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A NOVEL STUDY ON BIO-ECOLOGICAL AND GENETIC CHARACTERISTICS OF *ABIES DELAVAYI* SUBSP. *FANSIPANENSIS* DISTRIBUTED AT DIFFERENT ALTITUDES ON FANSIPAN-MOUNTAIN, LAO CAI PROVINCE, VIETNAM

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ABSTRACT

Abies delavayi subsp. *fansipanensis* is classified as a highly endangered plant in the Vietnam Red Data Book (2007) and a critically endangered species in IUCN red list (2012). This species distributed at the altitudes of 2600-2680 m and 2750-2950 m above sea level (a.s.l) on Fansipan-mountain, Lao Cai province, Vietnam. However, to date there is still a lack of information on the bio-ecological and genetics characteristics of *Abies delavayi* subsp. *fansipanensis* indifferent altitudinal conditions, which helps to conserve and promote this precious genetic resource in Vietnam. In this study, climate conditions, soil property, vegetation and genetic structure of two populations of *Abies delavayi* subsp. *fansipanensis* distributed in two altitudinal belts on Fansipan-mountain were investigated. The research results have shown that *Abies delavayi* subsp. *fansipanensis* is distributed in the ecological conditions including temperature: 1.6-29.3°C, rainfall: 26.8-500 mm, humidity: 65%-95%, sunshine: 90-130 h sunshine, pH: 4-5, and the total organic carbon value 34-73%. The importance value index (IVI) and the correlation coefficient of the relationship of tree height and diameter at breast height values of *Abies delavayi* subsp. *fansipanensis* distributed in the altitudes of 2600-2680 m a.s.l. were 4.72% and 0.2749, while those of this species in the altitudes of 2750-2950 m a.s.l. were 42.24% and 0.6824, respectively. The DNA analysisof *Abies delavayi* subsp. *fansipanensis* also indicated that the genome of individuals in the higher altitude is more diverse than those lived in the lower altitude. This is the first study of the bio-ecological and genetic structure characteristics of *Abies delavayi* subsp. *fansipanensis* distributed on Fansipan-mountain. The study provides valuable data for proposing solutions to conserve and promote this rare genetic resource for Vietnam as well as for the world.

Keywords: *Abies delavayi* subsp. *fansipanensis*, altitudinal conditions, bio-ecological characteristics, Fansipan-mountain, genetic characteristics.

INTRODUCTION

Vietnam has been recognized as one of the most biologically diverse countries in the world with over 15,986 plant species have been recorded (WCMC, 1992). Among them there are 448 plant species with 16 indigenous conifer species being evaluated as Vietnam threatened plant species and are listed in 2007 Vietnam Red Data Book. Hoang Lien National Park is located in the Hoang Lien mountain range with the Fansipan peak, which is considered as the highest mountain in Vietnam, at 3143 m in comparison with the sea level. According to Truong et al. (2011), there were 2434 plant species belonged to 892 genera and 209 families in the flora of the Hoang Lien National Park. These biological resources play a pivotal role in the socio-economic development and the improvement of human's livelihood, especially for the local people living around the Hoang Lien National Park.

Abies delavayi is a species in the second largest genus of *Pinaceae* family, which is grown on the high altitude mountains at elevations of 3000-4000 m (Farjon and Rushforth, 1989; Farjon, 1990). This species was found in the mountains in Yunnan-China, the neighbor border areas in the southeastern of Tibet, northern Myanmar, northeastern India (Farjon, 1990). In Vietnam, a species, namely as *Abies delavayi* subsp. *fansipanensis* which was considered as the subspecies of the *Abies delavayi* distributed only on the Fansipan mountain, Lao Cai province. There are several differences in the mature cones' color, seed scale shape, and the bract scale length relative to the seed scale length in the subspecies *Abies delavayi* subsp. *fansipanensis* compared to the other species. In addition, this subspecies is a large and erect tree with a tall of 15-20 m, and a base diameter of about 1 m, and possessors of 2-3 cm flat leaves which grow scattered on the high cliffs of the mountains in the primary forests (Xiang, 1997).

In the previous studies, Xian-Wen Yang et al. (2014) found 110 compounds divided into 49 terpenoids, 13 lignans, 20 flavonoids, three coumarins, and 25 other chemical constituents in *Abies delavayi*. Particularly, various compounds such as sesquiterpenoids, diterpenoids, triterpenoid, monoterpenoid, flavonoid, and phenols in this species were known as the potential cytotoxic bioassays against three tumor cell lines in human and NO production inhibition on RAW264.7 macrophages. According to, Cheng and Fu (1978), *Abies* is sensitive to temperature changes and common distributed in the subtropical mountainous regions in China. In addition, the paleoclimate researchers have been used fossils of *Abies* as the temperature indicator in studying Quaternary climatic history. These data indicate that *Abies delavayi* plays an important role in pharmaceutical research and application as well as studying the changes of climate and nature ecological systems in various countries in the world.

It is interesting to note that, *Abies delavayi* subsp. *fansipanensis* is reported to be restricted at the top of the Fansipan mountain in Vietnam with only a few individuals (Xiang, 1997), which is determined as a highly endangered plant reported in the Vietnam Red Data book (2007). In addition, it is also classified into the critically endangered plant according to IUCN Red List of Threatened species (Rushforth et al., 2011). A variety of reasons affecting the exist and the development of *Abies delavayi* subsp. *fansipanensis* was reported such as the distribution of this species in nature is not much, regeneration capacity is poor, whereas the situation of exploitation of forests for forest products and burning forest for agricultural production that decrease forest area. Furthermore, the construction of tourism roads to the Fansipan mountain to reduce the number of plant species including *Abies delavayi* subsp. *fansipanensis*. Therefore, it is needed to find out the resolutions to conserve and promote this genetic resource contributing to the biodiversity in Vietnam as the world.

In this study, we investigated the bio-ecological and genetic characteristics of *Abies delavayi* subsp. *fansipanensis* distributed on Fansipan-mountain belonged to Hoang Lien National Park in Lao Cai province, Vietnamvia various characteristics such as climate, weather, soil, population structure, vegetation structure and genetic structure. The study could be considered as the basis for proposing solutions to conserve and promote this rare genetic resource in Vietnam. In addition, the biological resources have a significant value for the socio-economic development and improving people's livelihood. The development of this plant might provide the important material resources for the pharmaceutical industry sector. Furthermore, the conservation of *Abies delavayi* subsp. *fansipanensis* will also help to reduce the situation of burning forest for cultivation, thus making an important contribution to the protection of ecosystems in the Hoang Lien National Park, Lao Cai province, Vietnam.

MATERIALS AND METHODS

Study area and plant materials: The study was conducted in the Hoang Lien National Park belonging to Hoang Lien Son Range with the Fansipan peak that is the highest mountain in the Indochinese Peninsula (comprising Vietnam, Laos and Cambodia) at 3143 m a.s.l. The national park was located in Lao Cai province with the coordinates of 22°15′00″N 103°30′00″E, and an area of 29845 ha (Fig.1). This is the exchange place of the sub-temperature climatic and the high tropical regions with high humidity throughout the years.

