

THE MOLECULAR CHARACTER OF *Tacca chantrieri* ANDRÉ IN BA VI NATIONAL PARK BASED ON CHLOROPLAST GENE REGION (*matK*)

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ABSTRACT

Tacca J.R.Forst. & G.Forst. is the only genus in Taccaceae that includes 19 species distributed around the world, of which have found 7 species in Vietnam. DNA barcoding is a rapid species identification and discovery method using short, standardized genes or DNA regions. In this study, we have sequences of chloroplast gene region (*matK*) from 30 *Tacca chantrieri* André collection individuals in Ba Vi National Park to investigate the genetic character and their phylogenetic inference in the genus *Tacca*. DNA was extracted from the tissue of leaves. The nucleotide sequence of *matK* was determined to be 747 bp and deposited in GenBank (ON337530-ON337559). All samples showed that their length and nucleotide sequence similarity were 100%. The analysis indicated the mean base compositions were nucleotide T(U) (39.9%), C (15.4%), A (29.9%), and G (14.9%). The GC content was found to be low (30.3%), compared to 69.8% of the AT content. Phylogenetic analyses using maximum likelihood (ML) indicated that all samples from Vietnam have a close relationship with *T. chantrieri* in GenBank with strong supporting values (98%). Genetic p-distances interspecific divergence within and among *Tacca* species varied from 0% to 5%, with a mean genetic distance of 2%. The gene (*matK*) can also be used as a DNA barcode to identify the *T. chantrieri* species in Vietnam.

Keywords: DNA barcodes, *matK* gene, *Tacca chantrieri*, phylogenetic tree.

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1. INTRODUCTION

A total of 19 species of *Tacca* (Taccaceae) have been identified by the World List of Monocotyledonous Plants with 7 species have been recorded in Vietnam, as follow: *Tacca chantrieri*, *T. integrifolia*, *T. palmata*, *T. leontopetaloides*, *T. plantaginea*, *T. subflabellata* and *T. khamhhoaensis* [1, 2, 3, 4]. *T. chantrieri* is a perennial herb that grows 50-80 cm tall. The body is long and has a lot of nodes. Petioles are 10-30 cm long and grow straight from the rhizome, with

pointed oval blades 25-60 cm long, 7-20 cm wide, glossy green, wavy borders. The purple-black flowers are clustered on a straight or curved handle 10-15 cm long; the canopy has four purple-brown bracts, the outer bracts oval, pointed, and the inner bracts oval, oblong at the base, with sterile filaments up to 25 cm long. Calyx stalks, 6 stamens, and lower gourds with lateral ovary attachment are all present in the flowers. The fruit does not open on its own; the seeds are crimson purple in hue and have three edges. July-August is the flowering season, while September-October is the fruiting season. This species may be found in the wild in Vietnam's northern regions, where it thrives in streams and damp woodlands. Its rhizomes have been used as folk medicine to treat gastric ulcers, enteritis, and hepatitis [3].

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Phytochemical investigations of this plant have resulted in the isolation of diarylheptanoids [5], and steroidal saponins [6, 7, 8], and sterol saponins [9]. In addition, these compounds show cytotoxic activities [5]. *T. chantrieri* distribution is restricted by overexploitation, habitat destruction, and habitat fragmentation [10].

To identify plant taxon, using a DNA barcode is considered one of the most effective tools available today. Compared to the mitochondrial gene (cytochrome c oxidase I), which has been used effectively to identify many animal groups [11, 12, 13], the synonymous substitution rate of this gene is very low in land plants [14]. Moreover, because of hybridization, polyploidy, and apomixis in plants, identifying DNA barcodes is more difficult. Therefore, the suggestion of using the *trnH-psbA* gene or using a combination of the *trnH-psbA* and *rbcL* genes as an appropriate tool for plant taxonomy was reported by Kress *et al.* (2005) [15], Kress and Erickson (2007) [16]. In the study of Lahaye *et al.* (2008), the authors analyzed and compared eight gene regions commonly used in plant taxonomy, including *trnH-psbA*, *matK*, *ycf5*, *rbcL*, *rpoB*, *ndhI*, *accD*, and *rpoC1* [17]. The results showed that the *matK* gene was considered a universal DNA barcode for flowering plants. The

