THAI NGUYEN UNIVERSITY
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RESEARCH ON CONSERVATION OF SOME ENDANGERED MEDICINAL PLANT SPECIES IN HOANG LIEN NATIONAL PARK, LAO CAI PROVINCE

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- Viet Nam national library:

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LIST OF PAPERS PUBLISHED

RELATING TO DISSERTATION

1. **Nguyen Hoang, Hoang Van Hung, Thao A Dia and Doan Thu Ha (2020).** Identification of some medicinal plants at risk based on the community in Hoang Lien National Park, Lao Cai province. TNU Journal of Science and Technology, 225 (16), pp. 13-18.
2. **Nguyen Hoang, Nguyen Thanh Nhung, Vuong Thi Huyen Trang, Tran Dang Khanh and Hoang Van Hùng (2023).** Molecular Analysis of Genetic Diversity and Genetic Relationship of Polygonatum kingianum Samples Collected in Northern Mountainous Regions in Vietnam. Eur. Chem. Bull., No12 (issue 8), pp. 9296-9304.
3. **Nguyen Hoang, Nguyen Thanh Nhung, Nguyen Thi Ngoc Minh, Vuong Thi Huyen Trang, Khuat Huu Trung, Tran Dang Khanh, Hoang Van Hung, (2023).** Morphological Traits and Nuclear Genetic Diversity of Coptis sp. in Hoang Lien National Park, Lao Cai Province, Vietnam. Journal of Advanced Zoology, 44 (03) pp. 1061-1068.

NTRODUCTION

1. The urgency of the dissertation

Hoang Lien National Park (HLNP) is unique in that there are residents (especially ethnic minorities) living in the core area of HLNP. The biodiversity of HLNP has played an important role in the livelihoods and production of the people here and is contributing to the socio-economic development, especially the tourism development of the market. Sa Pa commune in particular and Lao Cai province in general. Besides, tourism activities, construction projects, etc. has had a major impact on the ecosystem and species diversity in HLNP.

However, to date, research in HLNP has mainly focused on listing animal and plant species and introducing general environmental and ecological features. To date, there have been very few comprehensive research projects on medicinal plants, especially at-risk medicinal plant species in HLNP, Lao Cai province. The list of endangered medicinal plant species is mainly based on IUCN standards or the species identification standards of Vietnam's Red Book, while in many specific regions and ecological sub-regions Certain species at risk can be identified by many other criteria; And currently, there are few studies that determine the relationship between ecological and environmental factors affecting the distribution of endangered medicinal plant species. In particular, there have been no in-depth studies on genetic relationships using molecular biological markers for medicinal plants at risk of being threatened in HLNP, Lao Cai province.

Starting from the above requirements, it is necessary to conduct systematic and scientifically-based research from which to orient solutions for conservation. This is the reason for conducting the topic: ***"Research on conservation of some endangered medicinal plant species in Hoang Lien National Park, Lao Cai province"***.

2. Objectives of the study

2.1. Overall objectives

- Identifying some at-risk medicinal plant species in HLNP and conducting research to propose conservation solutions.

2.2. Detail objectives

- Assessing the current status of biodiversity and identifying some medicinal species at risk of being threatened in HLNP, Lao Cai province;
- Determining ecological and environmental factors affecting the distribution of endangered plant species in the study area;
- Evaluation of genetic relationships of some medicinal species at risk using molecular biological markers;
- Proposing some solutions to conserve some at-risk medicinal species in HLNP, Lao Cai province.

3. Scientific and practical significance

3.1. Scientific significance

The research results of the thesis have provided more data and scientific information about ecological and environmental conditions, distribution, genetic diversity, and the current state of the threat of extinction to serve research on conservation and development of medicinal plant genetic resources at HLNP.

3.2. Practical significance

The applied solutions will contribute to the sustainable conservation of medicinal plant resources in HLNP and promote the development, exploitation and effective use of precious medicinal plant genetic resources, increasing people's income.

4. New contributions of the thesis

- Using local knowledge, we have assessed the status of the threat of extinction of 15 rare medicinal species in HLNP, Lao Cai province, including 07 crictical endangered species, 05 endangered species, 03 vulnerable species to needs to be preserved.
- The level of genetic diversity has been evaluated and identified molecular markers (barcodes) of three medicinal plant species: Heliciopsis lobata (Merr.) Sleum, Coptis quinquesecta W. T. Wang and Polygonatum kingianum Coll et Hemsl.; Identify a number of ecological - environmental factors and plant species related to the distribution of these medicinal plants, thereby

proposing optimal solutions to preserve rare medicinal plants at HLNP.

Chapter 1
OVERVIEW

In this chapter, the thesis overviews the following issues:

- Overview of biodiversity conservation.
- Medicinal plant resources.
- Conservation activities of medicinal plants in the world and in Vietnam.
- Approaches to identifying species at risk.
- Overview of the research area and studies on medicinal plants in HLNP.
- Based on an overview of research issues, the thesis has proposed a research approach.

Chapter 2
CONTENTS AND METHODS OF THE STUDY

2.1. Objects and scope of the Study

2.1.1. Objects of the Study

Medicinal plants at HLNP are located in Sa Pa town, Lao Cai Province

2.1.2. Scope of the Study

The project focuses on researching the current status of medicinal plants in HLNP, thereby selecting a number of medicinal plants at risk to focus on studying ecological characteristics and evaluating genetic diversity by applying biotechnology. molecular learning. From there, orient solutions for medicinal plant conservation at HLNP.

2.2. Contents of the Study

2.2.1. Assessment of the endangered status of some medicinal plant species at HLNP

- Current status of biodiversity and medicinal plants in HLNP.
- Situation of exploitation and use of medicinal plants in HLNP
- Indigenous knowledge about the characteristics and uses of

some medicinal plants in HLNP

- Identifying some rare and endangered medicinal plant species in HLNP

2.2.2. The natural ecological characteristics of some medicinal plants at risk need to be preserved

- Natural ecological characteristics of Heliciopsis lobata (Merr.) Sleum.