Abies delavayi subsp. *fansipanensis* samples were collected on the eastern slopes of the Fansipan mountain in the tropical evergreen primary forest in two populations at different altitudes of 2600-2680 m (population A) and 2750-2950 m (population B) above sea level (a.s.l) with the areas were approximately of 2 km^2 and 1.2 km^2, respectively. The population A, is the mixed population of *Abies delavayi* subsp. *fansipanensis* with broad-leaved and coniferous-leaved trees, whereas the population B, is the domination of *Abies delavayi* subsp. *fansipanensis* in comparison with the other plant species. A total of 20 samples of *Abies delavayi* subsp. *fansipanensis* (where: 10 samples per population) from the national park was collected and then stored at -20°C prior to DNA analysis.

Climatic data analyses: Several environmental factors such as temperature, rainfall, humidity and sunshine hours that influence to plant vegetation and the distribution of *Abies delavayi* subsp. *fansipanensis* were investigated (Austin, 2002; Watt et al., 2011). The data of these factors from January to December, 2019 were employed by the Lao Cai province meteorological station, which reflect the characteristics of the climate in the study area throughout the years. The data were analyzed using SPSS v.16 software.

Soil sampling and properties analysis: A number of 60 and 30 soil samples were randomly collected in the population A and population B areas of *Abies delavayi* subsp. *fansipanensis*, respectively. In each sampling plot, three samples of soil and matter with 1,000 cm^3 of volume were collected from the horizon A and the forest floor, respectively. These samples in each sampling plot were mixed together, kept in plastic bags, and then transferred to the laboratory to sieve (2 mm) and oven-dry (50°C) before analysis. Various parameters in the soil samples were determined such as pH; the cation exchange capacity (Ca^{+2}, Fe^{+2}, Mg^{+2}); the total phosphorus; the total N (TN); and the total organic carbon (TOC). The pH value was measured by an electrical conductivity in 1:2 water solution (Martin and Thormas, 2015) whereas the cation exchange capacity was determined by the spectrophotometric method (Delphine et al., 2008). The total organic carbon and the total nitrogen in soil samples were analyzed by using the combustion-infrared method (Pavlos et al., 2015). The total phosphorus in soil was measured as the described in the study of Taylor (2000).

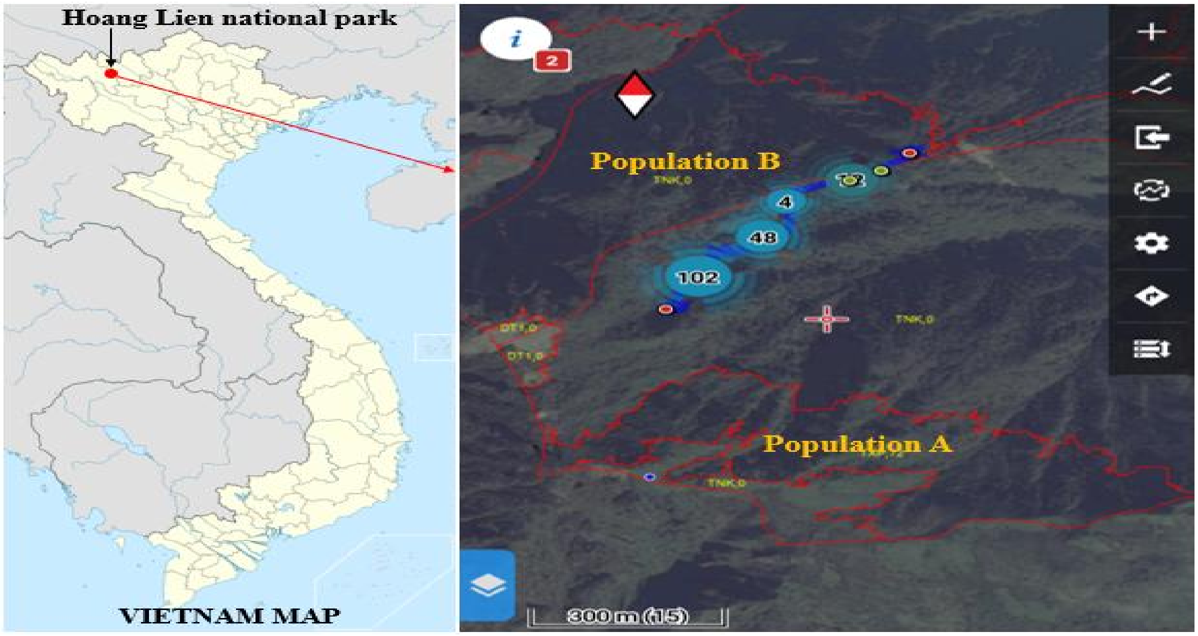


Fig.1. The study area in Hoang Lien National Park with two different populations of *Abies delavayi* subsp. *fansipanensis* distributed at the various altitudes (The red lines in the right part were borders of the study populations)

Vegetation structure and composition analyses: The vegetation was sampled along the soil samples' collection in population A and population B areas of *Abies delavayi* subsp. *fansipanensis* from the top of 2680 m and 2950 m down to 2600 m and 2750 m above sea level, respectively. The sampling was performed by establishing 60 and 30 plots of 20m × 20m size randomly on each position. The plant vegetation was determined for species richness, density and diversity of all the plots following standard methods (Whittaker, 1965; Sharma et al., 2009), therein species richness was the number of species per specified number of individuals, which was the species related diversity (Margalef, 1958). The tree basal area was calculated by using the circumference at breast height (cbh) with the formular πr^2, where r is the radius determined at 1.3m height. The basal area of a species was counted by multiplying density with the mean tree basal area. The sum of basal area of all species existing in the plot was total basal area. The relative dominance of a species was determined via the tree basal area. The sum of the relative frequency, relative density and relative dominance values was the importance value index (IVI) (Sharma et al, 2009; Phillips, 1959).

Growth parameters: Plant height of *Abies delavayi* subsp. *fansipanensis* was measured using clinometer while a flexible tape was used to determine the gift (G) of tree at 1.3 m above ground level. Diameter at breast height (dbh) was identified with the formular $dbh = G/\pi$. The relationship of plant height and diameter at breast height of *Abies delavayi* subsp. *fansipanensis* distributed in the altitude of 2600-2680 m and 2750-2950 m a.s.l. was investigated via the Pearson's correlations using the statistical software Minitab 16.

DNA extraction, polymerase chain reaction and genetic analyses: A total of 20 *Abies delavayi* subsp. *fansipanensis* samples were collected for genetic analysis, wherein, A1-A10 and B1-B10 letters were represented for the samples collected in the population A and population B, respectively. The young needle-leaf tissue samples of *Abies delavayi* subsp. *fansipanensis* were collected in the morning hours to avoid increasing of phenolic compounds for DNA isolation using modified CTAB method (Doyle et al, 1990; Sushma et al., 2017). Samples were chilled in liquid nitrogen, and were then ground to fine powder by a high-speed pulverizer. About 2 mg powder samples were soaked in 10 ml CTAB extraction buffer (2% w/v CTAB, 1.4 M NaCl, 20 mM EDTA, 100 mM Tris-Cl, pH 8.0) and 0.2% of β-mercaptoethanol at 65°C for 1 hour in a 50 ml polypropylene centrifuge tube, which was then cooled to room temperature. Subsequently, samples were extracted with 10 mL of Phenol : Chloroform : Isoamyl alcohol (v/v/v, 25:24:1) for 5 min and centrifuged at 10,000 rpm for 10 min at room temperature. DNA was purified from contaminants such as proteins and polysaccharides. In addition, RNase was added into each DNA sample (with a concentration of 5 µL RNase/ mg leaf tissues), followed by incubation at 37°C for one hour. The DNA samples were then extracted with Chloroform : Isoamyl alcohol (24:1) for 5 min and centrifuged at 10,000 rpm for 10 min at room temperature and checked for the quality by electrophoresis on 0.8% agarose gels.