matK gene can identify cryptic species. However, the application of DNA barcodes to plants has been impeded due to problems such as low variation between species. In a study by the Consortium for the Barcode of Life Plant Working Group (2009), the results recommended that the 2-locus combination of ribulose-1,5-bisphosphate carboxylase (*rbcL*) and maturase (*matK*) as the plant barcode, which can identify and discover overlooked plant species [18]. Currently, different researchers have used some region genes such as *ITS*, *18S*, *matK*, *psbA-trnH*, *rbcL*, *atpA*, *rbcL*, *trnL-F*, and *trnS-trnG* in the building of DNA barcodes for the identification of *Tacca* species [10, 19, 20, 21, 22].

In this study, we sequenced the chloroplast gene region (*matK*) to identify the genetic characteristics of *T. chantrieri* and their phylogenetic inference in the genus *Tacca* in Ba Vi National Park (Ba Vi NP). This study contributes to developing a DNA barcode database as a foundation for conservation, evolution, and biological systems.

2. MATERIALS AND METHODS

2.1. Collection sampling

Table 1. Population descriptions of all sampled populations of *T. chantrieri* of Ba Vi NP

Sample code	Locality	Latitude (N)	Longitude (E)	Altitude (m)	GenBank code
BV01	Ba Vi National Park	21°04'57.8"	105°22'007"	747	ON337530
BV02	Ba Vi National Park	21°04'57.8"	105°22'007"	747	ON337531
BV03	Ba Vi National Park	21°04'57.4"	105°22'55"	694	ON337532
BV04	Ba Vi National Park	21°04'56.5"	105°22'6.5"	698	ON337533
BV05	Ba Vi National Park	21°04'56.5"	105°22'6.5"	698	ON337534
BV06	Ba Vi National Park	21°04'55.6"	105°22'8.3"	686	ON337535

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BV07	Ba Vi National Park	21°04'57.4"	105°22'31"	687	ON337536
BV08	Ba Vi National Park	21°04'57.4"	105°22'31"	687	ON337537
BV09	Ba Vi National Park	21°03'6.52"	105°21'7.9"	1081	ON337538
BV10	Ba Vi National Park	21°03'6.52"	105°21'7.9"	1081	ON337539
BV11	Ba Vi National Park	21°03'6.52"	105°21'7.9"	1081	ON337540
BV12	Ba Vi National Park	21°03'6.52"	105°21'7.9"	1081	ON337541
BV13	Ba Vi National Park	21°03'6.52"	105°21'7.9"	1081	ON337542
BV14	Ba Vi National Park	21°03'6.52"	105°21'7.9"	1081	ON337543
BV15	Ba Vi National Park	21°03'6.52"	105°21'7.9"	1081	ON337544
BV16	Ba Vi National Park	21°04'51.5"	105°22'1"	700	ON337545
BV17	Ba Vi National Park	21°06'57"	105°24'53"	1076	ON337546
BV18	Ba Vi National Park	21°06'57"	105°24'53"	942	ON337547
BV19	Ba Vi National Park	21°05'15.8"	105°22'58"	942	ON337548
BV20	Ba Vi National Park	21°04'9.40"	105°22'52"	942	ON337549
BV21	Ba Vi National Park	21°04'9.61"	105°22'44"	993	ON337550
BV22	Ba Vi National Park	21°05'6.4"	105°22'56"	993	ON337551
BV23	Ba Vi National Park	21°03'6.37"	105°21'7.8"	993	ON337552
BV24	Ba Vi National Park	21°03'6.27"	105°21'7.6"	993	ON337553
BV25	Ba Vi National Park	21°05'6.15"	105°21'7.4"	997	ON337554
BV26	Ba Vi National Park	21°03'6.04"	105°21'7.1"	819	ON337555
BV27	Ba Vi National Park	21°03'59.3"	105°21'7.1"	985	ON337556
BV28	Ba Vi National Park	21°03'41.3"	105°21'25.8"	985	ON337557
BV29	Ba Vi National Park	21°03'56.6"	105°21'45.7"	972	ON337558
BV30	Ba Vi National Park	21°05'41.1"	105°21'8.56"	774	ON337559

In this study, 30 samples (young leaves) of *T. chantrieri* were collected and placed in plastic bags with silica gel and transferred to the

laboratory of Vietnam - Russia Tropical Centre, stored at -30°C for DNA extraction (Table 1).

