MINISTRY OF EDUCATION AND TRAINING

VIETNAM ACADEMY OF SCIENCE AND TECHNOLOGY

GRADUATE UNIVERSITY OF SCIENCE AND TECHNOLOGY

NGUYEN HUNG MANH

STUDY ON GENETIC, BIOLOGICAL, AND ECOLOGICAL CHARACTERISTICS OF VSF SUBSPECIES (*ABIES DELAVAYI* SUBSP. *FANSIPANENSIS*) (Q.P. XIANG, L.K. FU & NAN LI) RUSHFORTH) FOR CONSERVATION AND DEVELOPMENT AT HOANG LIEN NATIONAL PARK

SUMMARY OF ECOLOGICAL PHD THESIS

Code: 9.42.01.20

Hanoi - 2023

This study was achieved at Graduate University of Science and Technology - Vietnam Academy of Science and Technology.

Scientific Supervisor 1: Asso. Prof. Dr. Nguyen Van Sinh
Scientific Supervisor 2: Dr. Nguyen Thi Phuong Trang

Reviewer 1: Dr. Do Hoang Chung
Reviewer 2: Asso.Prof. Dr. La Viet Hong
Reviewer 3: Asso. Prof. Dr. Nguyen The Hung

The thesis was evaluated by The Board of Examiners at Graduate University of Science and Technology - Vietnam Academy of Science and Technology, at… hour …..day ……month ……year...

The thesis can be found at:
- Library of Graduate University of Science and Technology
- National Library of Vietnam

INTRODUCTION

1. Rationale of the study

VSF subspecies (*Abies delavayi* subsp. *Fansipanensis* (Q. P. Xiang, L.K.Fu & Nan Li) Rushforth (1999)) is a rare plant variety that is named in the Vietnam Red Book 2007 and also listed in Section IA of Decree No.84/2021/ND-CP of the Government of Vietnam.

The population of the subspecies is small and distributed only on eastern slope of the top of Fansipan Mountain (2.600 - 2.950 m) with a small number of regenerated trees. Therefore, research for conservasion and development of this rare plant variety is necessary to maintain, develop and protect the forest ecosystem in Hoang Lien National Park.

Based in reality, the PHD student proposed to carry out the project ***"Research on genetic, biologica and ecological characteristics of VSF subspecies (Abies delavayi* subsp. *fansipanensis* (Q. P. Xiang, L. K. Fu) & Nan Li) Rushforth) *for conservation and development at Hoang Lien National Park"*.** The success of this study will be the premise for research and proposion of solutions to conserve VSF as well as other rare plant varieties in the Hoang Lien National Park that are at risk of extinction, contributing to protecting ecosystems and forests and promoting the scientific and commercial value of native plant varieties.

2. Ojectives of the thesis study

2.1. General objective:

Developing a scientific and practical basis for the conservation and development of VSF subspecies (*Abies delavayi* subsp. *fansipanensis* (Q.P.Xiang, L.K.Fu & Nan Li) Rushforth) in Hoang Lien National Park.

2.2. Specific objectives:

1). To identify some biological characteristics (morphology, bud production, cone production) of VSF subspecies (*Abies delavayi* subsp. *fansipanensis* (Q. P. Xiang, L. K. Fu & Nan Li) Rushforth) in Hoang Lien National Park.

2). To identify some ecological characteristics in the natural distribution area of VSF subspecies in Hoang Lien National Park, including: Relative light intensity, temperature and humidity, terrain conditions (altitude, slope, aspect), soil conditions of layer A (K_2O, pH, Nitrogen, P_2O5, Ca^{2+}, Mg^{2+}, Fe^{2+}, Humus content).

3). To identify some characteristics of the vegetation structure and natural regeneration characteristics of VSF subspecies in its natural distribution.

4). To identify some genetic characteristics of VSF subspecies in Hoang Lien National Park.

5). To initially assessing the possibility of conservation of VSF subspecies in Hoang Lien National Park using breeding methods.

3. Main contents of the thesis study

1). Research on some biological characteristics (morphology, bud production, cone production) of VSF subspecies in Hoang Lien National Park.

2). Research on some ecological characteristics (relative light intensity, temperature and humidity, terrain conditions (altitude, slope, aspect), soil conditions of layer A (K_2O, pH, Nitrogen, P_2O_5, Ca^{2+}, Mg^{2+}, Fe^{2+}, Humus content) of VSF subspecies in Hoang Lien National Park.

3). Research on some characteristics of the vegetation structure and natural regeneration characteristics of VSF subspecies in Hoang Lien National Park.