Table 1. Primers used for the PCR amplification of DNA fragments.

Order	Regions	Primers	DNA sequence (3'-5')	Length (bp)
1	*rps*18-*rp*120	*rps*18-1	AGTCGATTTATTAGTGAGCA	584
		*rps*18-2	CTTCGTCGTTTGTTGATTAC	
2	*trn*L-*trn*F	*trn*L-1	TTGGCTTTATAGACCGTGAG	498
		*trn*L-2	CCAGGAACCAGATTTGAACT	
3	*nad*5	*nad5 ins 1*	GCATTCTGAGCTGGTTGGAT	979
		nad5 ins 2	GTGGGTGGGTATTCAGATGG	

The DNA fragments were amplified by using polymerase chain reaction (PCR) with specific primers designed following the genetic sequence of GenBank, as summarized in Table 1. The amplification reaction was optimized in a total volume of 20 µL, including 50 ng of the total DNA, 10 pM of each primer, 1 unit Phusion DNA polymerase, 1 mM deoxynucleotide triphosphates (dNTPs) and reaction buffers. A PCR program was of 33-35 cycles, including 95°C for 30 second, 56°C for 30 second, and 72°C for 40 second and then kept at 72°C for 5 min. The DNA was then loaded on 0.8% agarose and purified by GeneJET PCR Purification kit (Thermo scientific, USA), and analyzed by ABI 3500 Genetic analyzer following Sanger method utilizing using BigDye®Terminator v 3.1 Cycle Sequencing kit (Applied Biosystems, USA).

Sequence data were analyzed using the BioEdit software (Hall, 1999), in which the DNA sequences of *Abies delavayi* subsp. *fansipanensis* individuals in the two populations were compared to each other and compared with those of the same species A. delavayi and the other species A. concolor available in the GenBank database. The most appropriate sequence evolution model for each barcode sequence is determined using jModelTest software (Guindon and Gascuel, 2003; Darriba et al., 2012). Then, the pairwise distance between the sequences and the phylogenetic trees were analyzed following the Maximum likelihood methods (ML) using MEGA-X software with the bootstrap support values of posterior probabilities multiplied by 1000 replicates (Kumar et al., 2018).

RESULTS

Characteristics of climatic conditions in the study area: The variety of environmental conditions such as temperature, rainfall, humidity and sunshine hours in the study area was shown in Fig. 2. In general, the values of these factors were different changed during the research months in the Hoang Lien National Park. It is interesting to note that the temperature in the investigated area increased from January to April, then remained stable at a high degree until August, and decreased gradually in December. The weather is the warmest in June, July, August and is the coldest in December and January, and seem to cool in the whole year. Particularly, sometimes, snowing was appeared in the study area. It is obviously from the Figure 2A, the max temperature was changed from 19.5°C to 29.3°C whereas the min temperature was fluctuated in the range of 1.6°C and 16.9°C. There was a significant difference in the average rainfall in the study area between different months in the year 2019. The rainfall was found at 36.7 mm in February that was then slightly increased to an appropriate value of 142.6 mm in April. Interestingly, the rainfall was climbed considerably to 497.9 mm in July, and reached a highest rainfall of 778.5 mm in August. This figure suddenly dropped to about 200 mm of rainfall in September and then moderately decreased to appropriate values of 26.8 mm and 50.6 mm in November and December, respectively (Fig. 2A).

The modifications of humidity and sunshine hours in the investigated area were shown in Figure 2B. Obviously, a remarkable difference was reported in the average humidity between the different populations of *Abies delavayi* subsp. *fansipanensis* distributed in different study areas. The humidity changed from 64.6% to 88.4% in the distributed area of the mixed population of *Abies delavayi* subsp. *fansipanensis* with broad-leaved and coniferous-leaved trees, whereas the humidity of the distributed area of the population with the domination of *Abies delavayi* subsp. *fansipanensis* compared to the other plant species was fluctuated from 75.3% to 95.2%.

This shows that the higher the distribution of *Abies delavayi* subsp. *fansipanensis* on the mountains, the greater the monthly average humidity. In addition, a range from 92.3 to 230.3 hours was recorded as the total sunshine hour in each month of the research area. The sunshine hours were found at 92.3h, 103.3h, 108.0h, 117.8h, and 125.5h in Novembrer, January, July, October, and June, respectively. A figure of 145h to 177h was indicated in the total sunshine hours in May, March, September, and August. The higher values of sunshine hours over 200h for each month were reported in February (204.1h) and April (230.3h) (Fig. 2B). This indicated that the total sunshine hours in the research area was different between the months during the study process.

