SURVEYING THE ORIGIN AND BIOLOGICAL CHARACTERISTICS OF *STEPHANIA BRACHYANDRA* (DIELS) GENE SOURCES IN THAI NGUYEN

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ARTICLE INFO

Received: 19/6/2024

Revised: 16/10/2024

Published: 17/10/2024

KEYWORDS

Stephania brachyandra
Origin
Biological characteristics
Distribution
Gene sources

ABSTRACT

This study focuses on the biological characteristics of the genetic resources of the *Stephania brachyandra* in Thai Nguyen, one of Vietnam's valuable medicinal plants currently at risk of extinction. Investigation results indicate that *Stephania brachyandra* is commonly found at an average density of 32.57 plants per square kilometer, with distribution density varying across districts, being highest in Vo Nhai district and lowest in Phu Luong district. This species primarily thrives in rocky mountain forests and regenerating land after extension exploitation. The plant is predominantly found above attitudes 250 m, followed by altitudes between 200-250 m, and is least common at altitudes below 200 m. The average shrub cover density in the Open Top Chambers (OTCs) with *Stephania brachyandra* populations surveyed across the districts is 18%, significantly lower compared to other plant species such as: Mua, Sim, Don nem, and Tu chau. The average ground cover density is 15.8%, mainly consisting of weeds such as grass, daisies, bamboo grass, pig bristle grass, and betel leaf. Regarding soil, the thickness of the A0 horizon varies from 1 to 4 cm. The A horizon has an average thickness of 30.3 cm, and is reddish-brown, moist and friable, with low rock content. The B horizon has an average thickness of 60 cm, and is yellow-brown, moist, slightly compact, with higher rock content. The chemical composition of the soil ranges from medium to nutrient-poor.

KHẢO SÁT NGUỒN GỐC, ĐẶC ĐIỂM SINH HỌC NGUỒN GEN CÂY BÌNH VÔI NHỊ NGẮN TẠI THÁI NGUYÊN

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THÔNG TIN BÀI BÁO

Ngày nhận bài: 19/6/2024

Ngày hoàn thiện: 16/10/2024

Ngày đăng: 17/10/2024

TỪ KHÓA

Bình vôi
Nguồn gốc
Đặc điểm sinh học
Phân bố
Nguồn gen

TÓM TẮT

Nghiên cứu này tập trung vào các đặc điểm sinh học nguồn gen cây bình vôi nhị ngắn tại Thái Nguyên, một trong số những loài cây thuốc quý của Việt Nam và đang có nguy cơ tuyệt chủng. Qua kết quả điều tra cho thấy Bình vôi thường được tìm thấy ở mật độ trung bình là 32,57 cây/1 km^2, với mật độ phân bố dao động qua các huyện, cao nhất là ở huyện Võ Nhai và thấp nhất là ở huyện Phú Lương. Loài này chủ yếu sống ở các khu rừng núi đá và đất đang tái sinh sau khi bị khai thác mạnh mẽ. Loài cây này chủ yếu tập trung ở độ cao > 250 m, tiếp đến là ở độ cao từ 200-250 m và ít phổ biến nhất ở độ cao < 200 m. Tỷ lệ che phủ của cây bụi trung bình trong các ô tiêu chuẩn (OTCs) có phân bố của loài Bình vôi nhị ngắn ở các huyện đã được khảo sát là 18%, thấp hơn đáng kể so với các loài cây khác như Mua, Sim, Đơn nem và Tu châu. Mật độ che phủ trung bình của lớp thảm tươi là 15,8%, chủ yếu bao gồm cỏ dại như Cỏ, Cúc, Cỏ Tre, Cỏ Lông Lợn và Lá Lốt. Về đất đai, độ dày tầng A0 biến động từ 1-4 cm, tầng A có độ dày trung bình là 30,3 cm, màu nâu đỏ, ẩm và xốp, có hàm lượng đá thấp. Tầng B có độ dày trung bình là 60 cm, màu nâu vàng, ẩm, hơi chặt, có hàm lượng đá cao hơn. Thành phần hóa học của đất biến động từ trung bình đến nghèo chất dinh dưỡng.