4). Research on some genetic characteristics of VSF subspecies and its genetic relationship with some other varieties in the Pinaceae family based on sequencing of 05 gene regions including *trn*L-*trn*F, *rps*18-*rpl*20, *nad*5, *rbc*L and *trn*H-*psb*A

5). Research on cutting propagation, seed preservation of VSF subspecies and test cultivation of seedlings in the natural environment.

CHAPTER 1. OVERVIEW

1.1. Overview of study on *Abies* P.Miller, *Abies delavayi* Franch., *Abies delavayi* (*Abies delavayi* subsp. *fansipanensis* (Q.P.Xiang, L.K.Fu & Nan Li) Rushforth)

Abies is one of the genera with a large number of varieties of the Pinaceae family, with 48 varieties, distributed mainly distributed in North and Central America, Europe, North Africa, Asia (Southern Himalayas). , South China, Taiwan (China) and Vietnam (Lao Cai). These are plant varieties of high scientific and economic value, playing an important role in watershed and regional forest ecosystems, subtropical and temperate high-pressure regions.

Abies delavayi Franch was first found and published by Franchet in 1887, at an altitude of 3500-4000 m in the Cang Shan mountains near Dali in Yunnan province, China. This species has more than 100 chemical components, including anti-cancer compounds, 49 terpenoids, 13 lignans, 20 flavonoids, 3 counarins and 25 other chemical compounds [4].

VSF subspecies is classified as a subspecies of the *Abies delavayi*: *Abies delavayi* subsp. *fansipanensis* (Q.P.Xiang, L.K.Fu & Nan Li) Rushforth [5]. It is a large tree species that can grow up to 20 m tall, scattering at altitudes from about 2.600 m to 2.950 m on on eastern slope of the top of Fansipan Mountain, Hoang Lien Son range in the evergreen broadleaf forests, is now a wood species left over after mixed forest fires and grows in Dwarf Bamboo carpets.

**** Issues to be further studied for the VSF subspecies (Abies delavayi* subsp. *fansipanensis* (Q.P.Xiang, L.K.Fu & Nan Li) Rushforth) *in Vietnam*:**

- VSF subspecies has been included in the Vietnam Red Book 2007. In fact, this species is much more endangered, reflecting the small populations, strong classification, poor population with a low number of individuals in the population, poor natural regeneration from seeds. According to IUCN, the conservation classification of Abies delavayi in Vietnam must be evaluated as Endangered (EN).

1.2. Research on regeneration characteristics

- Most works focus on studying the regeneration situation under natural forest conditions (quantity and density of regenerated trees, characteristics of regenerated tree layers and the role of light for natural regeneration process) without mentioning regeneration in different vegetation states such as: grass cover, shrub cover, artificial secondary forest (forest after slash-and-burn cultivation and exhausted exploitation). Soil is an issue to be further studied.

Research methods and natural regeneration rules of forest vegetation around the world are the basis for preparing silvicultural techniques to manage forest resources sustainably. However, tropical vegetation is very diverse and complex, whose life is closely linked to the natural conditions in each geographical region. Therefore, further study on the rule of natural regeneration of forest ecosystems in different geographical regions is required.

1.3. Research on genetic characteristics

Previous studies around the world on genetic characteristics and phylogenetic relationship of varieties in the *Abies* branch are the scientific rationale for studying the genetic characteristics of Abies delavayi.

1.4. Conservation testing

Biodiversity conservation is considered a regular and long-term task to protect the country's rare and unique genetic resources,

preventing biodiversity degradation, contributing to the maintenance of the survival of some endangered species for future generations. Conservation methods for plants in the pine family published in previous studies are the basis for research on the conservation of VSF subspecies.

CHAPTER 2. RESEARCH OBJECTS AND METHODS

2.1. Research objects and locations

- Research object: VSF subspecies (*Abies delavayi* subsp. *fansipanensis* (Q.P.Xiang, L.K.Fu & Nan Li) Rushforth) belongs to the Pinaceae family.

2.2. Research location

- Hoang Lien National Park, Sapa district, Lao Cai province.

2.3. Research Methods

2.3.1. Literature review research methods

Selective absorption of advanced scientific achievements through information collection and scientific exchange related to the thesis content.

2.3.2. Field research

- Use of standard 20m x 20m plots and 4m x 4m slab plots for data collection and evaluation.

- Research on the biological and ecological characteristics of Abies delavayi by surveys at an altitude of 2.600-2.950 m, establishing standard plots to measure Abies delavayi of different diameter levels in the floristic composition: (1) Vegetation of broad-leaved and coniferous mixed trees at an altitude of 2.600- 2.700m; (2). Vegetation dominated by conifers (Abies delavayi) at an altitude of 2.700 - 2.950 m.

Evaluation criteria: Diameter (circumference) at breast height (at 1.3m from the base), length of young shoots of growing branches,

number of trees in standard plots with cones and number hats on each individual, light intensity, air humidity, temperature, vegetation type.