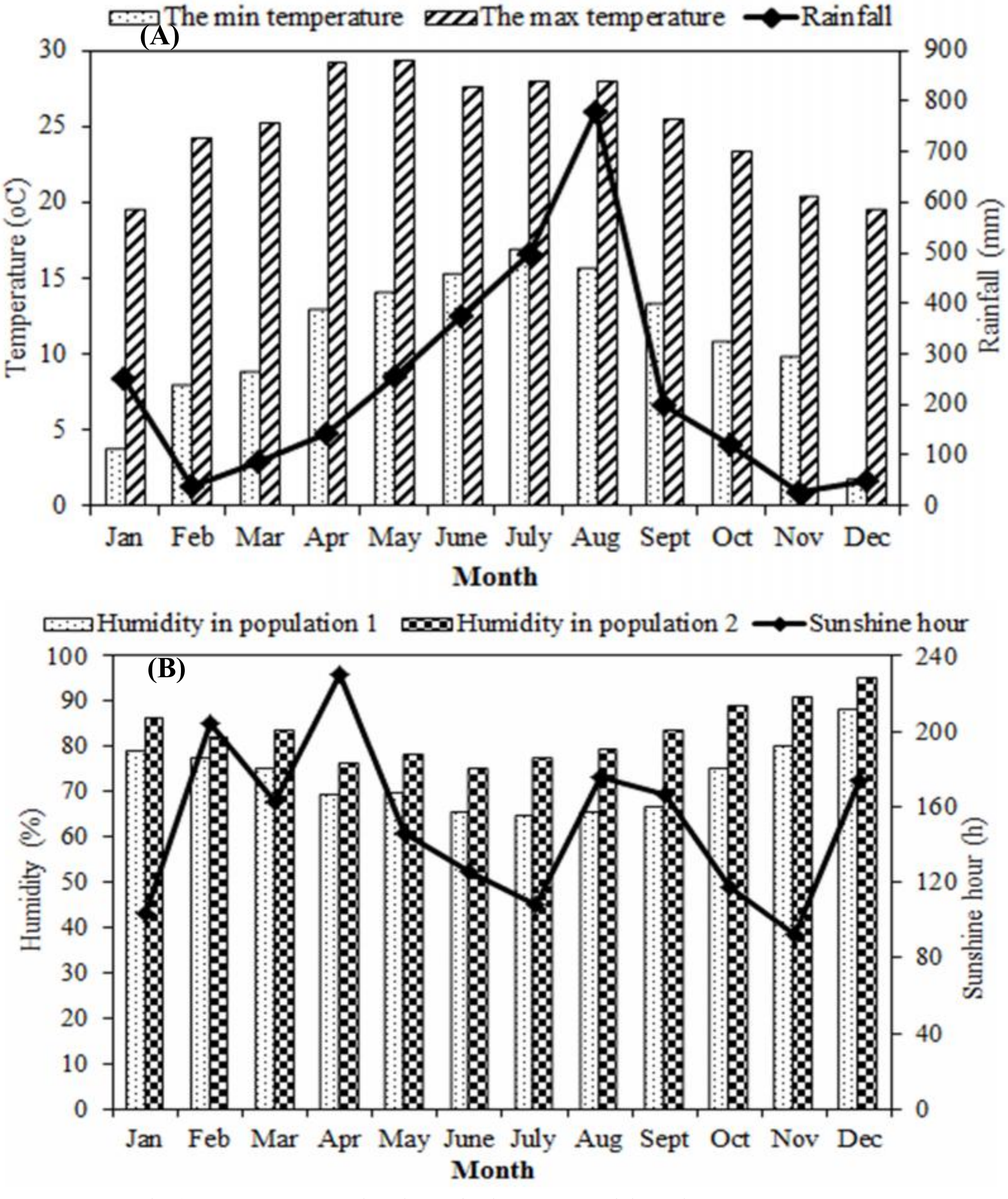


Fig.2. The characterization of climate conditions in the study area

Soil properties in the study area: The property of the soil collected in the investigated areas of two *Abies delavayi* subsp. *fansipanensis* populations was assessed via various parameters such as pH, the total nitrogen, the total phosphorus, the total organic carbon, and the cation exchange capacity (Ca^{+2}, Fe^{+2}, Mg^{+2}). pH values were found at 5.01 and 4.13 in the population A area and population B area, respectively. It is shown that the position of the population A had total nitrogen, total phosphorus and total organic carbon values were 2.53

(mg/g), 0.13%, and 72.50%, respectively, which were double higher than those of the population B position with the corresponding values of 1.23 (mg/g), 0.07%, and 34.08%, respectively. On the contrast, Fe^{2+} concentration in the soil samples collected in the population B area was 3341.42 (mg/kg) which was two times higher than the content of Fe^{2+} (1641.30 mg/kg) determined in the population A area. In addition, the values of Ca^{2+} and Mg^{2+} concentrations in the soil samples gathered in the population B field were 7.52 times and 2.85 times greater than those of the population A area (Table 2).

Table 2. Characteristics of the soil in the study area.

Parameters	Unit	Population A area	Population B area
pH	-	5.01 ± 0.05	4.13 ± 0.15
Total nitrogen	mg/g	2.53 ± 0.06	1.23 ± 0.14
Total phosphorus	%	0.13 ± 0.03	0.07 ± 0.01
Total organic carbon	%	72.50 ± 5.64	34.08 ± 3.04
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

Vegetation structure and composition: Table 3 showed the significant difference in the number of plant species, and their values of diversity, relative dominance, relative frequency and IVI between two populations of *Abies delavayi* subsp. *fansipanensis* distributed in various altitudes of 2600-2680 m a.s.l (A) and 2750-2950 m a.s.l. (B). It is estimated that a total of 1050 plants/ha with 24 plant species has been recorded from the population A whereas a figure of 625 plants/ha with 12 plant species were found in the population B. Particularly, the diversity and IVI values of *Abies delavayi* subsp. *fansipanensis* distributed in the population B were 8.03 and 8.95 times higher than those of the study plants distributed in the population A. In addition, the relative dominance and relative frequency of *Abies delavayi* subsp. *fansipanensis* were 3.51% and 4.93% of the population A, respectively; whereas the corresponding higher figures of 63.80% and 17.05% were found in the population B, respectively. Furthermore, there was a significant difference in the diversity, relative dominance, relative frequency and IVI between plant species belonged to two populations A and B. This illustrated that the difference in vegetation structure and composition between two populations of *Abies delavayi* subsp. *fansipanensis* distributed at the distinct altitudes of 2600-2680 m a.s.l and 2750-2950 m a.s.l in the Hoang Lien National Park. However, besides the distribution of *Abies delavayi* subsp. *fansipanensis*, nine other plant species have been determined in both studied populations, including: *Acer brevipes Gagn.*, *Camellia sp*, *Eurya distichophylla* Hermol, *Illicium tsai* L. C. Sm., *Prunus* sp1, *Rhododendron arboreum subsp. cinnamomum, Rhodoleia championii* Hook.f., *Rhododendron maddenii* Richard B., *Schefflera sp1*. This demonstrated that these plant species could be adapted to the environmental conditions in the national park at an altitude ranged from 2600 m to 2950 m above sea level.

Table 3. Structure and composition of species in the different populations of *Abies delavayi* subsp.*Fansipanensis.*

Species	Diversity (%)		Relative dominance (%)		Relative frequency (%)		IVI (%)	
	A	B	A	B	A	B	A	B
Abies delvayi subsp. *fansipanensis*	5.71	45.87	3.51	63.80	4.93	17.05	4.72	42.24
Acer brevipes Gagn.	6.83	4.00	6.56	1.61	5.63	3.41	6.34	3.01
Camellia sp	5.71	6.93	11.10	5.29	4.23	6.82	7.01	6.35
Eurya distichophylla Hermol.	5.08	5.60	2.07	3.02	6.34	10.23	4.50	6.28
Illicium tsai L. C. Sm.	5.24	2.13	4.38	2.10	4.23	5.68	4.62	3.31
Prunus sp1	1.90	2.67	0.81	0.61	2.82	5.68	1.85	2.98
Rhododendron arboreum subsp. cinnamomum	5.24	4.27	3.22	3.56	5.63	6.82	4.70	4.88
Rhodoleia championii Hook.f.	5.87	9.33	6.60	9.95	3.52	7.95	5.33	9.08
Rhododendron maddenii Richard B.	5.24	8.80	3.68	2.72	6.34	13.64	5.09	8.39
Schefflera sp1	2.38	2.13	1.16	0.76	4.23	3.41	2.59	2.10
Manglietia rufibarbata Dandy.	8.25	-	14.73	-	7.04	-	10.01	-
Schima wallichi (DC.) Korth.	1.90	-	7.48	-	5.63	-	5.01	-
Lithocarpus sp1	5.24	-	5.73	-	3.52	-	4.83	-

Ilex sp	4.13	-	5.29	-	3.52	-	4.31	-
Lithocarpus sp2	5.56	-	2.37	-	4.93	-	4.29	-
Acer campbelii var. *fansipanense* Gagn.	6.19	-	3.01	-	3.52	-	4.24	-
Rhododendron sp2	5.08	-	2.79	-	3.52	-	3.80	-
Castanopsis sp	2.38	-	3.54	-	3.52	-	3.15	-
Quecus sp	2.22	-	3.80	-	2.11	-	2.71	-
Cinnamomum durifolium Kost.	1.90	-	1.75	-	4.23	-	2.63	-
Schefflera sp2	1.90	-	1.59	-	3.52	-	2.34	-
Prunussp2	2.38	-	1.31	-	2.82	-	2.17	-
Symplocos sp1	1.90	-	1.75	-	2.11	-	1.92	-
Symplocos glauca Nooteb var. epapiela.	-	2.13	-	2.10	-	5.68	-	3.31
Pieris formosa	-	6.13	-	4.47	-	13.64	-	8.08

(Where: A is the mixed population of *Abies delavayi* subsp. *fansipanensis* with broad-leaved and coniferous-leaved trees distributed in the altitude of 2600-2680 m a.s.l and B is the dominant population of *Abies delavayi* subsp. *fansipanensis* in comparison with the other plant species distributed in the altitude of 2750-2950 m a.s.l.).