DOI: https://doi.org/10.34238/tnu-jst.10632

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1. Introduction

The term *Stephania brachyandra* (Diels) refers to a species of climbing plants with tuberous roots belonging to the genus *Stephania*, family Menispermaceae [1]. Globally, the genus *Stephania* comprises over 50 species, while in Vietnam, there are about 14 to 16 species widely distributed across the northern, central, and southern regions, commonly found in limestone mountainous areas such as Tuyen Quang, Hoa Binh, Cao Bang, Thai Nguyen, Thanh Hoa, Lam Dong, and Ba Ria – Vung Tau [1].

The scientific name is *Stephania brachyandra*, belonging to the family Menispermaceae. It is a climbing plant primarily distributed in China and Southeast Asian countries, including Vietnam. The *Stephania brachyandra* tuber contains various alkaloids, among which the compound rotundin (or L-tetrahydropalmatine) is present in large amounts [2], [3]. Traditionally, in ancient medicine, the *Stephania brachyandra* was used for its medicinal properties. The tuber was used in decoctions or alcohol infusions to treat insomnia, provide sedation, relieve headaches, and alleviate abdominal pain. Currently, rotundin is mainly used to treat insomnia and provide sedation. Natural rotundin has prominent advantages such as low toxicity, good drug tolerance, the ability to induce physiological sleep, and helping to restore memory [4]-[9]. Rotundin is a pharmaceutical compound extracted from the tuber of the *Stephania brachyandra*. According to healthcare industry statistics, Vietnam imports about 30 tons of rotundin annually, valued at approximately 180 billion VND [10], [11].

Currently, the demand of rotundin from the *Stephania brachyandra* tuber is increasing in Vietnam and worldwide. The Military Medical Academy has successfully researched the semi-synthesis of rotundin into rotundin sulfate for the production of injectable drugs. Commercial products containing rotundin and rotundin sulfate such as Brachyandra, Sen vong, Roxen, and Nightqueen have become increasingly available in the market. However, the source of *Stephania brachyandra* tubers in Vietnam is depleting due to unregulated and excessive exploitation. Therefore, it is essential to survey and identify *Stephania brachyandra* species with high rotundin content, then develop conservation and propagation plans for the *Stephania brachyandra*. The study "Surveying the origin, biological characteristics, yield, and quality of the genetic resources of the *Stephania brachyandra*" was conducted to preserve the genetic resources of the *Stephania brachyandra* in Thai Nguyen province.

2. Materials and methods

2.1. Materials

Study on the origin and biological characteristics of the genetic resource of *Stephania brachyandra* in Thai Nguyen.

2.2. Methods

2.2.1. Approach

Stephania brachyandra a rare plant, primarily within limestone mountains, hilly terrain, and lowland areas of limestone ranges. Therefore, research and conservation efforts focus mainly on natural forests. An interdisciplinary research approach is the most effective method, encompassing botanical classification, non-timber forest product surveys, biotechnology, and medicinal plant cultivation. From describing morphological characteristics to genetic traits and propagation and cultivation techniques, all methods are applied to conserve this species [12].

2.2.2. Surveying genetic resources by ecological region, altitude, and forest condition

Step 1: Conduct research involving local residents, forest rangers, and commune forestry officers to identify areas or locations where *Stephania brachyandra* is found.

Step 2: Perform field surveys using the transect survey method to determine the distribution

density by counting the number of *Stephania brachyandra* found along the total length and width of the survey transects. Collect soil samples and measure the size and morphological characteristics of the plants.

Step 3: Conduct a preliminary selection of mother plants for Rotundin analysis. Collect tuber samples and transport them to the analysis site for Rotundin content determination, with a total of 160 samples collected.

