J., Reiser O., Heilmann J. 2010. Cytotoxic steroidal saponins from the rhizomes of *Tacca integrifolia*. *Chemistry & Biodiversity* 7: 610-622.

18. Stevens PF. 2001 onwards. Angiosperm Phylogeny Website. http://www.mobot.org/MOBOT/research/APweb/

19. Tahir A., Hussain F., Ahmed N., Ghorbani A., Jamil A. 2018. Assessing universality of DNA barcoding in geographically isolated selected desert medicinal species of Fabaceae and Poaceae. *PeerJ* 6: e4499.

20. Tinley TL., Randall-Hlubek DA., Leal RM., Jackson EM., Cessac JW., Quada JC., Hemscheidt TK., Mooberry SL. 2003. Taccalonolides E and A: Plant-derived steroids with microtubule-stabilizing activity. *Cancer Research* 63: 3211-3220.

21. Trias-Blasi A., Vorontsova M. 2015. Plant identification is key to conservation. *Nature* 521: 161-161.

22. Vo Van Chi. 2012. *The Vietnamese Dictionary of Medicinal Plants.* Medical Publishing House, Hanoi, 485.

23. Vu Dinh Duy, Le Xuan Dac, Nguyen Dang Hoi, Pham Mai Phuong, Dang Ngoc Huyen, Tran Thi Thanh Huong, Bui Van Thanh, Nguyen Tam Thanh, Bui Van Thang, Luu Thi Phuong, Vu Thi Thu Hien, Nguyen Quoc Khanh. 2021. Application of DNA analysis approach contributes to the identification of several plant species in Truong Sa archipelago, Khanh Hoa province, Vietnam. Journal of Forestry Science and Technology 11: 3-10.

24. Vu Dinh Duy. 2019. Using ITS nuclear gene nucleotide sequences for identification species in *Dendrobium*. *Journal of Tropical Science and Technology* 18(7): 3-12.

25. Vu Dinh Duy, Tran Thi Viet Thanh, Phan Ke Loc, Nguyen Minh Tam, Nguyen Thi Thanh Huong, Nguyen Thi Hien, Phan Ke Long. 2020. Using ITS-rDNA and matK gene nucleotide sequences for identification ginseng species in *Panax* in Phu Xai Lai Leng, Ky Son, Nghe An. Journal of Biotechnology 18(1): 75-85.

26. White TJ., Bruns T., Lee S., Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols: a guide to methods and applications. (Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds). Academic Press, New York, USA, 315–322.

27. Yeng WS., Shen CK. 2019. Phylogeny of *Tacca* (Taccaceae) and traits in reproductive structures, with description of a new Bornean species. *Biodiversitas* 20 (11): 3096-3118.

28. Yokosuka A., Mimaki Y., Sakagami H., Sashida Y. 2002. New diarylheptanoids and diarylheptanoid glucosides from the rhizomes of *Tacca chantrieri* and their cytotoxic activity. *Journal of Natural Products* 65: 283-289.

29. Yokosuka A., Mimaki Y., Sakuma C., Sashida Y. 2005. New glycosides of the campesterol derivative from the rhizomes of *Tacca chantrieri*. *Steroids* 70: 257-265.

30. Yokosuka A., Mimaki Y., Sashida Y. 2004. Taccasterosides A-C, novel C28-sterol oligoglucosides from the rhizomes of *Tacca chantrieri*. *Chemical and Pharmaceutical Bulletin* 52: 1396-1398.

31. Zhang L., Li HT., Gao LM., Yang JB., Li DZ., Cannon CH., Chen J., Li QJ. 2011. Phylogeny and evolution of bracts and bracteoles in *Tacca* (Dioscoreaceae). *J Integr Plant Biol* 53: 901-911.

32. Zhang L., Li Q., Li D. 2006. Genetic diversity of *Tacca integrifolia* (Taccaceae) in the Brahmaputra valley, Tibet. *Biodiversity Science* 14 (1): 65-72.

