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Morphological and DNA barcode characteristics of *Dioscorea zingiberensis* C.H.Wright collected from Chu Yang Sin National Park, Dak Lak Province

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ABSTRACT

Introduction: Dioscorea zingiberensis is a medicinal herb with significant nutritional and pharmacological value. It is listed in both the Vietnam Red Data Book and the Red List of Medicinal Plants of Vietnam, and first identified in Chu Yang Sin National Park, Dak Lak Province. Objective: This study aimed to investigate the morphological and anatomical characteristics of Dioscorea zingiberensis C.H.Wright collected from Chu Yang Sin National Park, Dak Lak. Methods: Roots, stems, and leaves were identified based on morphological and anatomical analysis, combined with gene sequencing using the Sanger method. The obtained sequences were compared with reference sequences available in the GenBank database. Results: The results illustrated D. zingiberensis is an herbaceous, climbing plant with left-twining stems; leaves are heart-shaped, alternate, and bear two small spines; the fruit is a capsule; and the rhizome is horizontal, light brown, and bears numerous hard, fibrous roots. The microscopic observations of the stems, leaves, and roots revealed characteristic anatomical features of the genus Dioscorea, which help distinguish the species from closely related taxa. DNA sequencing results demonstrated a high degree of similarity between the studied sequence and the published D. zingiberensis sequences in GenBank, confirming accurate species identification. Conclusion: This study established a reference dataset on the morphology, anatomy, and DNA barcode of D. zingiberensis collected from Chu Yang Sin, contributing to the accurate identification, conservation, and sustainable utilization of Dioscorea genetic resources in Vietnam.

Keywords: *Dioscorea zingiberensis*, *DNA barcode, morphological, anatomical*

1. INTRODUCTION

The genus *Dioscorea* (Dioscoreaceae) is one of the largest genera of monocotyledonous plants, comprising approximately 600 species widely distributed across tropical and subtropical regions of the world. Several *Dioscorea* species possess high nutritional value (as edible yams) and exhibit significant medicinal properties [1]. They have been extensively used in traditional medicine systems in countries such as China, India, Thailand, Vietnam, and Mexico for the treatment of diabetes, arthritis, digestive disorders, and infectious diseases [2].

Dioscorea zingiberensis C.H. Wright is a representative species of the genus *Dioscorea*. It was first scientifically described by C.H. Wright in 1903 [2]. Taxonomically, this species belongs to the kingdom Plantae, phylum Tracheophyta, class Liliopsida, order Dioscoreales, family Dioscoreaceae, and genus *Dioscorea*. This species is a perennial herb originating from China, distributed mainly in the provinces of Henan, Guizhou, Hunan, Sichuan, and southern Shanxi.

In Vietnam, *D. zingiberensis* is also known by local names such as “Mài Gừng”, “Từ Gừng”, “Từ Tam Giác”, and “K'lóh” (Ba Na language). The species grows wild in mountainous forests and is mainly distributed in the Central and Central Highlands regions, particularly in Lam Dong, Dak Lak, Quang Ngai, Binh Dinh, and Phu Yen provinces [3]. *D. zingiberensis* had not been previously recorded in

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the flora of Chu Yang Sin National Park or Dak Lak Province. The species has been listed in the Vietnam Red Data Book (1996, 2007) and the Red List of Medicinal Plants of Vietnam (1996, 2001, 2006) as Endangered (EN) [4]. However, Nguyen Tap et al. [5] reported the presence of this species in Chu Yang Sin National Park in 2016, which is currently considered one of the most important *in situ* conservation areas for *D. zingiberensis* in Vietnam.

Although *D. zingiberensis* C.H. Wright has been recorded as part of the flora of Vietnam and is known for its nutritional and pharmacological values, there has been no systematic research on the botanical, ecological, or molecular genetic characteristics of this species. Therefore, this study aimed to investigate the morphological and anatomical characteristics and analyze the DNA barcode of *D. zingiberensis* C.H. Wright to establish a reference database for the accurate identification, conservation, and sustainable utilization of this valuable medicinal genetic resource in Dak Lak, Vietnam.

2. METHODS

2.1. Material

The *Dioscorea zingiberensis* C.H. Wright samples, including roots, stems, and leaves, were collected from Chu Yang Sin National Park, Dak Lak Province, in August 2025. Seedlings and voucher specimens were cultivated and preserved at the Applied Biomedical Research Institute, Buon Ma Thuot Medical University.