2.2.3. Supplementary investigation of the biological characteristics of the Stephania brachyandra

Table 1. *Summary of Survey Transects*

District	Dinh Hoa	Phu Luong	Vo Nhai	Dai Tu	Dong Hy
No. of Transects	8	7	10	4	4

The methods for soil sampling, preservation, and analysis were carried out according to proper standards. On each survey transect, five soil profiles were selected at locations where *Stephania brachyandra* was distributed. Soil profiles were then excavated and key indicators described, such as the average thickness of the soil layer, color, moisture, porosity, proportion of exposed rocks and rock fragments, as well as mechanical composition. The soil samples were then transferred to the Soil Analysis Laboratory of the Northern Mountainous Agriculture and Forestry Science Institute for analysis. Simultaneously, the biological characteristics of the *Stephania brachyandra* were also investigated, including the size of the stem and leaves, the structure and color of the flowers and fruits [12].

2.3. Data Analysis

The data resulting from this study were analyzed using SPSS and Microsoft Excel software.

3. Results

3.1. Distribution of the Stephania brachyandra species according to forest condition

3.1.1. Distribution of Stephania brachyandra species by forest condition

Based on the data from Table 2 and Figure 1, it can be observed that the number of *Stephania brachyandra* individuals across the survey transects is relatively low, averaging 32.57 plants per square kilometer (with a transect width of 20 m and a length of 639 km). The distribution density varies unevenly among districts, with the highest density in Vo Nhai district and the lowest in Phu Luong district. *Stephania brachyandra* are predominantly inhabit in rocky mountain forests and land mountain forests in recovering forest conditions, which have been heavily exploited. The weight of *Stephania brachyandra* tubers ranges from 0.3 to 4.2 kilograms per tuber. From the survey results, it can be concluded that *Stephania brachyandra* typically thrive in natural forest environments and are not concentrated in clearings. The survey results indicates that *Stephania brachyandra* are shade-tolerant species.

Table 2. *The distribution density of Stephania brachyandra in Thai Nguyen province*

District	No. of Survey Transects	Total Length (km)	No. of Plants Observed	Density (plants/km2)	Forest Condition
Dinh Hoa	8	143.9	73	25.36	Limestone mountains, IB, IC, IIA, IIB, IIIA1
Dai Tu	4	100.8	79	39.18	IIA, IIB, IIIA1
Dong Hy	4	94.8	55	29.01	IIA, IIB, IIIA1, Limestone mountains
Phu Luong	7	134.2	61	22.73	IIA, IIB, IIIA1, Limestone mountains
Vo Nhai	10	165.3	154	46.58	Limestone mountains, IIA, IIB, IIIA1, IIIA2, IIIB
Total	33	639.0	422	32.57	-

Source: Compilation of survey data

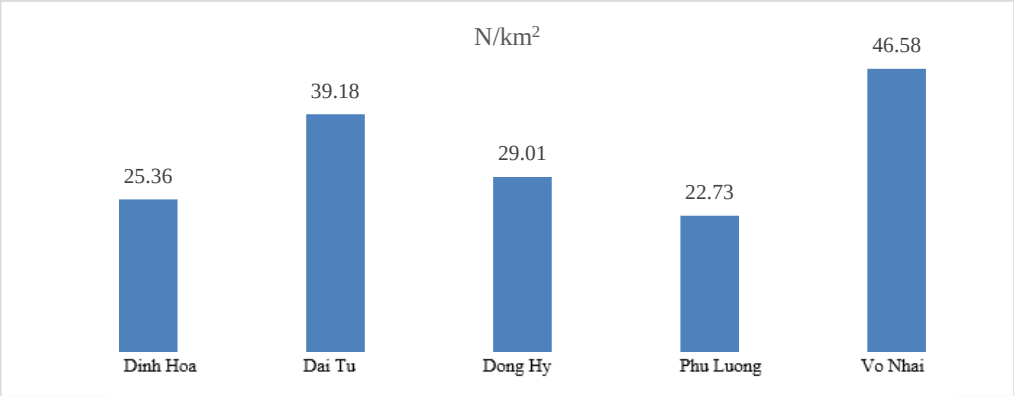

Figure 1. *Distribution density of Stephania brachyandra in Thai Nguyen province*

The larger tubers are predominantly concentrated in the rocky mountain forests of Vo Nhai and Dinh Hoa, where difficult terrain poses significant challenging. In recent years, the reason behind is the extensive exploitation of *Stephania brachyandra* tubers to sell to middlemen who then supply them to Rotudin production facilities. According to local residents, about 5-7 years ago, each household would harvest 500 - 2000 kg of tubers annually from natural forests, with some tubers weighing as much as 50 to 60 kilograms.