- Natural ecological characteristics of Coptis quinquesecta W. T. Wang.

- Natural ecological characteristics of Polygonatum kingianum Coll et Hemsl.

2.2.3. Results of identification and assessment of genetic diversity of some medicinal plant species at risk that need to be preserved

- Results of identification and assessment of genetic diversity of Heliciopsis lobata (Merr.) Sleum based on ITS sequence.

- Results of identification and genetic diversity assessment of Coptis quinquesecta W. T. Wang samples based on ITS sequences.

- Results of identification and genetic diversity assessment of Polygonatum kingianum samples Coll et Hemsl. based on the TrnL-TrnF IGS sequence.

2.2.4. Some solutions for preserving medicinal plants at HLNP

- Theoretical basis of proposed solution.

- Solutions.

2.3. Research Methods

2.3.1. Methods of investigation, survey and assessment of the endangered status of some medicinal plant species at HLNP

* To investigate the endangered status of medicinal plant species at HLNP according to IUCN and Vietnam's Red Book by using the method of inheriting secondary data from agencies, organizations and individuals.

* To investigate and assess the endangered status of medicinal plant species at HLNP based on the community using the following methods:

- Interview

+ Sampling: According to the stratified - random method (Russell Bernard H., 2017). The floors used are: (i) Households collecting medicinal plants; (ii) Households buy and sell medicinal

plants. Households are then selected by random drawing. A total of 70 households were selected and interviewed. Each household is a PRA (Participatory Rural Appraisal) group, including family members and work partners (3 - 5 people).

+ Collect information: According to the household survey method. Using a general form, information is collected including main groups: (i) social conditions, (ii) economic conditions, (iii) knowledge about medicinal plants (information about plants, exploitation situation and use, (iv) current status of conservation and development of medicinal plants.

- Group discussion: Using PRA tools: Determining the level of danger of medicinal plant species based on the community based on assessment of reserves (common/easy to see, rare/rarely seen, very rare/very rare and extremely rare/extremely rare) and user needs.

2.3.2. Methods of studying the natural ecological characteristics of some medicinal plants at risk that need to be preserved

Investigation of the vegetation, distribution and ecological conditions of a number of medicinal plants is investigated through the quadrats system (Gary J. Martin, 1997), carried out through the following steps:

- *Select samples and define quadrats*

According to the typical method, based on a 1:10,000 scale topographic map of the study area (Table 2.1).

Table 2.1. Describes the location of standard cells

Order	Quadrat's name	Location	Coordinates
1	Quadrat 1	Nui xe (Tram ton)	22º22’24’’N – 103º45’59’’S
2	Quadrat 2	Nui xe (Tram ton)	22º22’22’’N – 103^{0}45’57’’S
3	Quadrat 3	Nui xe (Tram ton)	22º22’20’’N – 103º45’59’’S
4	Quadrat 4	Nui xe (Tram ton)	22º22’14’’N – 103º46’11’’S
5	Quadrat 5	Nui xe (Tram ton)	22º22’16’’N – 103º46’09’’S

A quadrat has a size of 1,000 m^2 (20 x 50 m), selected by a stratified, random method based on the existing vegetation types in the study area. A total of 5 quadrats were set up.

- *Collect information*

Includes 2 main parts: (i) Information about ecological conditions, including: destruction level, altitude, slope, drying direction, plot location, soil type, soil pH, soil moisture, water

regime surface, distance to village, distance to nearest trail, exposed rock cover, boulder cover, crushed rock cover, rock-free soil, litter cover, canopy cover, fresh carpet cover, shrub cover, bamboo and bamboo cover, grass cover, birch tree abundance, vine abundance (ii) Useful tree information, including names and abundance of tree species medicinal herbs need research.

- *Process and analyze information*

Information is obtained through 2 sources: external industry (in the field), and internal industry. Information obtained from the field includes altitude, exposure direction, soil pH, surface water regime, soil type, etc. Information from the internal agriculture section is calculated based on data collected from the field such as the total number of species appearing in the research plot, the number of useful species (of each type), tree density, etc. This information is collected and encrypted.

The ecological survey data were compiled, coded and entered into the computer software PRIMER 5 (Plymouth Routines In Multivariate Ecological Research) into a database of ecological characteristics related to medicinal plants in HLNP. and analyzed using principal axis analysis PCA (Hoft M. et al., 1999).

- *Determine the scientific names of plant species*

Scientific names are determined according to expert methods and comparative morphological methods based on specimens at the herbarium of the Faculty of Forestry - Thai Nguyen University of Agriculture and Forestry, Institute of Tropical Biology, Hanoi National University. Noi, Institute of Ecology and Biological Resources. Scientific names are revised based on the documents Indochina Botanicals (Lecomte H. 1905-1952), Vietnamese Herbs (Pham Hoang Ho, 1999), Vietnamese Medicinal Plants Dictionary (Vo Van Chi, 2021) , List of Vietnamese forest trees (Ministry of Agriculture and Rural Development, 2000), 1900 useful tree species in Vietnam (Tran Dinh Ly, 1993).

2.3.3. Research methods to identify and evaluate genetic diversity of some medicinal plant species at risk that need to be preserved

2.3.3.1. Method for separating total DNA

Leaf DNA from medicinal plant samples was extracted and purified using the improved CTAB method based on the method of P. Doyle and Doyle (1987).