33. Zhao Y., Zhang L. 2015. The phylogeographic history of the selfpollinated herb *Tacca chantrieri* (Dioscoreaceae) in the tropics of mainland Southeast Asia. *Biochem Syst Ecol* 58:139–148.

34. Zhao YM., Zhang L. 2011. Using DNA barcoding in genus *Tacca* (Dioscoreaceae). *Plant Diversity and Resources* 33 (6): 674 – 682 (abstract English).

ĐẶC ĐIỂM DI TRUYỀN CỦA LOÀI RÂU HÙM (*Tacca chantrieri* André) VÀ SUY LUẬN PHÁT SINH LOÀI TRONG CHI RÂU HÙM (*Tacca*) TRÊN CƠ SỞ GIẢI TRÌNH TỰ VÙNG GEN NHÂN ITS-rDNA

Vũ Đình Giáp[1*], Phạm Mai Phương[2], Bùi Thị Tuyết Xuân[3], Bùi Văn Thắng[4], Trịnh Thị Thùy Linh[4], Vũ Kim Dung[4], Vũ Đình Duy[2*]

[1]Viện công nghệ HaUI, Trường Đại học Công nghiệp Hà Nội

[2]Trung tâm Nhiệt đới Việt – Nga

[3]Viện Sinh thái và Tài nguyên Sinh vật, Viện Hàn lâm Khoa học và Công nghệ Việt Nam

[4]Trường Đại học Lâm nghiệp

TÓM TẮT

Chi Râu hùm (*Tacca*) là chi duy nhất trong họ Râu hùm (Taccaceae) bao gồm 17 loài phân bố trên thế giới, trong đó 7 loài được ghi nhận ở Việt Nam. Mã vạch DNA là một phương pháp để xác định và phát hiện loài nhanh chóng dựa trên các gen hoặc vùng DNA ngắn, được tiêu chuẩn hóa. Trong nghiên cứu này, chúng tôi giải trình tự nucletide vùng gen nhân (*ITS-rDNA*) từ 9 cá thể Râu hùm (*Tacca chantrieri* André) thu tại 3 quần thể tự nhiên ở Việt Nam (Khu bảo tồn thiên nhiên Sông Hinh, Phú Yên; Vườn quốc gia Cúc Phương, Ninh Bình và Vườn Quốc gia Ba Vì, TP. Hà Nội) để điều tra đặc điểm di truyền và suy luận phát sinh loài của chúng trong chi *Tacca*. DNA tổng số được chiết xuất từ mô của lá. Trình tự nucleotide của gen *ITS-rDNA* được xác định với 674bp. Tất cả các mẫu thu thập tại ba địa điểm khác nhau cho thấy độ tương đồng về chiều dài và trình tự nucleotide của chúng là 100% dựa trên trình tự vùng gen *ITS - rDNA*. Phân tích đặc điểm trình tự nucleotide loài Râu hùm trong nghiên cứu chỉ ra nucleotide T(U) trung bình (16,9%), C (29,5%), A (20,3%) và G (33,2%). Hàm lượng nucleotide TA thấp (37,2%) so với GC (62,8%). Phân tích phát sinh loài sử dụng phương pháp khả năng tối đa (ML) chỉ ra rằng tất cả các mẫu trong nghiên cứu có mối quan hệ chặt chẽ với loài Râu hùm (*T. chantrieri*) với giá trị ủng hộ cao (bootstrap = 99%). Khoảng cách di truyền (p) giữa các loài trong chi *Tacca* thay đổi khá lớn từ 0% đến 19%, trung bình 10%. Kết quả nghiên cứu này đã chỉ ra vùng gen nhân (*ITS-rDNA*) là một công cụ hữu ích, làm mã vạch trong việc xác định nhận dạng loài *Tacca* ở Việt Nam.

Từ khóa: cây phát sinh loài, DNA mã vạch, Râu hùm, vùng gen nhân (ITS-rDNA).

Received : 21/10/2021
Revised : 23/11/2021
Accepted : 06/12/2021