Relationship of tree height and diameter at breast height: Tree height (h) and diameter at breast height (dbh) are pivotal parameters used in determining tree growth. The relationship between h and dbh of *Abies delavayi* subsp. *fansipanensis* distributed in the different altitudes was shown in Fig.3. The coefficient (R^2) was 0.2749 corresponded to the correlation between tree height and dbh of *Abies delavayi* subsp. *fansipanensis* distributed in the population at the altitude of 2600-2680 m a.s.l. with broad-leaved and coniferous-leaved trees. This can be explained that the individuals of *Abies delavayi* subsp. *fansipanensis* were randoomly scattered with their living different space conditions among the dominant of other species in this population. Joseph and Ray (2013) indicated that the competition for light and nutrients between plant species was decided their distribution, evolution, and natural selection. As results, the individuals that are competing for light are ussually higher than those with less competition for light. On the other hand, the higher coefficient ($R^2 = 0.6824$) was found in the correlation between tree height and dbh of the study plants in the dominant population of *Abies delavayi* subsp. *fansipanensis* compared with the other plant species at the altitude of 2750-2950 m a.s.l. In this area, the research plants grow in concentration at the ecological dominant layer so they have similar competition for light, nutrients and water conditions.

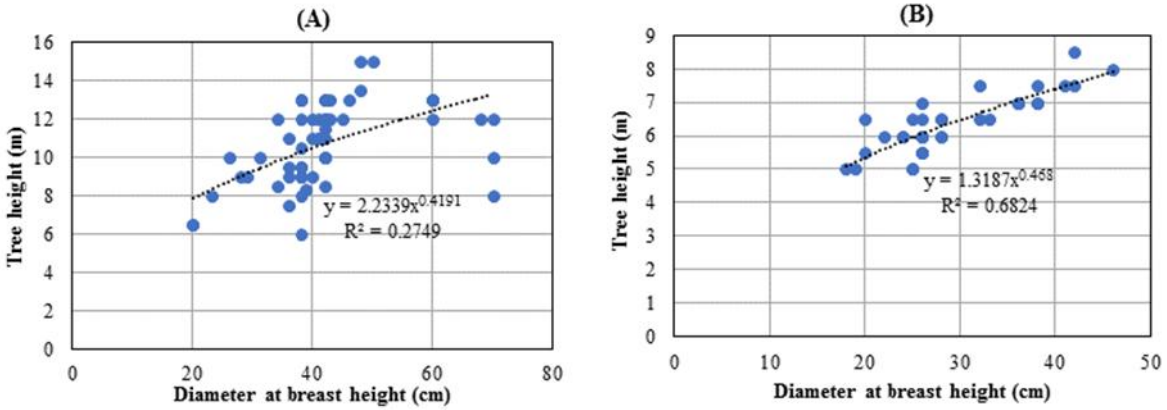
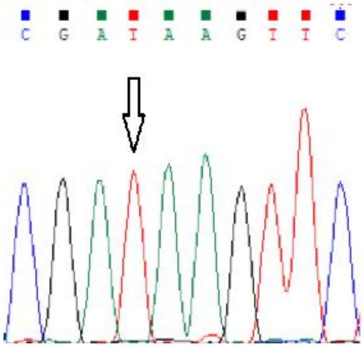


Fig.3. Relationships of tree height and diameter at breast height of *Abies delavayi* subsp. *fansipanensis*distributed in the altitudes of 2600-2680 m a.s.l.(A) and 2750-2950 m a.s.l. (B)

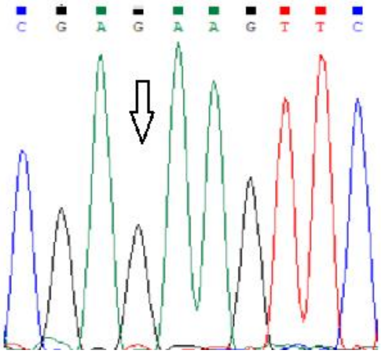
Genetic structure and characteristics

DNA sequence analysis: Total DNA was successfully isolated from the leaves of *Abies delavayi* subsp. *fansipanensis* distributed in different altitudes of 2600-2680 m and 2750-2950 m a.s.l. The DNA fragments were amplified by polymerase chain reactions using *rps*18 and *trn*L primers in the chloroplast genome and *nad*5 primers in the mitochondrial genome, respectively. DNA sequencing of *Abies delavayi* subsp. *fansipanensis* samples in two populations were analyzed and compared with those of *Abies delavayi* and *Abies concolor* available from GenBank.

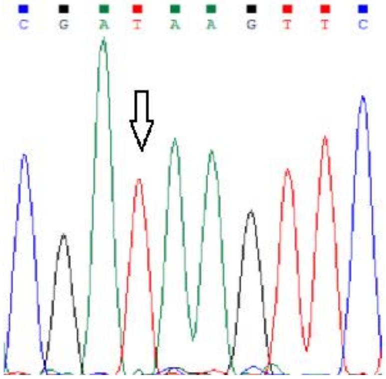
***rps*18-*rpl*20**



Nucleotide position at the 323rd of the samples: A1-5, A7-10

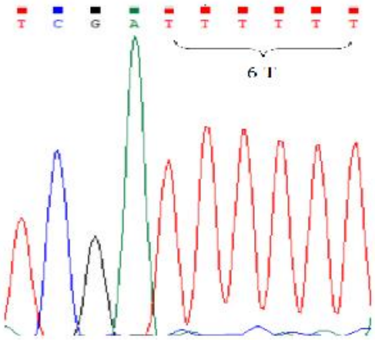


Nucleotide position at the 323rd of the samples: A6, B5, B6, B9, B10

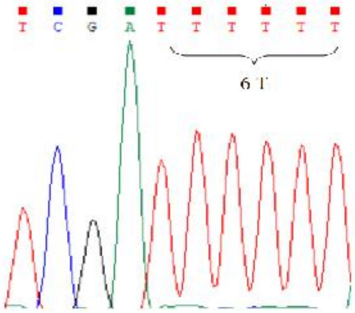


Nucleotide position at the 323rd of the samples: B1-4, B7, B8

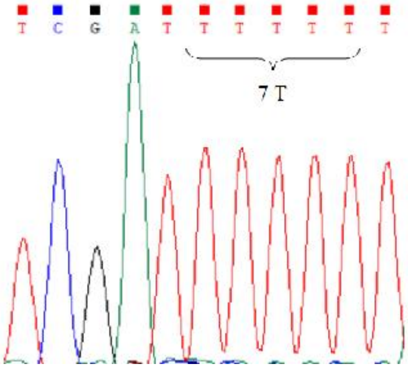
trnL-trnF



Nucleotide position at the 223rd of the samples: A1-10



Nucleotide position at the 223rd of the samples: B1, B3-10



Nucleotide position at the 223rd of the samples: B2

Fig.4. The nucleotide changes found in a few positions in the DNA sequence of *Abies delavayi* subsp. *fansipanensis* (A1-A10 and B1-B10 are the studied samples collected at the altitudes of 2600-2680 m and 2750-2950 m a.s.l, respectively)