Procedure:

- Prepare CTAB extraction buffer at 60°C.
- Grind 0.3 grams of leaf sample with a sterile ceramic mortar and pestle in liquid nitrogen until it becomes a fine powder (leaf sample, pestle, mortar are kept at - 80°C).
- Dissolve the crushed sample in 800µl CTAB buffer and 60µl SDS 10%. Extraction buffer composition: Tris-base 100 mM, EDTA 20 mM, NaCl 1.4 M, CTAB 2% và PVP 1%.
- Incubate the sample at 65°C in a thermostatic bath for 30 minutes.
- Cool at room temperature and add 200µl of 5M potassium acetate, mix well and incubate on ice for 45 minutes.
- Add an equal volume of chloroform - isoamylalcohol (24:1), shake gently until it forms an emulsion. Centrifuge at 11000 rpm for 30 minutes at 4^0C. Aspirate the above solution and transfer it to a new tube.
- Continue to extract a second time with chloroform: isoamylalcohol (24:1), obtaining the extract containing DNA.
- Precipitate DNA with isopropanol cooled at -20°C for 1 hour.
- Centrifuge at 11,000 rpm for 15 minutes at 4°C to collect the precipitate.
- Wash the precipitate with 70% ethanol, centrifuge the precipitate.
- Dry and dissolve DNA, remove RNA, dissolve DNA in TE buffer.

2.3.3.2. PCR method

Components of a PCR reaction with primer pairs ITS1/ITS2 and Trn-L/Trn-F are used to clone target gene segments of about 400 bp and 480 bp (White et al., 1990, Vijayan et al., 2010) has the following sequence:

ITS1: 5'-TCCGTAGGTGAACCTGCGG-3'

ITS2: 5'- CGA TAC TTG GTG TGA ATT GCA G -3'

Trn-L: 5'- GGT TCA AGT CCC TCT ATC CC-3'

Trn-F: 5'- ATT TGA ACT GGT GAC ACG AG-3'

Each PCR reaction includes the following components (Table 2.2):

Table 2.2. PCR reaction components

Order	Ingredient	Volume (µl)
1	The water is double distilled and deionized	9
2	Buffer Mg^+ 25 Mm	1,5
3	dNTPs 10 Mm	0,3
4	Taq ADN polymerase 5 U/µl	0,2
5	Forward primer 10 µM	1,5
6	Revverse primer 10 µM	1,5
7	DNA 50ng/µl	1
Total volume of a reaction		15,0

Program to run PCR

The PCR reaction was conducted in a 0.2ml Eppendorf tube and performed on a Mastercycler epgradient S machine according to the following cycle (Table 2.3)*:*

Table 2.3. PCR reaction cycle

Steps	Temperature (°C)	Time	Period
1	94	5 m	1
2	94	1 m	35
3	59	45 s	
4	72	50 s	
5	72	7 m	1
6	4	∞	1

After completing the PCR program, the PCR product was added with 4 µl loading dye and then electrophoresis tested the product.

2.3.3.3. Agarose gel electrophoresis method

- Weigh 0.6g of agarose into 40 ml of TAE 1X, heat to boiling so that the agarose dissolves completely. Cool at 45-50°C add 2,5µl Ethidium Bromide, pour into the prepared gel mold. After 30-60 minutes, when the gel has cooled and solidified, transfer the tray containing the gel plate to the electrophoresis machine and add TAE 1X running buffer into the electrophoresis chamber so that the buffer covers the gel plate about 0.5-1 cm.

- Sample application: PCR product is mixed with 4 µl loading

dye and applied to the wells on the gel.

- Run electrophoresis: After completing the electrophoresis sample, the electrophoresis machine is connected to the power supply. Set 120 V.

Observe: the gel is examined under an ultraviolet light, DNA will glow thanks to binding with EtBr.

- PCR products were purified according to the kit manufacturer's instructions (Promega, USA)

2.3.3.4. Sequencing method

Purified PCR products were bidirectionally sequenced using corresponding primers at Firt BASE company (Malaysia). The sequencing process was performed using an ABI/Prism 377 automatic sequencer.

2.3.3.5. Analysis of sequencing data

The obtained sequences were processed using the BioEdit program (Hall, 1999). Contigs from the forward and reverse sequences of each type were constructed. Neighbor-joining method was used to build phylogenetic tree using MEGA5.1 software. Bootstrap analysis (500 repetitions) was used to evaluate the reliability of the phylogenetic tree branches. Comparisons with sequences in the GenBank database were performed through BLASTN searches at the NCBI National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov).

2.3.4. Research methods in proposing conservation solutions

- Research ongoing conservation solutions at HLNP and research medicinal species using the method of inheriting secondary data at agencies, organizations and individuals.

- Based on the research results of the project on the 3 medicinal plant species studied to propose appropriate and feasible solutions.

Chapter 3
RESEARCH RESULTS AND DISCUSSION

3.1. Assessing the endangered status of some medicinal plant species in HLNP

3.1.1. Current status of biodiversity and medicinal plants in HLNP

3.1.1.1. Diversity of plant resources

HLNP is known as a center of biodiversity in the country and in the world. According to data from Hoang Lien National Park, initially 2,847 species of vascular plants belonging to 229 families were counted.

3.2.1.2. Diversity of medicinal plant resources

Based on the plant list at HLNP and documents on medicinal plants in Vietnam, the project has listed 1,025 species of 184 families belonging to 5 divisions used as medicine.

3.1.2. Situation of exploitation and use of medicinal plants in HLNP

Table 3.4. Frequency of taking medicinal plants and number of people/households taking medicinal plants of people in the HLNP area

Frequency of picking medicine plants (household)		**Number of people picking medicinal plants/household (person)**		
Often	No often	01	02	Trên 02
25	45	42	18	10

3.1.3. Indigenous knowledge about the characteristics and uses of some medicinal plants in HLNP

According to survey results of households living in the HLNP area, there are about 45 species of medicinal plants that people often go to collect..

3.1.4. Identifying some rare and endangered medicinal plant species in HLNP

3.1.4.1. Medicinal plant species at risk in HLNP according to IUCN

Data from investigation and research results in table 3.6 shows that: There are 64 species of medicinal plants in HLNP included in the IUCN list, of which: 03 data deficient species (DD), 48 least concern species (LC), 03 near threatened species (NT), 08 vulnerable species (VU) and 02 endangered species (EN).