2.3.3. Analysis of biological and ecological data

- Morphological comparison and expert methods are used to determine the scientific names of plant species (dominant species) in the distribution area of VSF subspecies.

Evaluation criteria: IVI index, relative light intensity, soil characteristics of the humus distribution area, digestible contents such as potassium, nitrogen, phosphorus, magnesium...).

2.3.4. Sample collection method for genetic research

Population A includes VSF individuals distributed at altitudes from 2.600-2.700m, population B includes VSF individuals distributed at altitudes from 2.700-2.950m.

Collection of 10 fresh leaf samples, denoted from A1 to A10 (10 samples randomly collected from 10 VSF individuals in population A) and from B1 to B10 (10 samples randomly collected from 10 VSF individuals in population B). Such leaf samples are stored on site in silica gel bags.

2.3.5. ADN analysis

2.3.5.1. Total DNA extraction: A total of 20 VSF subspecies leaf samples were ADN extracted using the CTAB method of Doyle and Suhma, total ADN is testsed by electrophoresis on a 0.8% agarose gel.

2.3.5.2. ADN cloning with PCR. Cloning of gene regions by PCR reaction, PCR products of each reaction are tested by electrophoresis on a 0.8% agarose gel.

2.3.5.3. Gene sequencing analysis

PCR products of 5 gene regions are purified with the GeneJETTM PCR Purification Kit made by Thermo Scientific company (USA). PP

Sanger is used with BigDye®Terminator v 3.1 Cycle Sequencing kit (Applied Biosystems, USA) on ABI 3500 Genetic Analyzer system.

2.3.5.4. Genetic data analysis

- Genetic data analysis is conducted with softwares including chromasPro, Clustal W and Mega 6.1 and creating tree with PP NJ by Naruya Saitou and Masatoshi Nei.

2.3.6. Propagation of Seed and Cuttings

VSF subspecies cuttings are treated with Viben - C 50BTN and IBA, then planted on the substrate: fine yellow sand and humus mixed with layer A soil, and waterred with mist every 5 days to maintain humidity 75%. Evaluation criteria: Percentage of cuttings with roots, average number of roots/cuttings and percentage of cuttings with young leaves.

CHAPTER 3. RESEARCH RESULTS AND DISCUSSION

3.1. Research results on some biological characteristics of VSF

3.1.1. Main morphological characteristics: Wooden trunk up to 20m high, pyramidal canopy, spiral leaves 1-3cm long, underside has 2 strips of moldy white stomatas, young buds at branch tips are brown, male cones grow solitary, female cones are almost sessile, cylindrical, 8-10cm long, 4-6cm wide, with many seed scales, each scale has 2 triangular seeds, each cone has 140-145 seeds. When ripe, the scales near the stem fall off first.

3.1.2. Research results on the characteristics of bud and cone production of VSF

- Buds of this subspecies usually appear from February to the end of May; and cones appear from mid-May, cones are ripen in December (seed scales begin to fall off); Correlations between bud formation (Hcn); The cone production (Nnon) and diameter class (D1.3) of VSF subspecies in the two distribution belts are shown in Figures 3.1, 3.2.

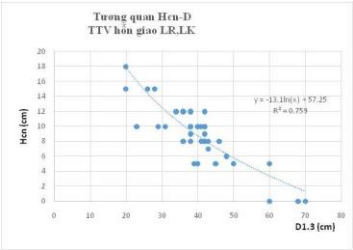


Figure 3.1

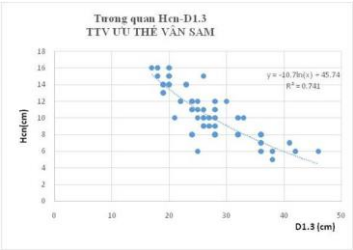


Figure 3.2

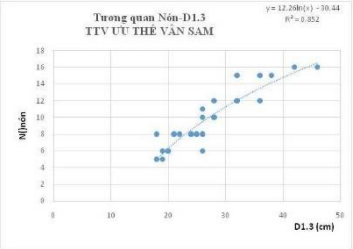


Figure 3.3

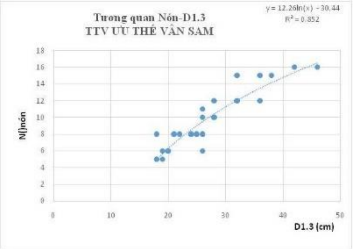


Figure 3.4

Figures 3.1, 3.2; 3.3, 3.4. Correlation (Hcn/D1.3) and (Hnon/D1.3) of VSF populations in 2 belts (2.600 -2.700 m &2.700 – 2.950 m), respectively