3.1.2. Distribution by ecological form

Based on the survey results, the study has classified the ecological types as follows: limestone mountain forests, natural upland forests, and regenerated forests after shifting cultivation. The research findings are summarized in Table 3.

Table 3. *Distribution of Stephania brachyandra by ecological forms*

No.	Ecological Form					
	Limestone Mountain Forests		Natural Upland Forests		Regenerated Forests	
	Quantity	Percentage (%)	Quantity	Percentage (%)	Quantity	Percentage (%)
1	184	43.60	236	55.93	2	0.47

(Source: Compilation of survey data)

The data from Table 3 indicates that the *Stephania brachyandra* species is primarily distributed in limestone mountain forest and natural upland forest ecological forms. Although the percentage of *Stephania brachyandra* detected in natural upland forests is higher than the percentage found in limestone mountain forests it is noted that limestone mountain forests in Thai Nguyen account for only about a quarter of the total natural forest area in the province.

3.1.3. Distribution by altitude

Table 4. *Distribution of Stephania brachyandra by altitude*

No.	Elevation levels (m)					
	< 200		200-250		> 250	
	Quantity	Percentage (%)	Quantity	Percentage (%)	Quantity	Percentage (%)
1	25	5.92	128	30.33	269	63.75

(Source: Compilation of survey data)

The aggregated data in Table 4 indicates that *Stephania brachyandra* is predominantly distributed at altitudes above 250 m, followed by altitudes of 200-250 meters, and least at altitudes below 200 meters. This phenomenon is due to the fact that in areas with lower altitudes, accessibility is convenient, leading to extensive exploitation by local residents. Consequently, only very small tubers weights, usually ranging from 0.2 to 0.4 kg, remain. As altitude increases, the

search and exploitation become more challenging, resulting in a higher number of Stephanie *brachyandra* remaining compared to lowland hilly areas.

3.1.4. Characteristics of shrubs and ground cover in areas where Stephania brachyandra is distributed

The survey data on regeneration, shrubs, and ground cover have been compiled in Table 5.

Table 5. *Average coverage of shrubs and ground cover in areas where Stephania brachyandra is distributed*

Description	Unit	Phu Luong	Vo Nhai	Dinh Hoa	Dong Hy	Dai Tu	Average
Shrubs	%	25	20	13	10	22	18
Ground cover	%	30	14	13	15	7	15.8

(Source: Compilation of survey data)

The data in Table 5 indicates that the average coverage of shrubs in the Out-of-Trial Control (OTC) areas where *Stephania brachyandra* is distributed across the surveyed districts is 18%. This is a relatively low coverage level, primarily influenced by species such as Mua, Sim, Don nem, Tu chau,… When evaluating for the richness of the shrub layer with such low coverage, the regeneration and growth potential of naturally regenerating *Stephania* seedlings are impacted. The average ground cover is 15.8%, with dominant species being weeds, grasses, bamboo grass, hairy grass, and betel leaves. When evaluating the diversity of the climbing plant and ground cover layer (Drude), this was also observed in the shrub layer. With such low coverage, it certainly affects the regeneration capacity and quality of *Stephania brachyandra* regeneration.

3.2. Soil physical and chemical properties

The results of the soil chemical analysis in areas where *Stephania brachyandra* is distributed have been compiled in Table 6. Based on the analysis parameters and assessment of the composition and pH level, the following characteristics are observed. The total nitrogen content in the soil ranges from 0.111% to 0.261%, with readily soluble nitrogen levels ranging from 3.997 mg/100 grams. The total phosphorus content in the soil varies from 0.021% to 0.088%, with readily soluble phosphorus levels fluctuating between 5.820 and 39.930 mg/100 grams. The total potassium content in the soil ranges from 0.082% to 0.524%, with readily soluble potassium levels ranging from 1.266 to 6.991 mg/100 grams. The pH index (KCI) of the soil varies from 4.98 to 6.24, indicating a neutral pH soil. The organic matter content in the soil ranges from 1.43 to 3.89%, which is an average indicator for plant growth and development. From these parameters, it can be observed that the soil in areas where *Stephania brachyandra* is distributed varies from moderately to poor in nutrient content. These characteristics may affect the growth and development of Stephania brachyandra.