2.2. Methods

Morphological: The morphological characteristics of *D. zingiberensis* were observed directly with the eyes and under a magnifying glass, and photographs were taken to document the diagnostic features. The scientific identification of the species was carried out based on the analyzed morphological traits compared with standard botanical descriptions and monographs on *Dioscorea* species [5].

Anatomical: Fresh samples of *D. zingiberensis* were washed after collection. Stem capsules, leaves, and petioles were cut into thin cross-sections and stained using the double-staining method with Carmine-Iodine dye, following the protocol of Applied Biomedical Research Institute, Buon Ma Thuot Medical University. The anatomical structures were examined and described under a light microscope, with five microsections observed for each organ.

DNA barcoding: Fresh leaf tissues were mixed with a lysis buffer supplemented with polyvinylpyrrolidone (PVP) and β-mercaptoethanol to neutralize phenolic compounds and reduce sample oxidation. The mixture was incubated at 65°C for 30 minutes with gentle inversion to facilitate cell lysis and release of genomic DNA. After pretreatment, DNA extraction was performed using the Thermo Scientific™ DNA extraction kit according to the protocol. DNA purity and concentration were assessed using a spectrophotometer at A260/A280. The matK chloroplast gene fragment was amplified by PCR using the forward primer (5'-CGATCTATTCATTCAATATTTC-3') and reverse primer (5'-TCTAGCACACGAAAGTCGAAGT-3'). PCR products showing the expected band size were purified and subjected to Sanger sequencing at Genlab (Vietnam). The obtained sequences were edited and analyzed using the BLASTn algorithm against the NCBI database to determine the species with the highest sequence similarity.

3. RESULTS

3.1. Morphological characteristics

Dioscorea zingiberensis C.H. Wright is a perennial climbing herb with a left-twining stem, 1.5 - 3 m long and 0.2 - 0.3 cm in diameter, dark green to brownish-green, with nodes bearing two sharp, curved spines. The rhizome grows horizontally, branched, nearly circular in cross-section, light yellowish-brown internally, with a distinctive aromatic odor; texture hard and fibrous. Roots arise in tufts from the rhizome, cylindrical, 10 - 20 cm long and 0.2 - 0.3 cm in diameter, light brown. The stem is smooth, glabrous, and longitudinally striated. Leaves are alternate; petiole 1 - 2 cm long; lamina cordate, 3 - 8 × 2.5 - 3.5 cm, acuminate at both apex and base; venation palmate with one main and four secondary veins; leaf surface glabrous, upper side dark green, underside light green. The fruit is a green, three-winged capsule containing three flattened seeds, each surrounded by a thin membranous wing (Figure 1).

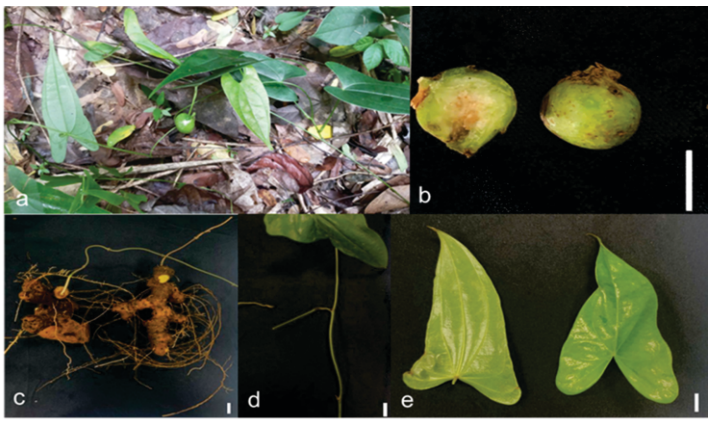

Figure 1. Morphological characteristics of *Dioscorea zingiberensis* C.H. Wright..
a. Habit; b. Longitudinal section of fruit; c. Rhizome; d. Stem; e. Leaf (scale bar = 1 cm)