No differences were found in the DNA sequence using *nad5* primers between *Abies delavayi* subsp. *fansipanensis* samples in two populations in comparison with those of *Abies delavayi* and *Abies concolor*. In the case of *rps*18-*rpl*20, it is interesting to note that G was found at the 323rd position in the sequence of one sample (A6) of the population A and of several samples including B5, B6, B9 and B10 of the population B while T was detected in the remained samples of these two populations of *Abies delavayi* subsp. *fansipanensis*. In terms of the *trnL-trnF* region, a T was added into the DNA sequence of the sample B2, whereas no differences were determined in the other samples of *Abies delavayi* subsp. *fansipanensis* populations (Fig. 4). These results showed that the DNA sequences of the three selected genome regions were identical in the majority of *Abies delavayi* subsp. *fansipanensis* individuals in the both populations, except for some nucletide positions found in a few individuals of the studied plants in these populations. The data obtained suggest that there was a little difference in the genome sequence of the *Abies delavayi* subsp. *fansipanensis* distributed on the altitudes of 2750-2950 m a.s.l. in comparison with those of the study plants distributed on the altitudes of 2600-2680 m a.s.l. This means that the DNA sequence of the *Abies delavayi* subsp. *fansipanensis* distributed on the altitudes of 2750-2950 m a.s.l. was more diverse than that of the investigated species distributed on the altitudes of 2600-2680 m a.s.l.

Genetic distance and phylogenetic tree

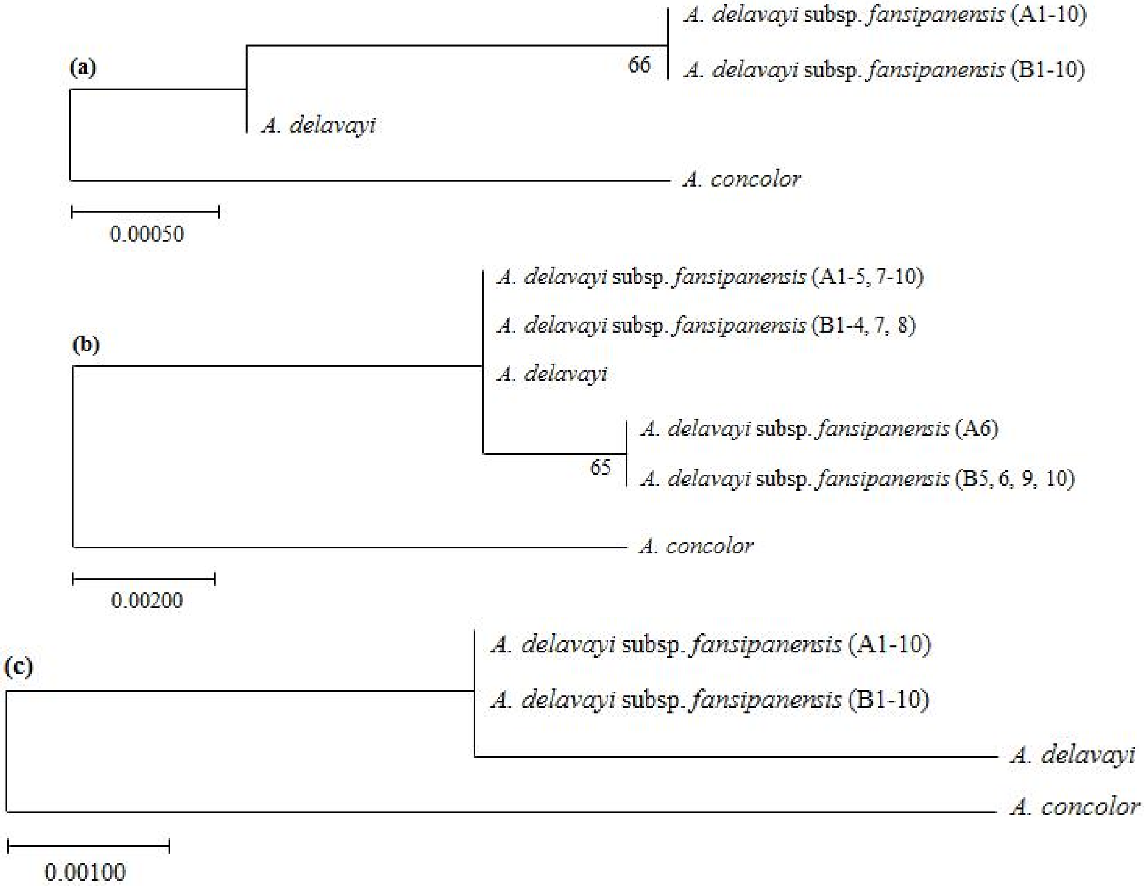


Fig.5. Phylogenetic trees of *Abies delavayi* subsp. *fansipanensis* distributed in the altitudes of 2600-2680 m (A1-10) and 2750-2950 m (B1-10) a.s.l.and other species based on nucleotide sequences of nad5 (a), rps18-rpl20 (b) and trnL-trnF primers in the genomes (Numbers below the branches are the bootstrap support values with posterior probabilities multiplied by 1000 replicates)

The genetic distances of the *Abies delavayi* subsp. *fansipanensis* were compared among the individuals distributed in the same population, between those existed in the different populations, and between this species in comparison with the other species as *Abies delavayi* and *Abies concolor*. Results illustrated that no genetic distances were found between the plants distributed between the population A and population B under DNA analysis using nad5 and trnL-trnF primers while the genetic distances between the individuals in two populations distributed in the altitudes of 2600-2680 m and 2750-2950 m a.s.l analyzing used rps18-rpl20 primers were changed from 0.00000-0.00223. Interestingly, the genetic distances between *Abies delavayi* subsp. *fansipanensis* and *Abies delavayi* analyzed by *nad5* and *trnL-trnF* primers were found at 0.00102 and 0.00248, respectively. Particularly, a figure dropped into the range from 0.00000 to 0.00223 was reported as the genetic distance of the studied plants in two populations, especially, the *Abies delavayi* subsp. *fansipanensis* plants distributed in the altitude of 2750-2950 m a.s.l and *Abies delavayi* under DNA analysis using rps18-rpl20. In comparison with *Abies concolor*, the genetic distances of the *Abies delavayi* subsp. *fansipanensis* were determined at 0.00307, 0.00746, and 0.01576 when measuring DNA used *nad5*, *trnL-trnF* and *rps18-rpl20* primers, respectively.