3.1.4.2. Medicinal species at risk at HLNP according to Vietnam's Red Book

According to the results of the investigation and research at HLNP, there are 42 species of medicinal plants at risk of extinction, specifically: 17 species will be vulnerable (VU), 19 species will be endangered (EN) and 06 species will be critically endangered (CR).

3.1.4.3. Medicinal species at risk at community-based HLNP

From the results of the household survey (Table 3.8), it was determined that in HLNP there are 18 medicinal plants at risk of extinction. All 18 medicinal species are plants that indigenous people often collect to make medicine. Among the 18 plants, there are 02 medicinal plants whose scientific names have not yet been determined: Bong dia bua (Dao language) and Keng pham (Dao language).

Table 3.8. List medicinal plant species at HLNP at risk according to community surveys

Order	Common names	Science name
1	Kim tuyen	Anoectochilus setaceus
2	Dang sam	Codonopsis javanica
3	Tam that hoang	Panax bipinnatifidus
4	Dia bay (Dao language)	Raphidophora sp.
5	Buong dia bua (Dao language)	Chưa xác định
6	Chua tay mhay (Dao language)	Illigera sp1.
7	Ban tay ma	Heliciopsis lobata
8	Hoang lien chan ga	Coptis quinquesecta
9	Hoang tinh hoa do	Polygonatum kingianum
10	Hoang lien gai	Berberis Wallichiana
11	Dia sieu xi (Dao language)	Ficus sp3.
12	Ngong uan mhay (Dao language)	Deris elliptica
13	Tac ke da	Drynaria bonii
14	Tho hoang lien	Thalictrum foliolosum
15	Keng pi (Dao language)	Luculia pinceana
16	Chan chim	Schefflera heptaphylla
17	Keng pam (Dao language)	Chưa xác định

18	No chau đeng (Dao language)	Schefflera chapana

Community-based determination of the level of danger of medicinal plant species is based on an assessment of reserves and user needs, matrixed according to the parameters in table 3.11.

Table 3.11. Assessing the level of endangered medicinal species based on the community

Demand use	Frequency of meeting and finding			
	Easily seen (1)	Rarely seen (2)	Very rarely seen (3)	Extremely rarely seen (4)
Much (4)	(5)	(6)	(7)	(8)
Little (3)	-	(5)	(6)	(7)
Very little (2)	-	-	(5)	(6)
Extremely little (1)	-	-	-	(5)

From the assessment results, a List of medicinal plant species in HLNP that are at risk according to community assessment is compiled (Table 3.12).

Table 3.12. List of medicinal plant species in HLNP that are at risk according to community assessment

Order	Common names	Science name	Score according to community reviews		Total score	Status
			Frequency of meeting	Demand use		
1	Kim tuyen	Anoectochilus setaceus	4	4	8	Critically endangered
2	Dang sam	Codonopsis javanica	4	4	8	Critically endangered
3	Tam that hoang	Panax bipinnatifidus	4	4	8	Critically endangered
4	Dia bay (Dao language)	Raphidophora sp.	4	4	8	Critically endangered
5	Buong dia bua (Dao language)	Chưa xác định	4	4	8	Critically endangered
6	Chua tay mhay	Illigera sp1.	4	4	8	Critically endangered

Order	Common names	Science name	Score according to community reviews		Total score	Status
			Frequency of meeting	Demand use		
	(Dao language)					
7	Ban tay ma	Heliciopsis lobata	4	4	8	Critically endangered
8	Hoang lien chan ga	Coptis quinquesecta	4	3	7	Endangerd
9	Hoang tinh hoa do	Polygonatum kingianum	4	3	7	Endangerd
10	Hoang lien gai	Berberis Wallichiana	4	3	7	Endangerd
11	Dia sieu xi (Dao language)	Ficus sp3.	3	4	7	Endangerd
12	Ngong uan mhay (Dao language)	Deris elliptica	3	4	7	Endangerd
13	Tac ke da	Drynaria bonii	2	4	6	Vulnerable
14	Tho hoang lien	Thalictrum foliolosum	3	3	6	Vulnerable
15	Keng pi (Dao language)	Luculia pinceana	3	3	6	Vulnerable
16	Chan chim	Schefflera heptaphylla	2	3	5	Low risk
17	Keng pam (Dao language)	Chưa xác định	3	2	5	Low risk
18	No chau đeng (Dao language)	Schefflera chapana	1	4	5	Low risk

From the above research results, the project selected 03 medicinal species to evaluate their natural ecological characteristics and genetic diversity to have a basis for conservation measures, which are:

- Heliciopsis lobata (Merr.) Sleum;
- Coptis quinquesecta;
- Polygonatum kingianum Coll et Hemsl.

General assessment part 3.1:

- HLNP is a center of biodiversity in the country and around the world, including many valuable and rare medicinal plants.
- People in the study area have been exploiting rare medicinal plants to the maximum, causing the risk of depletion without any solution for conservation.
- Based on the assessment of IUCN, Vietnam's Red Book, and community assessment, it has been shown that in HLNP, there are 15 medicinal species at risk of extinction, including 07 Critically endangered species, 05 endangered species, 03 Vulnerable species.
- Based on the above assessment, 03 medicinal species were selected to continue assessing natural ecological characteristics and genetic diversity as a basis for conservation measures, which are: Heliciopsis lobata (Merr.) Sleum, Coptis quinquesecta and Polygonatum kingianum Coll et Hemsl.

3.2. The natural ecological characteristics of some medicinal plants at risk need to be preserved

3.2.1. Natural ecological characteristics of Heliciopsis lobata (Merr.) Sleum

3.2.1.1. Botanical characteristics and uses

3.2.1.2. Ecological characteristics

Because only 1 individual of the Heliciopsis lobata (Merr.) Sleum was discovered in the study area, there is not enough basis to evaluate the relationship between ecological factors and other plant

species in the standard plot with the Heliciopsis lobata (Merr.) Sleum.