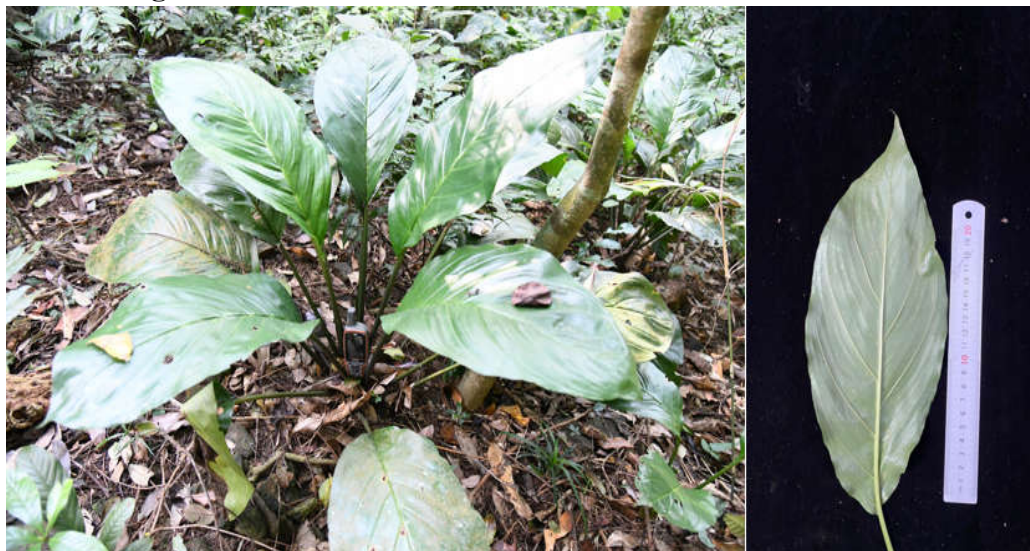


Figure 1. Adult plant of *T. chantrieri* species collected in Ba Vi NP

[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 20 ng/μl.

2.3. PCR amplification

The *matK* gene region was amplified through the following PCR cycling profile: an initial heating step at 94°C for 3 min; followed by incubating for 40 cycles of 94°C for 1 min, 55°C for 1 min, respectively, and 72°C for 1 min, and completed by incubating at 72°C for 10 min. All PCR reactions were performed in 25 μl volumes (2.0 μL of template DNA, 12.5 μL of 2X Taq Master Mix, 0.5 μL of each primer, and 9.5 μL of deionized water) using Gene Amp PCR Systems 9700. Double-stranded DNA was directly amplified by symmetric polymerase chain reaction (PCR) amplification using pairs of primers *matK-F* (5'-

ACCCTGTTCTGACCGTATCGC-3') and *matK-R* (5'- TCCATTTACATGATCCCATGACC-3') [23, 24].

2.4. Sequencing of the *matK* region

Sequencing was performed on an Avant 3100 automated DNA sequencer using the Dye Terminator Cycle sequencing kit (PE Applied Biosystems). Sequencing of the 30 studied samples used the primers *matK-F* and *matK-R*.