As can be seen from Fig.5, the phylogenetic trees of species were split into two major branches with the first branch was composed of two deeply divergent clades, whereas the second branch was the A. concolor in analysis the nucleotide sequences of *nad5*, *rps18-rpl20* and *trnL-trnF*. The first clade corresponds to A. delavayi and a subclade divided to *Abies delavayi* subsp. *fansipanensis* individuals (A1-10 and B1-10) distributed in the altitudes of 2600-2680 m and 2750-2950 m a.s.l with the bootstrap support values as 66 when analyzing phylogenetic tree using *nad5* nucleotide sequence. It is interesting to note that, the opposite splitting was reported in the first branch in the phylogenetic tree when investigating the nucleotide sequences of *trnL-trnF*, which including *Abies delavayi* subsp. *fansipanensis* individuals distributed in the altitudes of 2600-2680 m and 2750-2950 m a.s.l and a subclade as *A. delavayi*. Particularly, the first branch of the phylogenetic tree based on nucleotide sequence of *rps18-rpl20* was divided into *Abies delavayi* subsp. *fansipanensis* individuals as A1-5, A7-10, B1-4, B7, B8 and *A. delavayi*, and a subclade was found with several individuals of *Abies delavayi* subsp. *fansipanensis* as A6, B5, B9, B10. These data illustrated that there were a few individuals of *Abies delavayi* subsp. *fansipanensis* in the populations, especially, the individuals belonged to the population distributed in the higher altitude of 2750-2950 m a.s.l were differed considerably from the remained individuals existed in the same population and in the lower altitude of 2600-2680 m a.s.l. This indicated that the population of *Abies delavayi* subsp. *fansipanensis* distributed in the higher altitude on Fansipan-mountain was more diverse in genome than those lived in the lower altitude. It might be the wildfire status was frequently happening in the higher altitude of 2750-2950 m a.s.l in the past that fired many species, but a few species still existed included *Abies delavayi* subsp. *fansipanensis* due to natural selection which created the adaptation by the diversity in the genome of this species.

DISCUSSION

These data demonstrated that *Abies delavayi* subsp. *fansipanensis* distributed in the conditions with temperature ranged from 1.6°C to 29.3°C, the rainfall was dropped in the range of 26.8-778.5 mm, high humidity of 65-95%, and less sunshine hours of 92-230h. It is complied with previous studies of Gazoul & Le Mong Chan (1994) who reported the annual temperature in Sapa ranging from 1-29°C. In addition, Hoang Lien National Park was considered as the area with the seasonal climate characterized by "subtropical climate in summer" and "temperate climate in winter" (Kemp et al., 1995). According to Bennie et al. (2006), Marini Lorenzo (2007) and Khalid et al. (2016), environmental conditions play a pivotal role in the distribution and diversity of the plants. On the other studies indicated that the variation of environmental factors affected the changes of plant community composition (Heino, 2010; Mykrä et al., 2010; Victorero et al., 2018). This illustrated that the above climate conditions reflect the specific bio-ecological characteristics of *Abies delavayi* subsp. *fansipanensis* distributed in the Fansipan-mountain, Lao Cai province, Vietnam.

In the previous studies, Myklestad (2004) and Marini Lorenzo et al. (2007) demonstrated that chemical and physical soil properties affected both species richness and evenness of vascular plants. Taiz & Zeiger (1991) indicated that the availability of nutrients in soil to plants is determined by soil pH, which affected plant community composition and species richness (Laughlin and Abella, 2007). The changes in species diversity and production were impacted by soil nutrient contents and soil pH (Zhao et al., 2007; El-sheikh et al., 2010). In addition, the nutrient contents in the soil might influence the structure of plant communities (Peña-Claroset al., 2012; Becknell and Power, 2014), and the soil fertility is positively related to plant species richness (Dybzinski et al., 2008; Neri et al., 2012). This indicates that the distinction in the soil properties of the samples collected in the areas of two *Abies delavayi* subsp. *fansipanensis* populations reflect the difference in the distribution, diversity and the structure of plant vegetation of this species in the Fansipan-mountain.

According to Elmqvist et al. (2003) and Dorren et al. (2004), vegetation structure and species diversity are essential components for a long-term persistence of an ecosystem. In addition, knowledge of the biodiversity of the forest is crucial assess the consequences of their degradation and wider habitat loss caused by human activities, and to develop systematic strategies for their conservation and management, the ability to forecast future forest composition (Christelle et al., 2019). Moreover, determination of vegetation structure providing data on habitat suitability and ecosystem productivity, whereas measuring of species diversity plays a pivotal role in providing information on susceptibility to the ratio of native and exotic species, and the structure of trophic level needed for ecosystem resilience (Nichols and Nichols, 2003; Jones et al., 2004; Wang et al., 2004). This suggested that understanding the vegetation structure and composition of *Abies delavayi* subsp. *fansipanenis* in the study areas is the basis for proposing solutions to conserve and promote this rare genetic resource in the Fansipan-mountain, Vietnam.

The dominant plants possess linear diameter-height trajectories whilst suppressed plants own curve trajectories, with diameter growth decreasing more than height growth (Akihiro et al. (2013). In addition, the relationship of tree height and diameter plays an important role for understanding the tree growth dynamic patterns and estimating the accumulation of tree biomass. Furthermore, an important prediction from metabolic ecology relevant to tree growth is the declared universal scaling invariant in the ratio of plant height and diameter (Chen and Brockway, 2017). This illustrated that there were differences in plant height, diameter at breast height, and morphology of the individuals of *Abies delavayi* subsp. *fansipanensis* distributed in the altitude of 2600-2680 m a.s.l. in comparison with those of the plants lived in the altitude of 2750-2950 m a.s.l.

In the study of Perry and Lotan (1979) and Chapman and Crow (1981), forest fires can have a significant effect on gene frequencies and genetic drift. However, in the previous studies, Rajora and Pluhar (2002) indicated that there is little or no information on the genetic effects of natural or prescribed forest fires. In addition, it was not known whether the genetic effects of forest fires in comparison with harvesting practices. On the other hand, forest fires have been an integral part of the boreal forest ecosystems. Furthermore, the ecosystem-based natural disturbance regime is often proposed as a basis for forest management in many nations over the world. Nevertheless, genetic data to support this proposition is generally lacking. Therefore, this is considered as the first study of genetic structure characteristics of *Abies delavayi* subsp. *fansipanensis* distributed in the Fansipan-mountain, Vietnam. However, it is needed to further study of this species using more samples and genome sequences to accurately distinguish the two populations of *Abies delavayi* subsp. *fansipanensis* belong to the same or different subspecies. The study plays a pivotal role in assessing the genetic characteristics and the diversity of *Abies delavayi* subsp. *fansipanensis* species distributed on the Fansipan-mountain, which contributes to the plant species biodiversity and application potential serving for social needs in Vietnam and the world. In addition, the conservation of *Abies delavayi* subsp. *fansipanensis* will also help to reduce the situation of burning forest for cultivation, thus making an important contribution to the protection of environment and ecosystems.