Table 6. *Results of soil chemical analysis in areas where Stephania brachyandra is distributed*

No.	Sample code	Analysis indicators							
		$pH_{(Kcl)}$	OM (%)	N_{ts} (%)	P_{ts} (%)	K_{ts} (%)	Ndt (mg/100g)	P_{dt} (mg/100g)	K_{dt} (mg/100g)
1	1	5.82	1.980	0.188	0.059	0.159	5.210	31.220	1.421
2	2	5.77	1.590	0.261	0.054	0.113	4.112	22.550	1.266
3	3	5.67	2.050	0.140	0.021	0.193	4.200	9.033	4.667
4	4	5.91	2.570	0.222	0.076	0.177	3.994	7.556	3.551
5	5	5.98	2.310	0.205	0.044	0.211	4.900	22.412	3.556
6	6	5.88	2.860	0.211	0.056	0.128	4.577	21.431	1.998
7	7	5.79	2.760	0.189	0.031	0.112	4.612	13.552	2.331
8	8	5.22	2.570	0.199	0.066	0.099	3.997	27.912	1.227
9	9	4.98	2.980	0.168	0.088	0.082	4.669	29.990	3.926
10	10	5.62	2.770	0.194	0.077	0.121	4.055	26.662	3.542
11	11	5.77	2.810	0.197	0.087	0.133	5.221	9.662	2.314

No.	Sample code	Analysis indicators							
		$pH_{(Kcl)}$	OM (%)	N_{ts} (%)	P_{ts} (%)	K_{ts} (%)	Ndt (mg/100g)	P_{dt} (mg/100g)	K_{dt} (mg/100g)
12	12	5.75	2.490	0.231	0.048	0.122	4.400	8.941	1.890
13	13	5.48	2.550	0.163	0.032	0.146	4.337	12.881	2.188
14	14	5.81	1.750	0.221	0.062	0.524	5.341	42.720	2.816
15	15	6.24	1.880	0.140	0.087	0.441	5.233	35.623	2.832
16	16	5.77	0.890	0.133	0.036	0.351	6.300	39.160	2.631
17	17	5.89	1.420	0.113	0.033	0.355	6.123	49.552	2.446
18	18	5.73	2.530	0.140	0.033	0.178	4.300	23.221	4.291
19	19	5.46	2.230	0.111	0.049	0.221	4.667	12.331	3.995
20	20	5.87	2.160	0.135	0.041	0.229	4.900	39.930	3.225
21	21	4.88	2.440	0.180	0.032	0.243	4.881	37.991	2.099
22	22	5.71	2.120	0.237	0.038	0.279	4.200	5.820	1.998
23	23	5.87	2.160	0.180	0.067	0.278	5.994	6.889	3.445
24	24	5.95	2.080	0.148	0.036	0.268	6.100	7.560	2.331
25	25	4.99	2.370	0.231	0.077	0.099	6.033	7.998	4.668
26	26	5.73	2.210	0.168	0.077	0.331	6.300	12.480	5.321
27	27	5.68	2.490	0.152	0.042	0.367	5.331	11.097	6.991
28	28	5.67	2.470	0.111	0.059	0.211	4.445	10.570	5.221
29	29	5.44	3.560	0.111	0.068	0.267	4.337	10.661	5.443
30	30	5.96	2.550	0.271	0.077	0.223	5.032	36.871	4.993

(Source: Compilation of survey data)

3.3. The results of additional surveying on the biological characteristics of Stephania brachyandra

The results of additional surveying on the biological characteristics of *Stephania brachyandra* are summarized from Table 7 and images of the leaves, flowers, fruits, and seeds of *Stephania brachyandra*. From the data in Table 7, we can observe several important characteristics of *Stephania brachyand.*