3.2. Anatomical characteristics

Stem: The cross-section of the stem is nearly circular. The cortex occupies approximately one-fourth of the stem radius, while the stele occupies about three-fourths. The epidermis consists of a single layer of rectangular to polygonal cells, with the outer wall cutinized. The collenchyma, composed of several layers of polygonal cells, is mainly concentrated at the protruding corners. The parenchyma consists of 5 - 6 layers of irregularly polygonal, thin-walled cells with intercellular spaces and small secretory canals. The endodermis is represented by 2 - 4 layers of polygonal cells, with the outer walls lignified, forming a continuous ring surrounding the vascular system. The vascular system is composed of primary xylem and phloem bundles arranged in concentric rings, increasing in size from the periphery toward the center. Each vascular bundle is collateral and open. The xylem vessels are V-shaped, consisting of small polygonal protoxylem elements with lignified walls and larger circular to oval metaxylem elements. The phloem consists of small polygonal cells with cellulose walls, arranged irregularly. The perimedullary region contains lignified sclerenchymatous parenchyma composed of round to slightly oval cells. The central pith is made up of thin-walled parenchymatous cells, circular to oval in shape, loosely arranged (Figure 2).

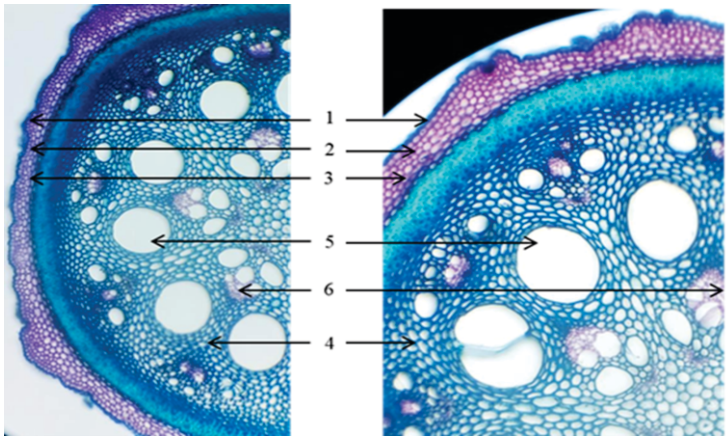

Figure 2. A Cross-section of the stem (magnification: 100x)
1. Epidermis; 2. Collenchyma; 3. Parenchyma; 4. Sclerenchyma; 5. Xylem; 6. Phloem

Leaf: The cross-section of the leaf is axially symmetrical, comprising two distinct regions as the midrib and the lamina.

The petiole (Figure 3a): From the outside inward, the petiole consists of an outer epidermis formed by a single layer of rectangular cells, followed by 2-3 layers of angular collenchyma composed of rectangular to polygonal cells of irregular sizes arranged compactly. The parenchymatous cortex includes 4-5 layers of polygonal cells of varying sizes, forming a continuous band. Below the cortex is a sclerenchymatous ring, consisting of 3-4 layers of thick-walled, lignified cells with collapsed lumina, forming a dark green stained continuous band. The vascular bundles are arranged in a semicircular pattern, with xylem

located on the inner side and phloem on the outer side. The phloem is composed of irregularly arranged polygonal cells with cellulose walls, while the xylem consists of vessels that are nearly circular, varying in diameter, arranged in radial rows. The xylem parenchyma cells are smaller, polygonal, with lignified walls, interspersed among the vessels.

The lamina (Figure 3b): The upper and lower epidermis each consist of a single layer of rectangular to polygonal cells of unequal sizes, with cellulose walls and a cutinized outer wall. The palisade parenchyma is composed of 1-2 layers of columnar cells arranged perpendicularly to the upper epidermis. The spongy parenchyma consists of multiple layers of irregularly shaped parenchymatous cells, varying in size and loosely arranged, forming large intercellular spaces.

The midrib (Figure 3c, d): The upper surface is slightly concave, the lower surface is round and convex. The upper epidermis is a single layer of rectangular to polygonal cells with cutinized outer walls. Beneath it lies several layers of parenchyma with circular to polygonal cells of irregular sizes. The sclerenchyma consists of 2 - 3 layers of polygonal, thick-walled, lignified cells forming an incomplete ring around the vascular bundles. The vascular bundles are arranged in a semicircular pattern, with xylem on the inner side and phloem on the outer side. The phloem consists of irregularly arranged polygonal cells with cellulose walls, while the xylem includes circular vessels of varying diameters, arranged in radial rows, interspersed with smaller polygonal lignified xylem parenchyma cells. The lower collenchyma comprises 4 - 5 layers of polygonal cells of irregular sizes. The lower epidermis is a single layer of polygonal cells with thick cutinized outer walls.