2.5. Phylogenetic analysis

Chromas Pro 2.1.6 software (Technelysium Pty Ltd., Tewantin, Australia) was used to edit the sequences [25]. Sequence alignments were made with Bioedit v7.0.5.2 [26]. We used MEGA 7.0 to analyze our data [27]. 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 [28]. 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 30 leaf tissues of *T. chantrieri* in Ba Vi National Park. Results of DNA electrophoresis on 1% agarose gel showed that

each sample had single, sharp, and bold bands indicating successful DNA extraction (figure 2). The purity of extracted DNA was excellent, as evident in DNA concentrations A260/A280 ratio ranging from 1.732 to 2.0, which also suggested that the preparations were sufficiently free of proteins and polyphenolic/polysaccharide compounds. The DNA concentration ranged from 640 to 980 ng/μl. The extracted DNA was suitable for PCR amplification of plant barcode genes. The DNA concentration was diluted for the PCR reaction to 20 ng/μl. The primer pair *matK-F/matK-R* was successfully cloned for 30 samples at a primer temperature of 55°C (Figure 3). The PCR product was approximately 800 bp in length. Electrophoresis on a 1.5% agarose gel showed a high-quality PCR product, with only a single bright band qualified for nucleotide sequencing.

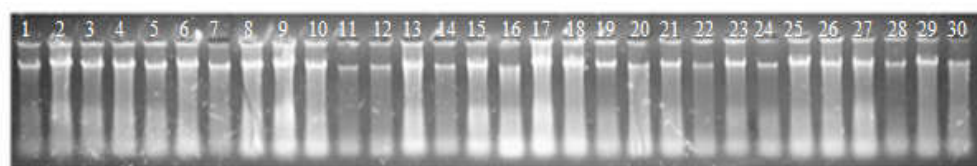


Figure 2. Electrophoresis of total DNA from 30 samples of *T. chantrieri* using 1% agarose gel

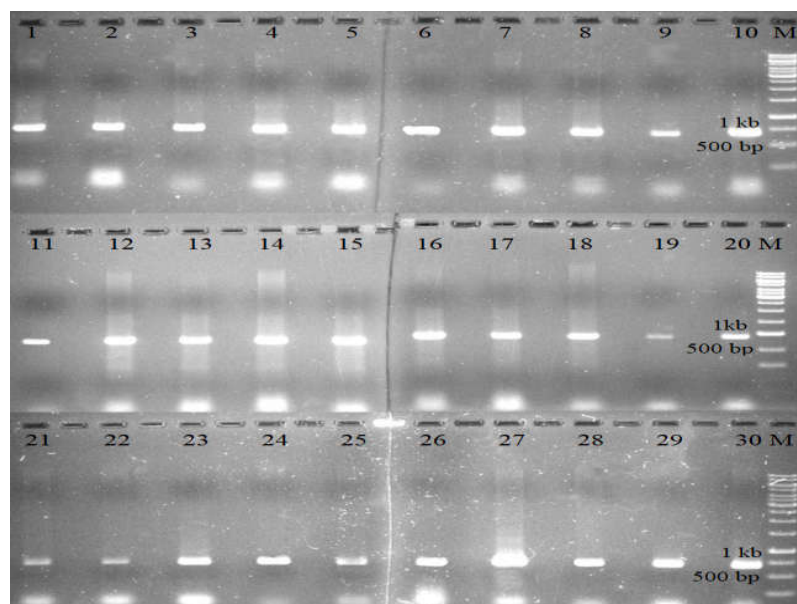


Figure 3. PCR products from 30 samples of *T. chantrieri* were electrophoresed on 1.5% agarose gel (M: DNA ladder 100 bp; 1-30: No. samples)

3.2. Characteristic analysis of *matK* sequences

All the studied samples were successfully amplified for *matK* gene regions with a high sequencing rate of 100%. Chromas Pro2.1.6 software was used to display the results and edit the sequences [25]. After removing the two ends, we identified that the remaining size of each sample was 747 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* (MK153234). The sequences for the *matK* gene region from *T. chantrieri* in Ba Vi NP 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 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, *matK* sequences of *T. chantrieri* consisted of 747 nucleotide positions. The mean base compositions were 39.9, 15.4, 29.9, and 14.9% for T (U), C, A, and G, respectively. The GC content was low (30.3%) compared to 69.8% of the AT content. This difference showed a low TA content at all three codon positions. These values were 63.8%, 67%, and 78.3% for the 1st, 2nd and 3rd codons, respectively (Table 2).