3.2. Research results on some ecological characteristics in the research area

3.2.1. Soil terrain

Table 3.1. Terrain characteristics in the research area

TTOTC	Slope (%)	Altitude (m)	Longitude (E)	Latitude (N)
1.	17	2,601	103.780786	22.310810
2.	22	2,603	103.780753	22.310804
3.	25	2,608	103.780741	22.310785
4.	18	2,619	103.780705	22.310746
5.	32	2,630	103.780642	22.310741
6.	42	2,633	103.779230	22.310222

7.	35	2,636	103.779241	22.310234
8.	26	2,638	103.779161	22.310146
9.	18	2,638	103.779155	22.310157
10.	42	2,636	103.779133	22.310215
11.	38	2,642	103.779303	22.310221
12.	35	2849	103.46.676	22.18377
13.	45	2866	103.46.671	22.18368
14.	41	2932	103.46.574	22.18408
15.	36	2937	103.46.574	22.18423

Table 3. 2. Soil characteristics of layer A in the research area

No.	**Unit**	**Average analysis results**	
		Mixed vegetation of broad-leaved and coniferous trees	**The dominant vegetation of VSF**
pH	-	5,01 ± 0,05	4,13 ± 0,15
Total K_20	mg/g	0,42±0,11	0,35±0,05
Easy-to-digest K_20	mg/g	0,402±0,17	0,22±0,05
Total nitrogen	mg/g	2,53 ± 0,06	1,23 ± 0,14
Easy-to-digest nitrogen	mg/g	0,34 ± 0,03	0,25±0,15
Total P_2O_5	%	0,13 ± 0,03	0,07 ± 0,01
Easy-to-digest P_2O_5	%	0,02±0,01	0,01±0,01
Humus	%	72,50 ± 5,64	34,08 ± 3,04

No.	Unit	Average analysis results	
		Mixed vegetation of broad-leaved and coniferous trees	The dominant vegetation of VSF
Ca^{2+}	mg/kg	199,80 ± 10,23	1502,80 ± 57,78
Fe^{2+}	mg/kg	1641,30 ± 60,56	3341,41 ± 107,59
Mg^{2+}	mg/kg	113,10 ± 4,38	322,40 ± 26,94

3.2.2. Climate conditions in the research area

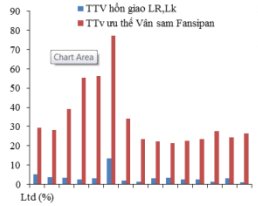


Figure 3.5

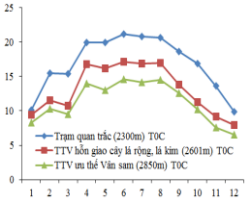


Figure 3.6

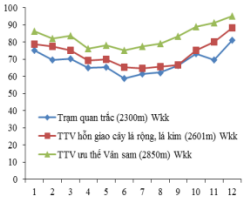


Figure 3.7

Figures 3.5; 3.6; 3.7 respectively: Relative light intensity; temperature; Air humidity in the research area

3.3. Structural characteristics of vegetation and Natural regeneration of VSF in the natural distribution area

Table 3.3. Summary of indicators of dominant plant varieties of both vegetation types in the research area

Variety	Relative abundance (%)		Relative dominance (%)		Relative frequency (%)		IVI (%)	
	A	B	A	B	A	B	A	B
1. Abies delvayi subsp. *fansipanensis* (Q.P. Xiang, L.K. Fu & Nan Li) Rushforth	5,71	45,87	3,51	63,8	4,93	17,05	4,72	42,24
2. Acer brevipes Gagnep.	6,83	4	6,56	1,61	5,63	3,41	6,34	3,01
3. Camellia sp.	5,71	6,93	11,1	5,29	4,23	6,82	7,01	6,35
4. Eurya distichophylla Hermol.	5,08	5,6	2,07	3,02	6,34	10,23	4,5	6,28
5. Illicium tsai L. C. Sm.	5,24	2,13	4,38	2,1	4,23	5,68	4,62	3,31
6. Prunus sp1	1,9	2,67	0,81	0,61	2,82	5,68	1,85	2,98
*7. Rhododendron aboretum var. cinnamomum (*Wall. Ex G. Don) Lindl.	5,24	4,27	3,22	3,56	5,63	6,82	4,7	4,88
8. Rhodoleia championii Hook.f.	5,87	9,33	6,6	9,95	3,52	7,95	5,33	9,08
9. Rhododendron maddenii Richard B.	5,24	8,8	3,68	2,72	6,34	13,64	5,09	8,39
10. Schefflera sp1	2,38	2,13	1,16	0,76	4,23	3,41	2,59	2,1
11. Magnolia cathcartii (Hook.f. & Thomson) Noot., Blumea 31(1): 88 (1985)	8,25	-	14,73	-	7,04	-	10,01	-
12. Schima wallichi (DC.) Korth.	1,9	-	7,48	-	5,63	-	5,01	-
13. Lithocarpus sp1	5,24	-	5,73	-	3,52	-	4,83	-