3.2.2. Natural ecological characteristics of Coptis quinquesecta W. T. Wang

3.2.2.1. Botanical characteristics and uses

3.3.3.2. Ecological characteristics

Analyze the relationship between ecological and environmental factors related to the appearance of Coptis quinquesecta W. T. Wang. The results in Figure 3.9 show that Coptis quinquesecta W. T. Wang has a close relationship with exposure direction, exposed rock cover, soil moisture, and crushed rock cover.

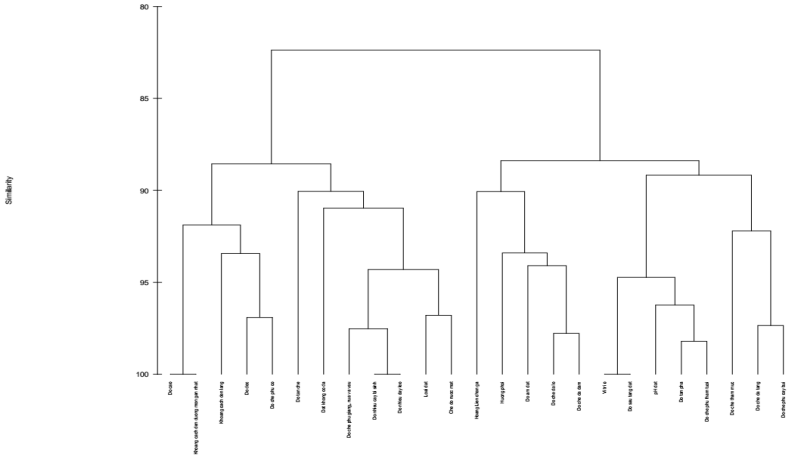


Figure 3.4. The relationship between ecological and environmental factors with the Coptis quinquesecta W. T. Wang

When processing the data compiled from the survey results, an analysis of the relationship between species with Coptis quinquesecta W. T. Wang was conducted. The results in Figure 3.11 show that Coptis quinquesecta W. T. Wang has a close relationship with some

of the following species: Pentapanax fragrans, Amentotaxus yunnanensis, Heliciopsis lobata, Lycopodium cernuum L. (Lycopodiella), Lycopodium hamiltonii, Panax bipinnatifidus, Panax stipuleanatus, Ophiorrhiza sp1., Podocarpus neriifolius.

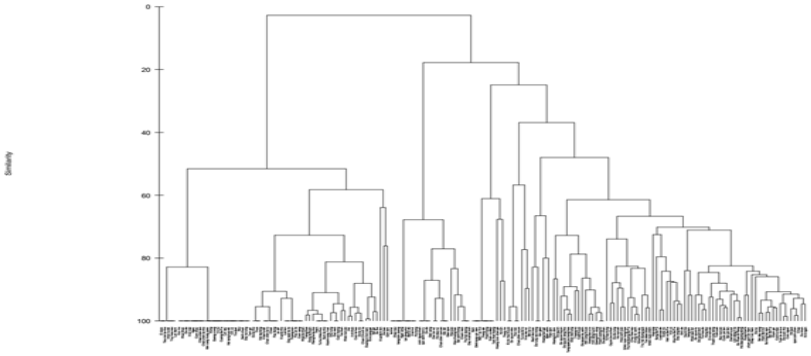


Figure 3.5. Species relationships with Coptis quinquesecta W. T. Wang

3.2.3. Natural ecological characteristics of Polygonatum kingianum Coll et Hemsl.

3.2.3.1. Botanical characteristics and uses

3.2.3.2. Ecological characteristics

Analyze the relationship between factors related to the appearance of Polygonatum kingianum Coll et Hemsl. The results in Figure 3.14 show that Polygonatum kingianum Coll et Hemsl. closely related to the following factors: litter cover, rock cover, shrub cover, plot location, soil depth, soil pH, destruction, fresh litter cover, direction exposure, soil moisture, exposed rock cover and crushed rock cover.

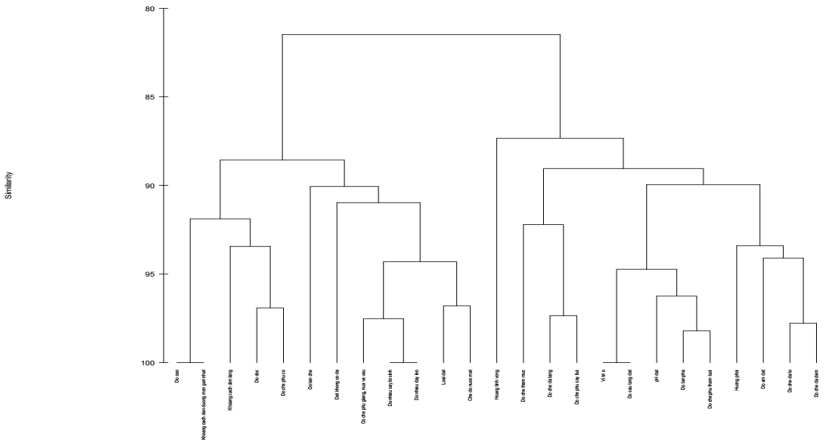


Figure 3.8. The relationship between ecological and environmental factors with Polygonatum kingianum Coll et Hemsl.

When processing the data compiled from the survey results, an analysis of the relationship between species with Polygonatum kingianum Coll et Hemsl was conducted. The results presented in Figure 3.16 show that Polygonatum kingianum Coll et Hemsl. has a close relationship with some of the following species: Acorus verus (A. calamus L.), Rhododendron veichiamum, Smilax granulicaulis, Mahonia nepalensis, Smilax chapaensis, Rhododendron hemsleyaneum,...