Table 2. Nucleotide base compositions (%) for the *matK* sequences of *T. chantrieri*

	Codon position	Base				Length (bp)
		T(U)	C	A	G	
<i>Tacca chantrieri</i>	All positions	39.9	15.4	29.9	14.9	747
	1st positions	36.5	20.5	27.3	15.7	249
	2nd positions	34.9	16.9	32.1	16.1	249
	3rd positions	48.2	8.8	30.1	12.9	249

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 (BV) and 15 species of the genus *Tacca* in the GenBank (Table 3 and Figure 4). The mean genetic distance was 2% ranging from BV/*T. chantrieri* (0%) to *T. plantaginea*/*T. artocarpifolia* (5%). The species pairs: *T. plantaginea*/*T. artocarpifolia* (5%) showed the highest genetic distances, whereas the 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 low genetic distances among species in the genus *Tacca*.

The maximum likelihood tree of sequence divergences (K2P) in the *matK* region reflects the above findings. It shows that all 15 species in the genus *Tacca* were distinctly separated and characterized by a high bootstrap value and a branch length of 0.005 (Figure 4). The ML tree showed a clear separation of samples (BV) into one clade together with *T. chantrieri* (MK153234) with a bootstrap value of 98%. BV/*T. chantrieri* had

highly identical *matK* sequences. The results one species. showed that this species pair was determined as

Table 3. Different genetic distances among species in genus *Tacca* based on *matK* analysis

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. BV	-															
2. <i>T. chantrieri</i> MK153234	0.00	-														
3. <i>T. subflabellata</i> JF956654	0.02	0.01	-													
4. <i>T. plantaginea</i> JF956647	0.03	0.03	0.04	-												
5. <i>T. leontopetaloides</i> JF956642	0.03	0.03	0.03	0.04	-											
6. <i>T. amplipecta</i> JF956614	0.02	0.02	0.01	0.04	0.04	-										
7. <i>T. integrifolia</i> JF956637	0.01	0.01	0.01	0.03	0.03	0.00	-									
8. <i>T. havilandii</i> MK153235	0.01	0.01	0.01	0.03	0.03	0.01	0.01	-								
9. <i>T. borneensis</i> MK153231	0.01	0.01	0.01	0.03	0.03	0.01	0.01	0.01	-							
10. <i>T. bibracteata</i> MK153225	0.02	0.02	0.02	0.03	0.03	0.02	0.02	0.02	0.02	-						
11. <i>T. sumatrana</i> MK153224	0.01	0.01	0.02	0.03	0.03	0.02	0.01	0.01	0.01	0.02	-					
12. <i>T. cristata</i> MK153223	0.01	0.01	0.01	0.03	0.03	0.01	0.01	0.00	0.01	0.02	0.01	-				

13. <i>T. reducta</i> MK153216	0.01	0.01	0.02	0.03	0.03	0.02	0.01	0.01	0.01	0.02	0.00	0.01	-			
14. <i>T. palmata</i> MK153217	0.02	0.02	0.03	0.03	0.03	0.03	0.02	0.02	0.02	0.02	0.02	0.02	0.02	-		
15. <i>T. maculata</i> MK153197	0.03	0.03	0.04	0.04	0.01	0.04	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	-	
16. <i>T. artocarpifolia</i> KU308827	0.03	0.03	0.04	0.05	0.01	0.04	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.01	-