Variety	Relative abundance (%)		Relative dominance (%)		Relative frequency (%)		IVI (%)	
	A	B	A	B	A	B	A	B
14. Ilex sp.	4,13	-	5,29	-	3,52	-	4,31	-
15. Lithocarpus sp2.	5,56	-	2,37	-	4,93	-	4,29	-
16. Acer campbelii var. *fansipanense* Gagnep.	6,19	-	3,01	-	3.52	-	4,24	-
17. Rhododendron sp.	5,08	-	2,79	-	3,52	-	3,8	-
18. Castanopsis sp.	2,38	-	3,54	-	3,52	-	3,15	-
19. Quercus sp.	2,22	-	3,8	-	2,11	-	2,71	-
20. Cinnamomum durifolium Kost.	1,9	-	1,75	-	4,23	-	2,63	-
21. Schefflera sp2.	1,9	-	1,59	-	3,52	-	2,34	-
22. Prunus sp2.	2,38	-	1,31	-	2,82	-	2,17	-
23. Symplocos sp1.	1,9	-	1,75	-	2,11	-	1,92	-
24. Symplocos glauca Nooteb var. e*papiela*.	-	2,13	-	2,1	-	5,68	-	3,31
25. Pieris formosa (Wall.) D. Don	-	6,13	-	4,47	-	13,64	-	8,08

Table 3. 4. Summary of important plant varieties in the natural distribution area of VSF

No	Name of variety		Name of family	Average IVI (%)
	Common name	Scientific name		
1	VSF	*Abies delvayi* subsp. *fansipanensis* (Q. P. Xiang, L.K. Fu & Nan Li) Rushforth	Pinaceae	23,48

No	Name of variety		Name of family	Average IVI (%)
	Common name	Scientific name		
2	Hồng quang	*Rhodoleia championii* Hook.f.	Hamameliaceae	7,205
3	Đỗ quyên	*Rhododendron maddenii* Richard B.	Ericaceae	6,74
4	Chè rừng	*Camellia sp*	Theaceae	6,68
5	Súm	*Eurya distichophylla* Hermol.	Theaceae	5,39
6	Đỗ quyên	*Rhododendron arboreum subsp. cinnamomum* Wall. ex Lindl.	Ericaceae	4,79
7	Thích	*Acer brevipes* Gagn.	Aceraceae	4,675
8	Hồi	*Illicium tsai* L. C. Sm.	Illiciaceae	3,965
9	Đào rừng	*Prunus* sp1.	Rosaceae	2,415
10	Ngũ gia bì	*Schefflera* sp1.	Araliaceae	2,345

3.3.2. Characteristics of VSF subspecies population structure

Table 3. 5. Distribution of number of trees by diameter class (D1.3, cm)

$D_{1.3}$, cm	**Mixed vegetation LR,LK (2.600m)**		**Dominant vegetation VS (2.900m)**	
	N _tree	Rate %	N _tree	Rate %
10-20	2	3,51	15	20
20-30	4	7,02	**43**	**57,33**
30-40	**22**	**38,60**	13	17,33
40-50	**21**	**36,84**	4	5,33

50-60	4	7,02	-	-
60-70	4	7,02	-	-
Tổng	**57**	**100**	**75**	**100**

3.3.2.3. Characteristics of natural regeneration of VSF subspecies

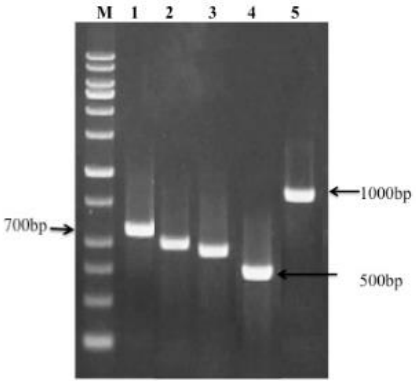
- Distribution characteristics of VSF regenerated seedlings: random distribution according to holes and strips.