The plant has a twining vine stem, typically evergreen and long-lived. The length of the stem ranges from 1.3 to 4.5 meters, with some cases reaching up to 6 meters. The stem diameter at the root collar is usually between 0.5 and 0.8 cm. The leaves of the plant are alternate and lanceolate with petiole length ranging from 3.3 to 5.4 cm and leaf blade length from 9 to 12 cm, and width from 5 to 7.5 cm. *Stephania brachyandra* bears flowers in axillary panicles, often found in leaf axils or on deciduous branches. Male flowers typically have 5-6 sepals and 3-4 petals, while female flowers usually have 1 sepal and 2 petals. The fruit of *Stephania brachyandra* is ovoid, broad, and slightly flattened, ranging in size from 0.6 to 0.8 cm. When ripe, the fruit turns red. The seeds have hard shell husks, dark brown to black in color, with approximately 3900 - 4000 seeds per 100 g. The weight of 1000 fresh seeds with husks is 122.61 grams, and the kernel weight after removing impurities is 23.84 grams. The tubers of *Stephania brachyandra* have dark brown outer skin, and the tubers reveal an interior of grayish-white coloration. The biological characteristics of *Stephania brachyandra* in this study do not differ significantly from descriptions in forest botanical literature. However, some aspects such as stem length, leaf size, and duration of fruit ripening appear to be longer than traditionally described.

Table 7. *Summary of additional survey results on the biological characteristics of Stephania brachyandra*

No.	Location	Absolute Height (m)	Slope (degrees)	Forest Status	Vine Length (m)	Stem Diameter (cm)	Stem Bark	Leaf (shape/arrangement)	Petiole Length (cm)	Leaf Blade Length (cm)	Leaf Blade Width (cm)	Flower (flower color/flower type)	Fruit Type	Number of Seeds/Fruits
1	Phu Luong	190-297	25-39	IIA, IIB, IIIA1, rocky forest	1.5-3.3	0.6-0.8	Smooth	Alternate/lanceolate	4.1-5.4	9-12	5-7	Pale yellow cluster	Oval	1
2	Dinh Hoa	180-292	24-39	IIA, IIB, IIIA1, rocky forest	1.3-3.5	0.5-0.8	Smooth	Alternate/lanceolate	4.2-4.6	10-12	6-7.5			
3	Vo Nhai	180-290	25-39	Rocky forest, IIA, IIB, IIIA1	1.8-4.5	0.5-0.8	Smooth	Alternate/lanceolate	4.1-4.4	11-12	6-7	Pale yellow cluster	Oval	1
4	Dong Hy			IIA, IIB, IIIA1, rocky forest	1.6-3.9	0.5-0.8	Smooth	Alternate/lanceolate	4.1-4.6	10-12	6-7			
5	Dai Tu	190-295	20-39	IIA, IIB, IIIA1, rocky forest	2-3.6	0.6-0.8	Smooth	Alternate/lanceolate	3.3-4.7	10-12	6-7		Oval	

(Source: Compilation of survey data)

4. Conclusion

The average number of *Stephania brachyandra* specimens per survey route is 32.57 plants per square kilometer, with varying distribution densities across districts, highest in Vo Nhai district and lowest in Phu Luong district. *Stephania brachyandra* primarily inhabits rocky mountainous forests and land regenerating lands that have been heavily exploited. This species is predominantly found at altitudes above 250 m, followed by altitudes between 200-250 m, and least common at altitudes below 200 m. The average bush cover within the monitored Outside-Testing-Controls (OTCs) with *Stephania brachyandra* populations in surveyed districts is 18%, considerably lower compared to other species like Mua, Sim, Don nem, and Tu chau. The average ground cover of the herbaceous layer is 15.8%, consisting mainly of weeds such as grass, daisy, bamboo grass, pig grass, and betel leaf. Regarding soil, the A0 layer varies from 1-4 cm thick, the A layer has an average thickness of 30.3 cm, reddish-brown, moist, and friable, with a low stone content. The B layer has an average thickness of 60 cm, yellowish-brown, moist, slightly compacted, with a higher stone content. Soil chemical composition ranges from average to nutrient-poor, affecting the growth and development of *Stephania brachyandra*.

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