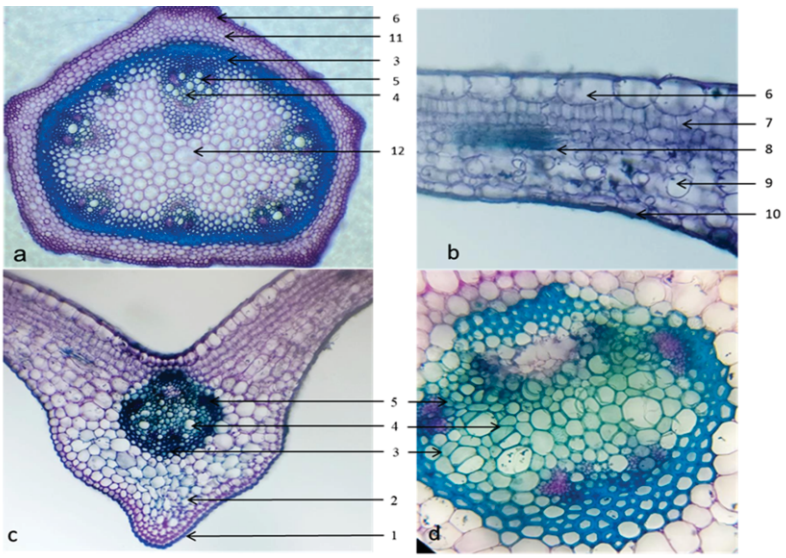

Figure 3. A Cross-section of leaf (magnification: 40x, 100x)
a: Petiole (40x); b: Leaf blade (100x); c: Midrib (40x); d: Central part of the midrib (100x)
1. Epidermis; 2. Medullary parenchyma; 3. Sclerenchyma; 4. Xylem; 5. Phloem; 6. Upper epidermis; 7. Palisade parenchyma; 8. Midrib; 9. Spongy parenchyma; 10. Lower epidermis; 11. Collenchyma; 12. Parenchyma

Root (Figure 4): The microscopic cross-section of the root has a nearly circular. The cortex occupies approximately two-thirds of the root radius, while the stele accounts for the remaining one-third. The root hair layer consists of a single layer of polygonal cells with cellulose walls impregnated with suberin, closely arranged, and bearing scattered root hairs. The epidermis is composed of 2 - 3 layers of rectangular to slightly polygonal cells, compactly arranged in straight rows. The cortical parenchyma includes two regions as outer and inner cortex. The outer cortex, consisting of 7 - 9 layers of irregularly polygonal cells with cellulose walls, loosely arranged. The inner cortex, comprising 3 - 4 layers of polygonal cells, regularly arranged in concentric rows. The endodermis is characterized by U-shaped thickened walls. The pericycle is a single layer of rectangular cells with cellulose walls, evenly sized and alternating with endodermal cells. The vascular system consists of alternating primary xylem and phloem bundles arranged in a ring. Each primary xylem bundle consists of nearly circular, lignified vessels,

radially differentiated, with metaxylem elements 4 - 5 times larger than protoxylem elements. The primary phloem is composed of irregularly polygonal cells with cellulose walls, forming small clusters between the xylem arms. The pith parenchyma is made up of irregularly polygonal, lignified cells of various sizes, loosely arranged in the central region.