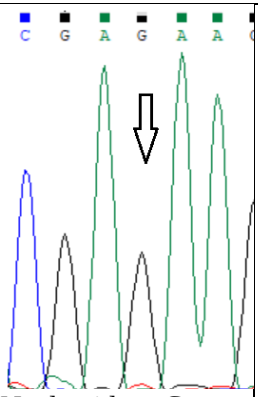
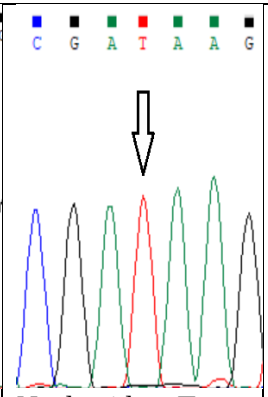
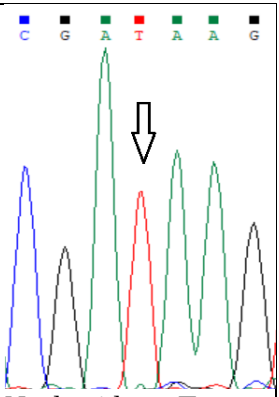
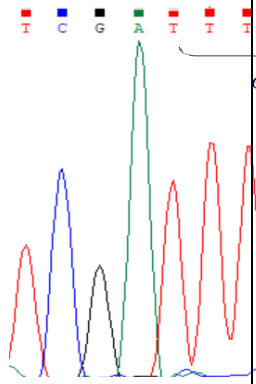
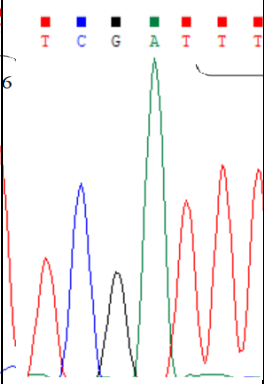
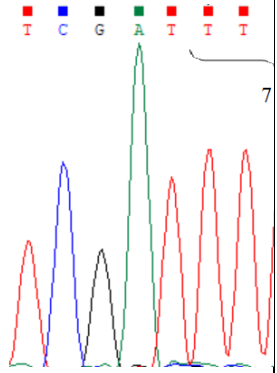
- The rate of appearance of naturally regenerated VSF seedlings with towering height (Hvn - m <1m) in the study area is very low at 30% (9 OTC/30 OTC)

- The rate of regenerated seedlings with good growth quality is 15/25 trees, accounting for 60%, which is very good for the conservation and development of this variety in the research area.

3.4. Genetic characteristics of VSF subspecies

Results of amplification of 5 gene regions (*rps*18-*rp1*20, *trn*L-*trn*F, *trn*H-*psb*A, *rbc*L and *nad*5) by PCR



 Nucleotide G at position 323 in samples A6, B5, B6, B9, B10	 Nucleotide T at position 323 of samples A1-A5, A7-A10	 Nucleotide T at position 323 of samples B1-B4, B7, B8
 Samples from A1-A10 (6 nucleotides T from position 224 to 229)	 Samples B1 and B3-B10 (6 nucleotides T from position 224 to 229)	 Sample B2 has an additional Nucleotide T at position 223 (total 7 Nucleotides T)

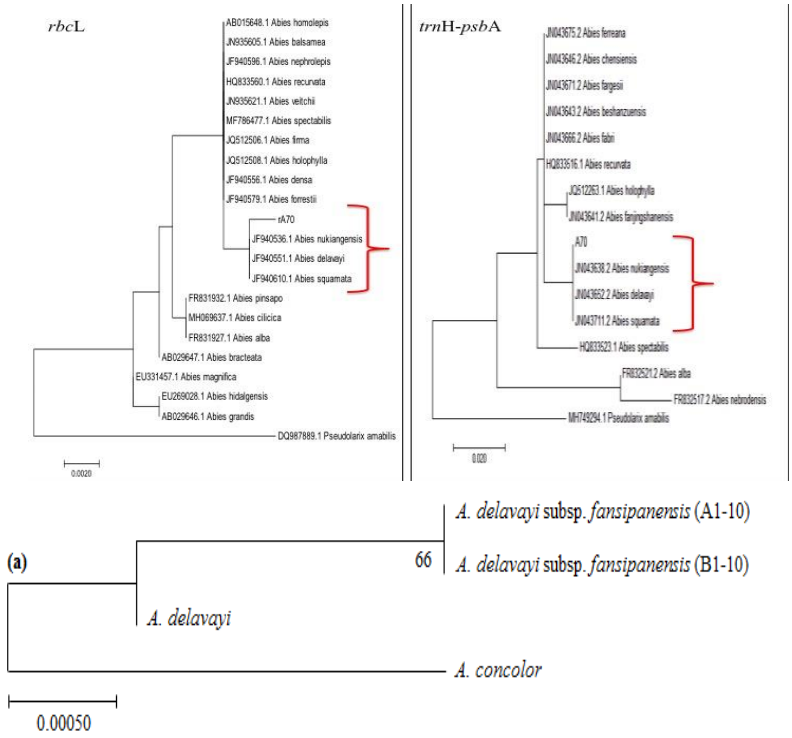
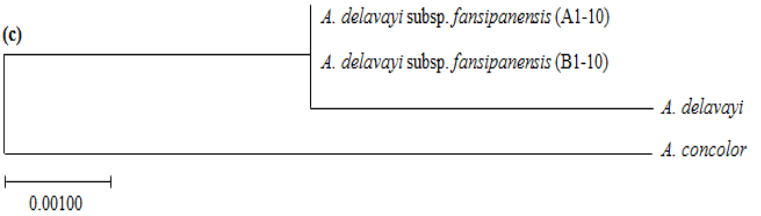