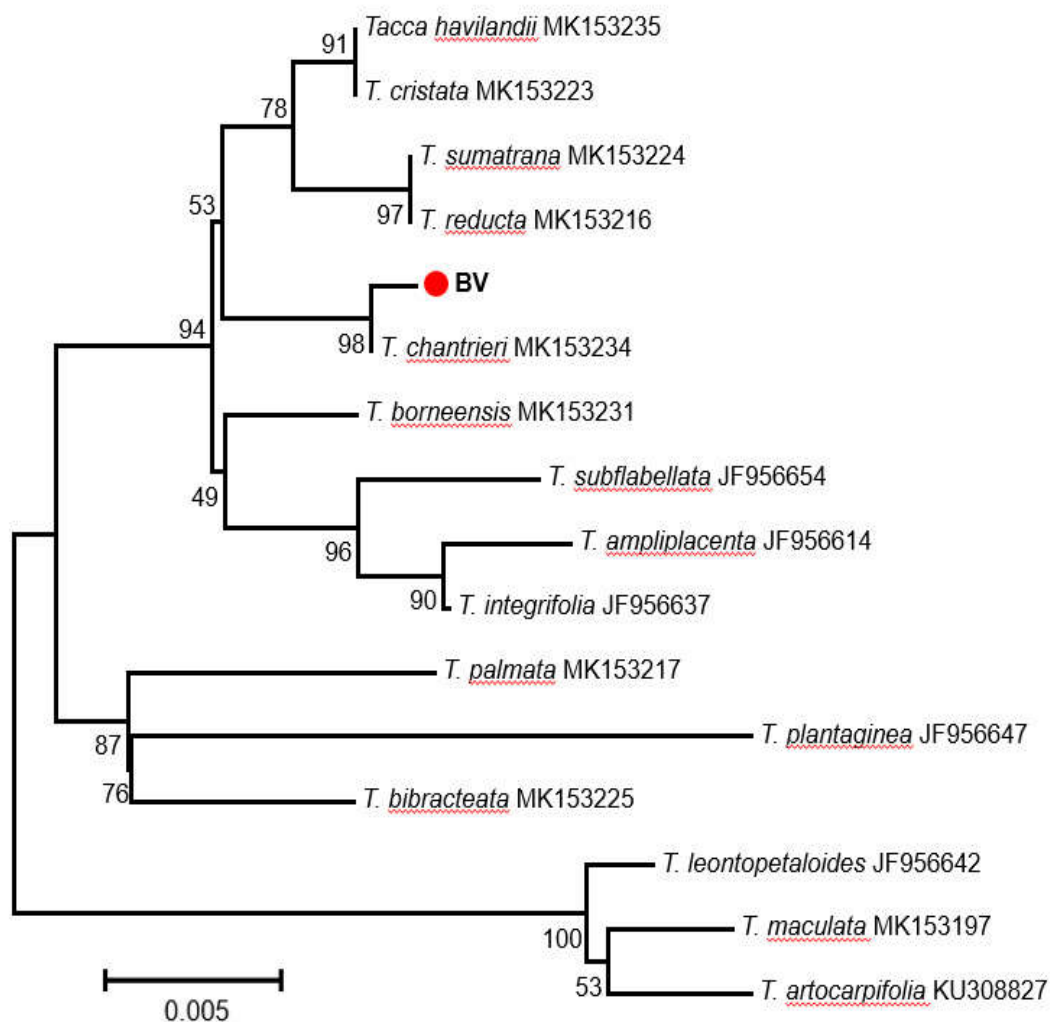


Figure 4. Phylogenetic relationships among *Tacca* species based on the sequence of chloroplast gene (*matK*) using the Maximum likelihood (ML) tree. The numbers above branches represent bootstrap

Accurate species identification is essential for managing and conserving species [29]. Species identification based on morphology is mostly uphill and less accurate. However, molecular-based methods such as DNA barcoding are rapid and accurate for specific identification [30, 31, 32]. 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 was evaluated among 36 accessions representing 6 species of *Tacca* [33]. 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* [10]. Phylogenetic analysis of 16 *Tacca* species utilizing nuclear *ITS* and plastid *matK* gene areas [21]. Our research indicates that the genetic distance between the species *Tacca* (2% *matK* gene) is less than that between nuclear gene regions (10% *ITS-rDNA* gene) [22]. Our results agree with the previous finding, confirm the core barcode's effectiveness, and suggest using *matK + ITS-rDNA* gene region as DNA barcode sequences in the genus *Tacca* in Vietnam

4. CONCLUSIONS

In the current study, we sequenced chloroplast gene region nucleotides (*matK*) to identify *T. chantrieri* in Vietnam, constructed

phylogenetic trees of the genus *Tacca*, and suggested using *matK* gene region to identify *Tacca* species in Ba Vi NP. The findings there will be significant in the study of evolution, systematics, and conservation of the species.

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