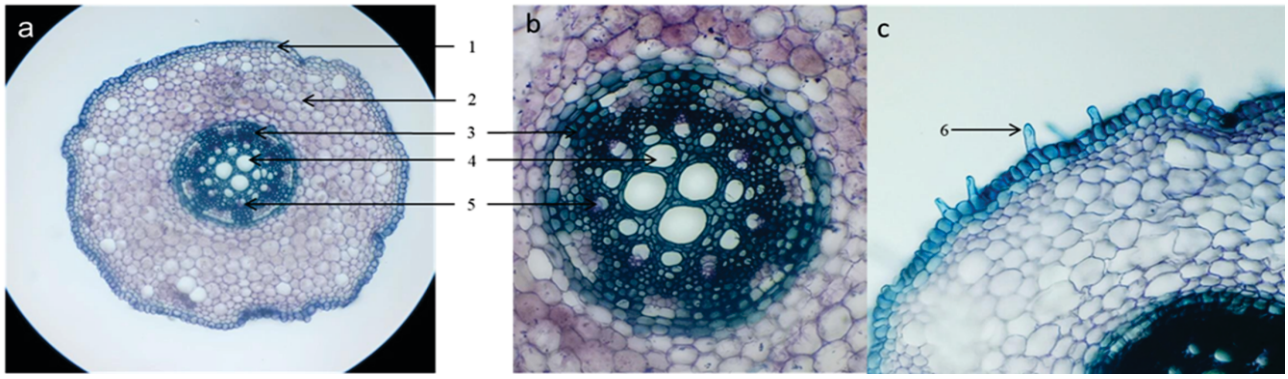

Figure 4. A Cross-section of root (magnification: 40x, 100x)
a: General view of the entire root cross-section (40x); b,c: Enlarged portions of the cross-section (100x)
1. Epidermis; 2. Parenchyma; 3. Sclerenchyma; 4. Xylem; 5. Phloem; 6. Root hairs

3.3. DNA barcoding

The result of *Dioscorea zingiberensis* C.H. Wright sequencing (586 bp)

TCAACGTCCTCTGGATTCTTTCTTGAGCGAACGCATT
TCTATGGAAAAATAGAGCAGTTTCTAGTAGCGTGTCG
TAATTATTTTCAGAAGACCCAATGGTTCTTCAAGGAC
CCTTTCATGCATTATGTTCGATATCAAGGAAAAGCAA
TTCTGGTGTCAAAGGGAACTTATCTTTTGATGAAGA
AATGGAGATCTTACCTTGTTAATTTCTGGCACTATTAT
TTTGAATTTTGGTCTCACCCGCATAGGATTCAGATAA
ACCAATTATCAAATTATTCTTTCTGTTTTCTGGGTTATC
TTTCAAATGTACTAATAAATCTTTCCGTGGTAAGGAG
TCAAATGCTAGAAATTTTTTTTTTAATAGATACTCTTA
CTAAGAAATTCGATACCATAGTTTCAGTTATTGCTCTT
ATTCGATCATTGTCTAAACCGAAATTTTGTACCGTATC
TGGTCATCCCATTAGTAAGTCAATATGGACAAATTTAT
CAGATTTGGATATTATTCATCGATTTGGTCGGATATGT
AGAAATCTTTCTCATTATCATAGTGGATCCTCAAAAA
AACAGAGTTTGTATC

The matK gene obtained from *Dioscorea zingiberensis* had a length of 586 base pairs (bp). The sequence was analyzed using BLASTn against the NCBI GenBank database. The comparison results (Table 1) show that the obtained sequence had a 99.48 - 99.65% similarity with reference sequences of *D. zingiberensis* available on GenBank, including accessions OQ526000.1, NC_027090.1, HQ637598.1, HQ637594.1, MH660261.1, and others, with E-value as 0.0 and 100% query coverage.

Table 1. Local alignment results obtained from BLAST of *Dioscorea zingiberensis*

Species	Max score	Total score	Query cover	E-value	Per. Ident.
Dioscorea zingiberensis OQ526000.1	1055	1055	100 %	0.0	99.65 %
Dioscorea zingiberensis NC_027090.1	1055	1055	100 %	0.0	99.65 %
Dioscorea zingiberensis HQ637598.1	1055	1055	100 %	0.0	99.65 %
Dioscorea zingiberensis HQ637594.1	1055	1055	100 %	0.0	99.65 %
Dioscorea zingiberensis HQ637599.1	1055	1055	100 %	0.0	99.65 %
Dioscorea zingiberensis MH660261.1	1055	1055	100 %	0.0	99.65 %
Dioscorea zingiberensis HQ637596.1	1050	1050	100 %	0.0	99.48 %
Dioscorea zingiberensis HQ637592.1	1050	1050	100 %	0.0	99.48 %
Dioscorea zingiberensis AY973831.1	1050	1050	100 %	0.0	99.48 %

This tree was constructed using a SNP matrix generated by kSNP 4.0 and inferred with IQ-TREE v2.4.0. Support bootstrap values > 89 are shown for each clade using 1,000 ultrafast bootstraps. Figure 5 demonstrates that our sequenced sample clusters closely with Distichochlamys zingiberensis

accessions deposited in GenBank, indicating a high level of genetic similarity and confirming the species identity of the sample.

These results confirm that the DNA sequence obtained from the studied sample corresponds to *D. zingiberensis* C.H. Wright, consistent with its morphological and anatomical identification. The successfully sequenced gene region was also deposited for future reference and comparative genetic analysis of the genus *Dioscorea* in Vietnam.