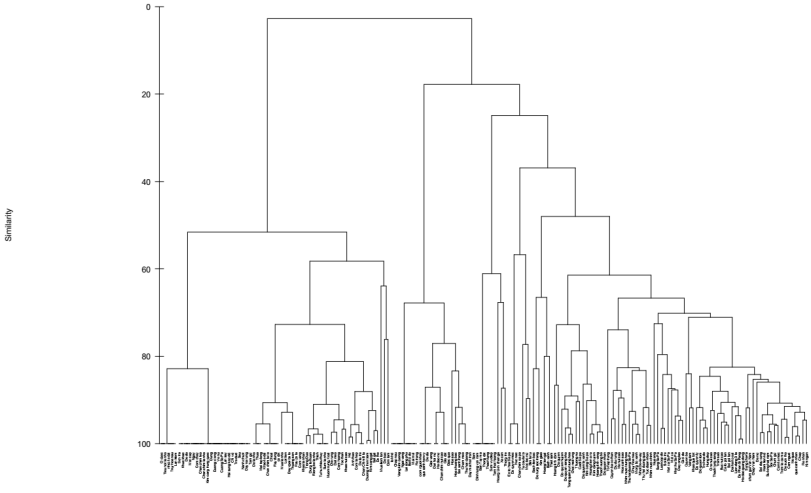


Figure 3.10. Species relationships with Polygonatum kingianum Coll et Hemsl.

General assessment section 3.2:

From the assessment of the natural ecological characteristics of three at-risk medicinal plant species that need to be preserved, a number of ecological and environmental factors and plant species have been found that are related to the distribution of plant species. this medicine. This is an important basis for implementing in-situ or ex-situ conservation. Specifically:

- Heliciopsis lobata (Merr.) Sleum is a very rare tree in the HLNP area. Heliciopsis lobata (Merr.) Sleum only appeared in 1 out of 5 quadrats, reaching a rate of 20 %, with a total of 1 individual detected.

- Coptis quinquesecta W. T. Wang is a fairly rare tree in the HLNP area. Coptis quinquesecta W. T. Wang appeared in 2 out of 5 quadrats, reaching a rate of 40%, with a total of 5 individuals discovered. Coptis quinquesecta W. T. Wang is closely related to the factors of exposure direction, exposed rock coverage, soil moisture, and crushed rock coverage. Coptis quinquesecta W. T. Wang has a close relationship with a number of species such as Pentapanax fragrans, Amentotaxus yunnanensis, Heliciopsis lobata, Lycopodium cernuum L. (Lycopodiella), Lycopodium hamiltonii, Panax

bipinnatifidus, Panax stipuleanatus, Ophiorrhiza sp1., Podocarpus neriifolius.

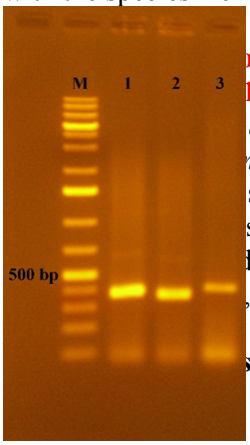
- Polygonatum kingianum Coll et Hemsl. is a quite rare tree in the HLNP area. Polygonatum kingianum Coll et Hemsl. appeared in 02 out of 05 quadrats, reaching a rate of 40%, with a total of 09 individuals detected. Polygonatum kingianum Coll et Hemsl. closely related to the following factors: litter cover, rock cover, shrub cover, plot location, soil depth, soil pH, destruction, fresh litter cover, direction exposure, soil moisture, exposed rock cover and crushed rock cover and is closely related to many other medicinal species.

3.3. Results of identification and assessment of genetic diversity of some medicinal plant species at risk that need to be preserved

3.3.1. Results of identification and assessment of genetic diversity of Heliciopsis lobata (Merr.) Sleum based on ITS sequence

3.3.1.1. Results of identification of Heliciopsis lobata (Merr.) Sleum based on ITS sequence

The results showed that the obtained gene sequence was similar to the sequence of Heliciopsis lobata species (Genbank code OQ220917.1) with 85% coverage, 98.32% similarity rate. This result proves that the samples collected in Lao Cai have a close relationship with the species Heliciopsis lobata.



ophoresis images of Heliciopsis lobata (Merr.)
1.5% agarose gel with primer pair ITS1/ITS2

assessment of genetic diversity of Heliciopsis
n based on ITS sequence

studying the genetic diversity of the human gene
s lobata (Merr.) Sleum will provide additional
le sequence of the gene region, serving as a basis
, replication and conservation of these medicinal

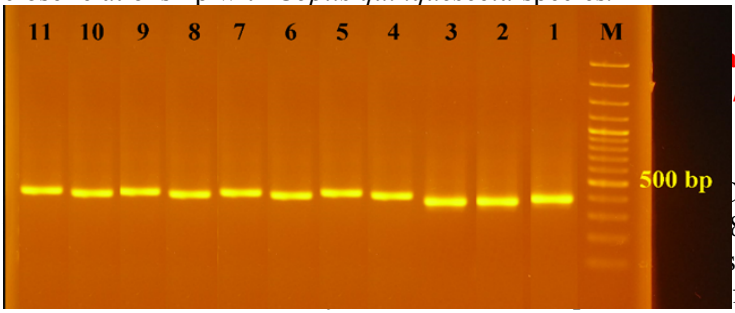
se genetic distance between Heliciopsis lobata
and reference species using the Kimura-2
parameter model