Figure 3



3.5. Results of propagation, seed preservation and test cultivation of VSF in the natural environment

3.5.1. Cutting propagation VSF

- IBA treatment increases the rate of rooting, the number of roots and the rate of cuttings for production of young leaves in VSF propagation

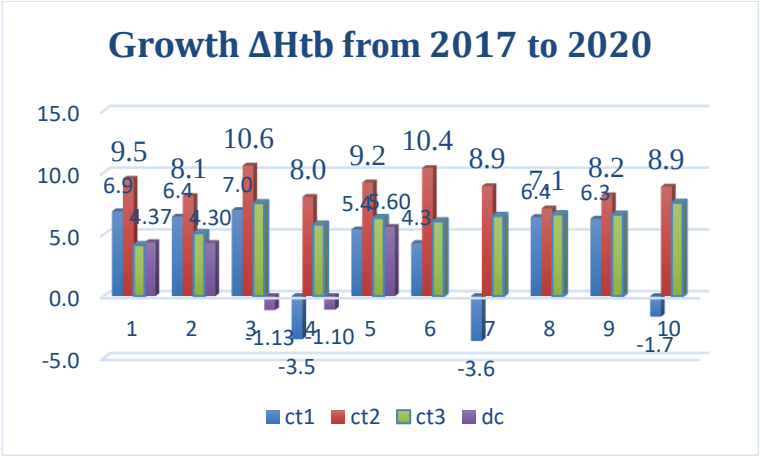
and the highest value obtained with the treatment formula of 1500 (mg/L) IBA.

- After 12 months of testing, VSF cuttings on fine yellow sand substrate had a rooting rate of 50.67%, with an average number of roots of 2.59 (roots/cuttings) and a rate of young leaf development (47.37%), meanwhile, the results obtained on the humus substrate mixed with layer A soil were 34.46%; 2.59 (roots/cuttings) and 69.23% corresponding to the rate of rooted cuttings, average number of roots and the rate of cuttings for production of young leaves.

3.5.2. VSF propagation tests with seeding

- Seeds stored at -10^{o}C (CT 3) give the highest germination rate among experimental formulas with 75/90 seeds germinated (83,33%);
- Formula 2: Seeds are preserved in sealed plastic bags at normal temperature (CT 2), giving the lowest germination rate among the 3 experimental formulas with 7/90 seeds germinated (7.80%);
- Formula 1: Seeds stored in the refrigerator at a temperature of 4-5^{o}C gives relatively high germination results with 56/90 seeds sprouting at 62.22%.

3.5.3. Results of test cultivation of seedlings in the natural environment. Figure 3.52 shows the growth (ΔHtb) of Fansipan spruce seedlings (1 year old sown from seeds) after 3 years of *test cultivation* in the natural environment both inside and outside their natural distribution area, ranging from 4.3-10.6cm, among which, 5 trees showed average growth (ΔHtb) less than zero, that is, 5 trees died (accounting for 14.29%, of which, 3 dead trees under Formula 1 and 2 dead trees in the control group), 30 alive trees (accounting for 85.71%).



CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

Regarding biological characteristics, VSF subspecies produces young buds from February to May; Cones appear from mid-May and ripen in December. Bud producing has a close correlation with diameter class at 1.3m (inversely proportional) at low and high belts, diameter class D1,3 from 20-50cm produces the strongest buds. Meanwhile, cone production of this subspecies has a loose (proportional) correlation in the low belt and tight correlation in the high belt, cone production cycle is erratic.

Regarding ecological characteristics, this taxon has a narrow distribution area, currently only discovered about 100 hectares at an altitude of 2.600-2.950m above sea level in Sapa. This is a subtropical and temperate alpine climate zone. This taxon has not been found at altitudes below 2,000m, in tropical climates. The data obtained on the terrain, soil, light regime, temperature, air humidity, and soil chemical composition of the habitat have determined the habitat of this

subspecies. They often grow on soil or rocky mountain slopes, and they cannot survive in drainage areas, and in stagnant or damps.