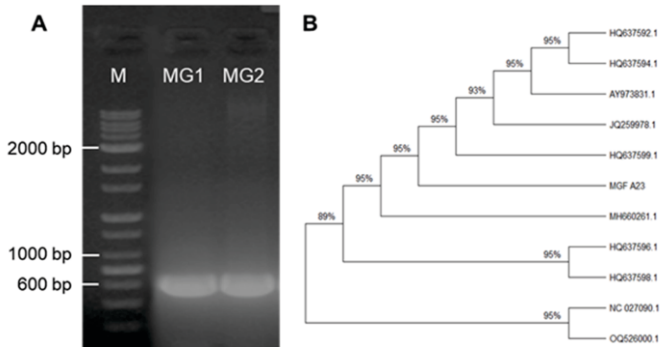

Figure 5. PCR amplification and phylogenetic analysis of *D. zingiberensis*
A. Agarose gel electrophoresis of the PCR-amplified *matk* gene;
B. The phylogenetic tree of the genomic sequences

4. DISCUSSIONS

The morphological characteristics of *Dioscorea zingiberensis* collected from Chu Yang Sin National Park are consistent with previous descriptions from China and Vietnam [2, 3]. The plant exhibits a perennial, left-twining climbing habit, alternate cordate leaves, and a horizontal rhizome with fibrous roots, features typical of the genus *Dioscorea* [6]. The presence of two curved spines at the nodes and a distinctive aromatic rhizome agrees with diagnostic traits described by Zhang et al. [2] and Tap et al. [5]. Minor variations in leaf size and color intensity observed in the Chu Yang Sin population may result from ecological adaptation to the montane environments of Dak Lak Province. These morphological traits confirm the accurate identification of *D. zingiberensis* as an endemic and endangered taxon occurring naturally in the Central Highlands of Vietnam [5].

The anatomical structures of the stem, leaf, and root of *D. zingiberensis* exhibit typical monocotyledonous features consistent with those of other *Dioscorea* species [7, 8]. The stem possesses concentric collateral vascular bundles and a distinct sclerenchymatous ring, while the leaf shows dorsiventral symmetry with differentiated palisade and spongy mesophyll layers [9]. These results are consistent with previous anatomical on *D. opposita* and *D. alata* (Table 2), which also revealed U-shaped endodermal thickening and well-developed sclerenchyma supporting tissue [2]. The root anatomy with radially arranged xylem and phloem bundles conforms to the diarch pattern observed in *Dioscorea* [6]. Anatomical markers can serve as diagnostic characters for distinguishing *D. zingiberensis* from morphologically similar taxa such as *D. nipponica* and *D. alata*, which differ in bundle arrangement and cortical organization [10].

Table 2. The morphological characteristics of *D. zingiberensis* C.H. Wright and species of *Dioscorea*

Morphological characteristics	*D. zingiberensis*	*D. nipponica*	*D. alata*	Notes
Monocot stem Structure	Present	Present	Present	All show typical monocot organization
Collateral vascular bundles	Yes	Yes	Yes	Arrangement differs, but type is conserved
Presence of sclerenchyma in stem	Yes	Yes	Yes	A diagnostic feature of *Dioscorea* stems
Dorsiventral leaf structure	Yes	Yes	Partially (weak)	D. alata tends toward isobilateral but still shows dorsal-ventral polarity

Morphological characteristics	*D. zingiberensis*	*D. nipponica*	*D. alata*	Notes
Cuntinized epidermis on leaves	Yes	Yes	Yes	Common to most *Dioscorea* species
Root endodermis thickening	U-thickening	U/O-thickening	U-thickening	Type differs in degree, but thickening present in all
Vascular bundles are arranged radially in the root	Yes	Yes	Yes	A genus-level character
Pith composed of parenchyma	Yes	Yes	Yes	Present in all species

Molecular identification based on the matK gene (586 bp) confirmed the taxonomic of *D. zingiberensis*. The DNA sequence displayed 99.48 - 99.65% similarity with reference sequences in GenBank (OQ526000.1, NC_027090.1, HQ637598.1, HQ637594.1, MH660261.1), aligning closely with findings by Sun et al. [7] and Girma et al. [6], who reported that chloroplast DNA regions (matK, rps16) are reliable for discriminating *Dioscorea* species. This result also supports previous phylogenetic studies emphasizing the use of chloroplast markers in Dioscoreaceae [10]. The local sequence obtained in this study thus provides valuable molecular evidence for taxonomy, conservation genetics, and resource authentication of *D. zingiberensis* populations in Vietnam.