	OQ220917.1 _Heliciopsis _lobata	EU642791.1 _Virotia_neu rophylla	EU642792.1 _Virotia_lept ophylla	OQ355468.1 _Heliciopsis _lobata	EU642789.1 _Heliciopsis _lobata	EU642793.1 _Athertonia_ diversifolia	EU642794.1 _Catalepidia _heyana	EU642788.1 Malagasia_alt icola	KT006051.1 _Lomatia_tin ctoria	EU642802.1 _Macadamia _jansenii	EU642801_ Macadamia_t ernifolia	EU642798.1 _Macadamia _integrifolia	BTM-ITS1
OQ220917.1_Heliciopsis_lobata													
EU642791.1_Virotia_neurophylla	0.065												
EU642792.1_Virotia_leptophylla	0.064	0.018											
OQ355468.1_Heliciopsis_lobata	0.051	0.059	0.059										
EU642789.1_Heliciopsis_lobata	0.051	0.056	0.056	0.000									
EU642793.1_Athertonia_diversifoli	0.103	0.061	0.061	0.078	0.079								
EU642794.1_Catalepidia_heyana	0.108	0.082	0.086	0.080	0.077	0.111							
EU642788.1Malagasia_alticola	0.148	0.116	0.116	0.115	0.112	0.144	0.028						
KT006051.1_Lomatia_tinctoria	0.129	0.109	0.113	0.107	0.100	0.136	0.098	0.124					
EU642802.1_Macadamia_jansenii	0.143	0.109	0.113	0.116	0.112	0.149	0.124	0.148	0.151				
EU642801_Macadamia_ternifolia	0.143	0.109	0.113	0.116	0.112	0.149	0.124	0.148	0.151	0.000			
EU642798.1_Macadamia_integrifol	0.143	0.109	0.113	0.116	0.113	0.149	0.125	0.148	0.151	0.007	0.007		
BTM-ITS1	1.167	1.301	1.292	1.256	1.264	1.336	1.238	1.373	1.208	1.213	1.213	1.229	

3.3.2. Results of identification and genetic diversity assessment of Coptis quinquesecta W. T. Wang samples based on ITS sequences

3.3.2.1. Results of identification of Coptis quinquesecta W. T. Wang samples based on ITS sequences

This result proves that the samples collected in Lao Cai have a close relationship with *Coptis quinquesecta* species.



W. T.
/ITS2
Coptis
Cai and
8. This
samples
ference

samples, it is quite high (Table 3.19).

Table 3.19. Pairwise genetic distance between Coptis quinquesecta samples using the Kimura-2 parameter model

	KX714731.1	KX714729.1	X714727.1	KX714733.1	KY780750.1	KC815158.1	HL1	HL2	HL3	HL4	HL5	HL6	HL7	HL8	HL9	HL10	HL11
KX714731.1																	
KX714729.1	0.003																
X714727.1	0.003	0.007															
KX714733.1	0.007	0.010	0.010														
KY780750.1	0.045	0.049	0.049	0.053													
KC815158.1	0.045	0.049	0.049	0.053	0.000												
HL1	1.685	1.711	1.661	1.599	1.546	1.546											
HL2	1.720	1.748	1.695	1.629	1.574	1.574	0.003										
HL3	1.685	1.711	1.661	1.599	1.546	1.546	0.000	0.003									
HL4	1.685	1.711	1.661	1.599	1.546	1.546	0.000	0.003	0.000								
HL5	1.685	1.711	1.661	1.599	1.546	1.546	0.000	0.003	0.000	0.000							
HL6	1.685	1.711	1.661	1.599	1.546	1.546	0.017	0.013	0.017	0.017	0.017						
HL7	1.685	1.711	1.661	1.599	1.546	1.546	0.017	0.013	0.017	0.017	0.017	0.000					
HL8	1.652	1.676	1.629	1.570	1.519	1.519	0.013	0.017	0.013	0.013	0.013	0.003	0.003				
HL9	1.685	1.711	1.661	1.599	1.546	1.546	0.010	0.013	0.010	0.010	0.010	0.013	0.013	0.010			
HL10	1.720	1.748	1.695	1.629	1.574	1.574	0.013	0.010	0.013	0.013	0.013	0.010	0.010	0.013	0.003		
HL11	1.685	1.711	1.661	1.599	1.546	1.546	0.010	0.013	0.010	0.010	0.010	0.013	0.013	0.010	0.000	0.003	

3.3.3. Results of identification and genetic diversity assessment of Polygonatum kingianum Coll et Hemsl. based on the TrnL-TrnF IGS sequence

3.3.3.1. Results of identification of Polygonatum kingianum Coll et Hemsl. based on the TrnL-TrnF IGS sequence

Comparison results with sequences available on Genbank show that the obtained samples have 100% similarity with the species Polygonatum kingianum (code OQ532971.1).

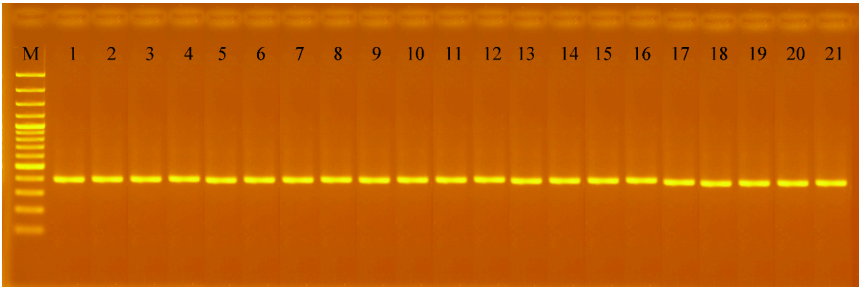


Figure 3.16. Electrophoresis image of 1.5% agarose gel sample with primer pair trnL/trnF

3.3.3.2. Results genetic diversity assessment of Polygonatum kingianum Coll et Hemsl. based on the TrnL-TrnF IGS sequence

Results of sequence analysis of 21 Polygonatum kingianum samples Coll et Hemsl. showed that the average nucleotide composition of the trnL-F region was 36.22% A, 12.7% C, 17.57% G and 33.51% T. The number of nucleotides in each sequence segment varied between samples. This difference may be due to insertion or deletion at some positions in the gene segment (Ha et al., 2022).