Based on the composition and structure of the vegetation in the distribution area of the VSF subspecies, along with the results of test cultivation in natural forests, it is possible to determine that VSF is a light-loving plant when it is young, when mature they often occupy the ecologically dominant layer; Research results on the regeneration characteristics of VSF show that this species has few trees that regenerate naturally due to thick litter that prevents seeds from coming into contact with the soil and the canopy cover (mixed vegetation area) or tall shrubs (area of vegetation dominated by Fansipan spruce) prevent light from providing for photosynthesis of understory seedlings.

Regarding genetic characteristics, the VSF subspecies has the closest genetic relationship with A. delavayi and A. nukiangensis with a genetic distance of 0.001. Some Nucleotide positions are different in the 5 gene regions rbcL and trnH-psbA; rps18-rpl20; nad5; The detected trnL-trnF of 20 VSF samples is not only important in accurately determining their taxonomic position but also indicates the genetic diversity of populations distributed in highland belts (2.950m) is higher than the population in the low belt (2.600m) - this is an important orientation for selecting seed sources adapted to climate change. However, it has not yet been confirmed whether the above differences in Nucleotides have genetic significance or are just differences due to geographical distance. The rbcL and trnH-psbA gene region sequences of the spruce fansipan species have been registered to Genbank with accession codes MK783132 and MK783131, respectively.

Regarding conservation propagation, during propagation by cuttings, plant growth regulator IBA with different concentrations was used. The results of IBA treatment increased the rate of rooting cuttings, the number of roots on cuttings and the rate of cuttings developing young leaves for VSF. The best results were obtained with treatments of 1,500 (mg/L) IBA; Analytical results affecting the results of IBA-treated cuttings suggest the following grafting process: First of all, to root the cuttings, use fine yellow sand medium; Then transfer the rooted cuttings to a humus substrate mixed with layer A soil at a ratio of (65% and 35%); Seed propagation results show that post-harvest preservation of VSF seeds not only has a great influence on the germination ability of the seeds but also affects the growth of seedlings obtained from them. Storing VSF seeds at -10^{o}C gives the highest germination rate (83.3%) and best growth (ħvn: 5.1 cm/5 months);

The results of test cultivation of seedlings from VSF seeds in the natural environment show that a relatively high survival rate (approximately 86%) can be achieved if planted in an area with lots of light and a slope of about 20%, canopy cover is about 0.35 and little fresh carpet of shrubs.

RECOMMENDATIONS

1. It is necessary to re-evaluate the conservation level based on the thesis data.

2. It is necessary to urgently conduct research on regeneration solutions such as regeneration with natural seeds in the field, regeneration with cuttings, etc. to have many seedlings to plant.

NEW CONTRIBUTION OF THE THESIS

- The thesis has made a new contribution to science with quantitative data related to VSF subspecies including: Ecological characteristics of

the distribution area and ecological needs of VSF seedlings, current status of VSF populations, quality and the current distribution status of naturally regenerated seedlings with a height of <1m; Genetic characteristics of 20 VSF individuals randomly distributed from 2.600 - 2.950m (with 5 different genetic regions) and close relationships with some species in the Pinaceae family.

- New contributions to science and practice on success rates and techniques for propagating VSF by cuttings at the species' natural distribution location.
- New contributions to science and practice documenting the ability and technique of preserving VSF seeds.
- New contributions to the practice of techniques for growing VSF seedlings in the natural environment (in situ and ex situ).
- Sequencing of 5 gene regions rbcL, trnH-psbA; rps18-rpl20; nad5; trnL-trnF VSF species.

LIST OF PUBLISHED WORKS

(1) H. M. Nguyen1, 2, *, V. S. Nguyen1, 2, V. N. Le3, T. T. H. Huynh4 and T. H. Do5, A novel study on bio-ecological and genetic characteristics of VSF subspecies distributed at different altitdes on Fansipan - Mountain, Lao Cai province, Vietnam, Journal of Animal & Plant Sciences, 2022, 32(5), 1331-1346,

(2) Nguyen Hung Manh, Lai Thi Thu Hang, Nguyen Thi Hong Mai, Nguyen Thi Phuong Trang*, Nguyen Van Sinh, *Determining the characteristics of the rbcL and trnH-psbA gene regions of the spruce fansipan subspecies (Abies delavayi subsp. fansipanensis* (Q. P. Xiang, L. K. Fu & Nan Li) Rushforth) in Vietnam, Journal of Science and Technology, 2021, 63 (3), 28

(3). Nguyen Hung Manh *, Nguyen Van Sinh, Lai Thi Thu Hang, Phi Cong Thuong, Le Van Nhan, Vuong Trong Kha, Le Tu Anh, Research

on breeding of VSF subspecies (*Abies delavayi subsp. fansipanensis* (Q. P. Xiang, L. K. Fu & Nan Li) Rushforth) in Hoang Lien National Park, Journal of Agriculture and Rural Development, 2020, 21, 112-116.