There are several limitations in this study. Firstly, the *D. zingiberensis* specimens were collected in August, outside the documented flowering season of the species (typically May to June). Floral and inflorescence characters are critical taxonomic traits in Dioscorea, the absence of reproductive material prevented a complete morphological diagnosis and limited direct comparison with formal identification keys. Nevertheless, the vegetative morphology (leaf arrangement, nodal spines, and rhizome structure) together with detailed anatomical characteristics of the stems, leaves, and roots, combined with high-resolution DNA barcoding, provides strong evidence supporting the accurate identification of *D. zingiberensis*. Secondly, anatomical observations were conducted on a small number of individuals, which may not fully capture the range of intra-population variation. Finally, DNA barcoding in this study employed only a single chloroplast marker (matK); incorporating additional chloroplast and nuclear loci could enhance phylogenetic resolution and improve species delimitation. Therefore, we will conduct further studies to integrate reproductive morphology, expanded anatomical sampling, and multilocus molecular markers to provide a more complete understanding of *D. zingiberensis* populations from Chu Yang Sin.

5. CONCLUSIONS

This study presents the first integrated analysis of morphological, anatomical, and DNA sequence characteristics of *Dioscorea zingiberensis* C.H. Wright collected from Chu Yang Sin National Park, Dak Lak Province. The plant's diagnostic features and anatomical structure were consistent with previously described *Dioscorea* species, and DNA barcode analysis confirmed 99.65% similarity with GenBank reference sequences. These findings confirm the presence of *D. zingiberensis* in Dak Lak, expanding its known distribution and providing essential data for conservation planning. The established morphological, anatomical, and genetic datasets serve as a foundational reference for the accurate identification, conservation, and sustainable use of *Dioscorea* germplasm resources in Vietnam. Future research should focus on population genetics, secondary metabolite profiling, and pharmacological activity to support the conservation and medicinal utilization of this valuable species.

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Đặc điểm hình thái và mã vạch DNA của cây Mài gừng (*Dioscorea zingiberensis* C.H.Wright) thu tại Vườn quốc gia Chư Yang Sin, tỉnh Đắk Lắk

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TÓM TẮT

Đặt vấn đề: Mài gừng là dược liệu có giá trị dinh dưỡng và dược liệu quan trọng, được đưa vào sách đỏ và chỉ lục cây thuốc đỏ Việt Nam, lần đầu tiên được xác định tại Chư Yang Sin, tỉnh Đắk Lắk. Mục tiêu: Nghiên cứu nhằm khảo sát đặc điểm hình thái, giải phẫu thực vật và phân tích mã vạch DNA của cây Mài gừng (Dioscorea zingiberensis C.H.Wright) thu hái tại Chư Yang Sin, tỉnh Đắk Lắk. Phương pháp: Các mẫu nghiên cứu bao gồm rễ, thân và lá được định danh bằng phương pháp so sánh hình thái và vi phẫu, kết hợp phân tích trình tự gen bằng phương pháp Sanger và đối chiếu với các trình tự gen trên cơ sở dữ liệu Genbank. Kết quả: Mài gừng là loài thân thảo, dây leo; thân quấn về bên trái; lá hình tim, mọc cách có hai gai nhỏ; quả nang; thân rễ nằm ngang, màu nâu nhạt, nhiều rễ chùm và cứng. Vi phẫu thân, lá và rễ đã ghi nhận đặc điểm cấu trúc mô đặc trưng của chi Dioscorea nhằm phân biệt loài với các taxon gần gũi. Kết quả giải trình tự DNA cho thấy đoạn gen nghiên cứu có mức độ tương đồng cao với các trình tự Dioscorea zingiberensis đã công bố trên GenBank, khẳng định tính chính xác trong định danh loài. Kết luận: Nghiên cứu đã xác lập cơ sở dữ liệu về hình thái, vi phẫu và mã vạch DNA của cây Mài gừng thu nhận tại Chư Yang Sin; góp phần quan trọng cho công tác định danh, bảo tồn và phát triển bền vững nguồn gen dược liệu Dioscorea tại Việt Nam.

***Từ khóa**: Dioscorea zingiberensis, mã vạch DNA, giải phẫu, hình thái*

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