Table 3.22. Pairwise genetic distance between samples of Polygonatum kingianum Coll et Hemsl. using the Kimura-2 parameter model

	HTD_1	HTD_2	HTD_3	HTD_4	HTD_5	HTD_6	HTD_7	HTD_8	HTD_9	HTD_10	HTD_11	HTD_13	HTD_14	HTD_15	HTD_16	HTD_17	HTD_18	HTD_19	HTD_20	HTD_21	OQ532971.1	MW566447.1	MZ286326.1	OQ532966.1	ON872712.1	MZ286324.1
HTD_1																										
HTD_2	0.000																									
HTD_3	0.000	0.000																								
HTD_4	0.000	0.000	0.000																							
HTD_5	0.000	0.000	0.000	0.000																						
HTD_6	0.000	0.000	0.000	0.000	0.000																					
HTD_7	0.000	0.000	0.000	0.000	0.000	0.000																				
HTD_8	0.000	0.000	0.000	0.000	0.000	0.000	0.000																			
HTD_9	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000																		
HTD_10	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000																	
HTD_11	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.000	0.003	0.003																
HTD_13	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000															
HTD_14	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.000	0.003	0.003	0.000	0.000														
HTD_15	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000													
HTD_16	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000												
HTD_17	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000											
HTD_18	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000										
HTD_19	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.000	0.003	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000									
HTD_20	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000								
HTD_21	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000							
OQ532971.1	1.033	1.037	1.044	1.029	1.022	1.033	1.050	1.041	1.045	1.053	1.048	1.037	1.045	1.048	1.022	1.015	1.026	1.045	1.022	1.022						
MW566447.1	1.033	1.037	1.044	1.029	1.022	1.033	1.050	1.041	1.045	1.053	1.048	1.037	1.045	1.048	1.022	1.015	1.026	1.045	1.022	1.022	0.000					
MZ286326.1	1.033	1.037	1.044	1.029	1.022	1.033	1.050	1.041	1.045	1.053	1.048	1.037	1.045	1.048	1.022	1.015	1.026	1.045	1.022	1.022	0.000	0.000				
OQ532966.1	1.030	1.034	1.041	1.026	1.019	1.030	1.047	1.038	1.043	1.050	1.045	1.034	1.043	1.045	1.019	1.012	1.023	1.043	1.019	1.019	0.003	0.003	0.003			
ON872712.1	1.030	1.034	1.041	1.026	1.019	1.030	1.047	1.038	1.043	1.050	1.045	1.034	1.043	1.045	1.019	1.012	1.023	1.043	1.019	1.019	0.003	0.003	0.003	0.000		
MZ286324.1	1.030	1.034	1.041	1.026	1.019	1.030	1.047	1.038	1.043	1.050	1.045	1.034	1.043	1.045	1.019	1.012	1.023	1.043	1.019	1.019	0.003	0.003	0.003	0.000	0.000	

General assessment of section 3.3:

The study conducted an assessment of the genetic diversity of three highly endangered and rare medicinal plant species, Heliciopsis lobata (Merr.) Sleum, Coptis quinquesecta W. T. Wang and Polygonatum kingianum Coll et Hemsl. by molecular biological markers. Specifically:

- Provided valuable barcodes to develop molecular markers to distinguish medicinal plant species in HLNP from other types and for phylogenetic studies of those plants in Lao Cai.

- The level of genetic diversity of the above tree species is relatively low, so ex-situ conservation is difficult.

3.4. Some solutions for preserving medicinal plants at HLNP

3.4.1. Theoretical basis of proposed solution

In the study of plant diversity conservation in general, and medicinal plants in particular, there are many influencing factors. It is necessary to consider and select factors that have a direct and decisive impact in order to find an effective solution. First, it is necessary to determine the current status of the research area of medicinal species that are at high risk of extinction to distinguish

between very endangered and endangered species that need to be conserved immediately.

Next, from high-risk species, it is necessary to have results to determine ecosystem diversity, species diversity and genetic diversity. This is also the scientific basis for choosing solutions to prevent degradation and preserve them.

Different ecosystems have different species diversity, and a species in different ecosystems has different genetic diversity. The application of molecular biological indicators to genetic diversity research to determine the level of genetic diversity is important in identifying species at risk of extinction due to their high genetic diversity. Low transmission often has poor adaptability to environmental conditions.

In addition, it is necessary to consider the specific socio-economic conditions of the area needing conservation. Of particular note are population density, farming practices and exploitation of medicinal plants... to have appropriate solutions for the conservation of existing endangered species.

3.4.2. Proposed Solutions

3.4.2.1. Group of solutions on policy mechanisms

3.4.2.2. Human resource development solution group

3.4.2.3. Science and technology solution group

3.4.2.4. Solution group on Information - Education - Communication and attracting the community to participate in biodiversity conservation

CONCLUSIONS AND RECOMMENDATIONS

1. Conclusions

1.1. Based on the assessment of the current situation by community surveys, the risk of extinction of 15 rare medicinal medicinal species in HLNP, Lao Cai province has been identified,

including 07 critically endangered species; 05 endangered species and 03 vulnerable species will be endangered.

1.2. Identifying a number of ecological - environmental factors and plant species related to the distribution of medicinal plants: *Heliciopsis lobata* (Merr.) Sleum, *Coptis quinquesecta* W. T. Wang and *Polygonatum kingianum* Coll et Hemsl.

1.3. The level of genetic diversity has been evaluated and the molecular identification markers (barcodes) of three species Heliciopsis lobata (Merr.) Sleum, Coptis quinquesecta W. T. Wang and Polygonatum kingianum Coll et Hemsl have been identified.

1.4. Based on the research results, optimal solutions have been proposed to preserve three medicinal plant species at high risk of extinction in Hoang Lien National Park: Heliciopsis lobata (Merr.) Sleum, Coptis quinquesecta W. T. Wang and Polygonatum kingianum Coll et Hemsl.

2. Recommendations

2.1. It is necessary to continue conservation research at HLNP, especially research related to medicinal plants.

2.2. There needs to be practical and special activities to conserve endangered species in HLNP, which should focus on educational activities and raising people's awareness in conservation and environmental protection activities.

2.3. HLNP management activities should be viewed and evaluated from both aspects: value and threat from human exploitation activities.

2.4. It is necessary to conduct further research on endangered medicinal plant species, especially genetic studies using molecular biological markers.