Conclusions: *Abies delavayi* subsp. *fansipanensis* distributed on the Fansipan mountain range, Lao Cai province, Vietnam with the environmental conditions were determined including the temperature ranged from 1.6°C to 29.3°C, the rainfall fluctuated in the range of 26.8 mm and 500 mm, the humidity changed from 65% to 95%, and the sunshine hours were modified from 90 h to 230 h. In addition, soil pH was 4-5 whereas the total organic carbon value was dropped in the range of 34-73%. The IVI and R^2 values of *Abies delavayi* subsp. *fansipanensis* in the mixed population of this species with broad-leaved and coniferous-leaved trees were 4.72% and 0.2749, whereas those of the study plants in the dominant population of *Abies delavayi* subsp. *fansipanensis* compared to other specieswere 42.24% and 0.6824, respectively. The results showed the difference in vegetation structure and composition, and genetic characteristics of *Abies delavayi* subsp. *fansipanensis* population distributed in the higher altitude of 2750-2950 m a.s.l compared to those of the study plants distributed in the lower altitude of 2600-2680 m a.s.l. Our study provides valuable data about bio-ecological and genetic structure characteristics of *Abies delavayi* subsp. *fansipanensis* distributed in two altitudinal belts on Fansipan-mountain, Lao Cai province, which serves as important scientific bases for proposing solutions to conserve and promote the rare genetic resource of *Abies delavayi* subsp. *fansipanensis* in Vietnam as well as in the world.

Author Contributions: Hung Manh Nguyen and Van Sinh Nguyen conceived and designed the experiments; Hung Manh Nguyen performed the experiments; Thi Thu Hue Huynh and Hung Manh Nguyen analyzed the data; Van Nhan Le contributed reagents/materials/analysis tools; Van Nhan Le; Hung Manh Nguyen and Thi Huong Do wrote the paper.

Conflicts of Interest: The authors declare no conflicts of interest.

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Supplementary Materials

Figure 1. Status of dominant vegetation structures mixed between broad leaves trees and conifers (*Abies delavayi* subsp. *fansipanensis*) at the altitude of 2600m a.s.l.

Figure 2. Status of dominat vegetation structures by conifers (*Abies delavayi* subsp. *fansipanensis*) at the altitude of 2750m a.s.l.

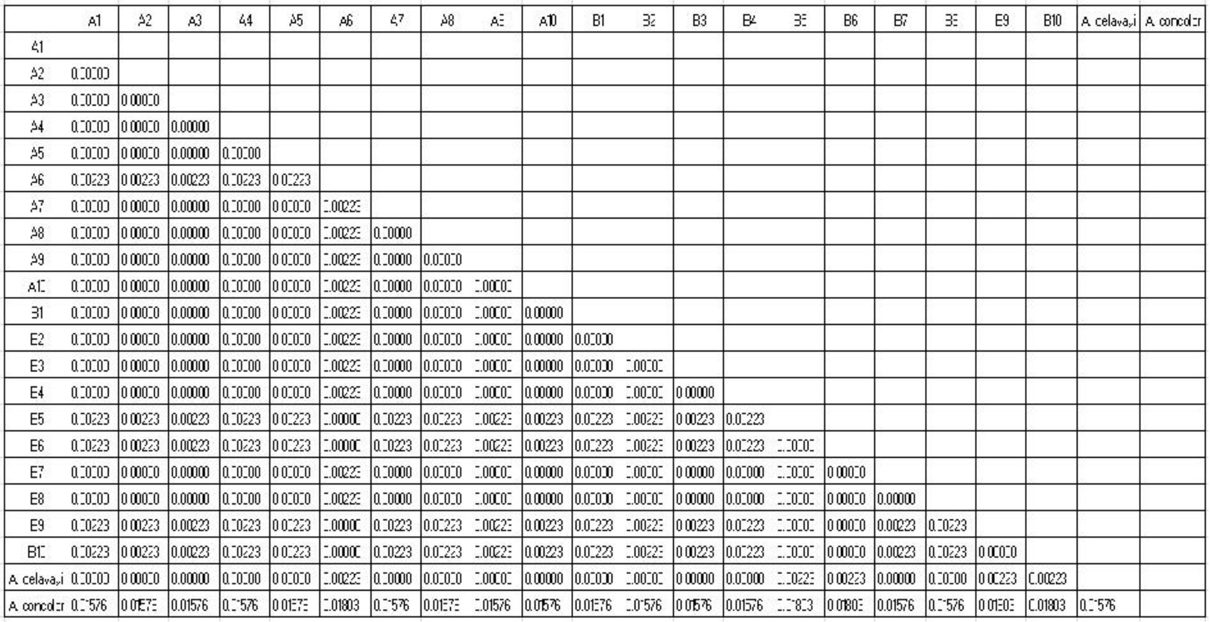
Figure 3. Status of dominat vegetation structures by conifers (*Abies delavayi* subsp. *fansipanensis*) at the altitude of 2900m a.s.l.

Table 1. The genetic distances of the *Abies delavayi* subsp. *fansipanensis* distributed in the altitudes of 2600-2680 m and 2750-2950 m a.s.l in comparison with *Abies delavayi* and *Abies concolor*

(a) Base on *nad*5 region

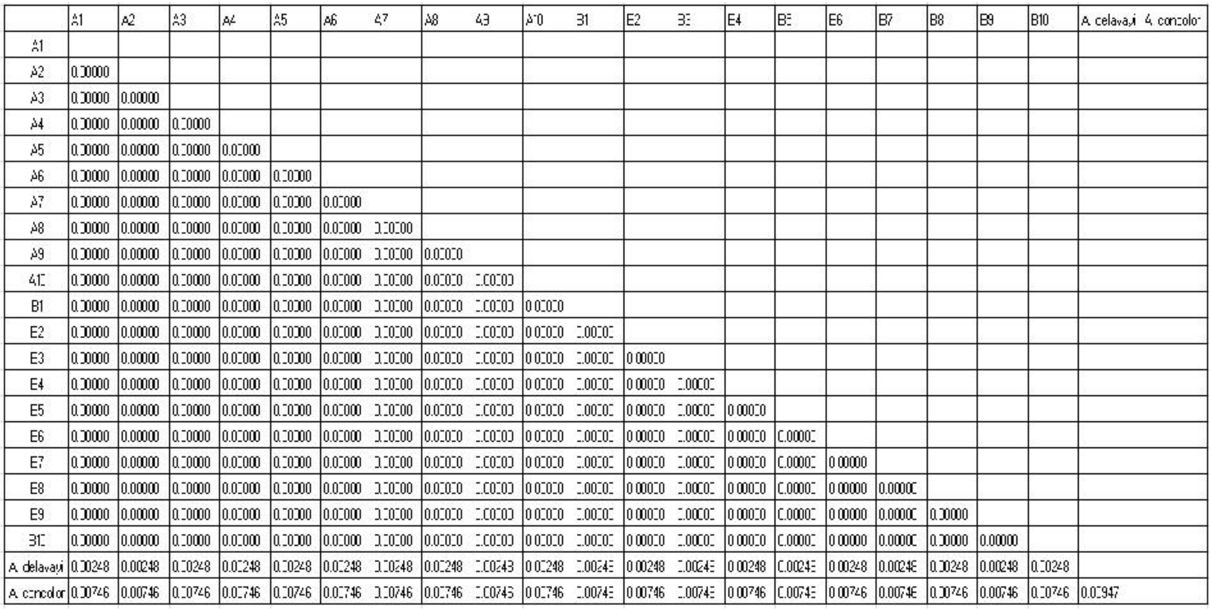
	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	A. delavayi	A. concolor
A1																						
A2	0.00000																					
A3	0.00000	0.00000																				
A4	0.00000	0.00000	0.00000																			
A5	0.00000	0.00000	0.00000	0.00000																		
A6	0.00000	0.00000	0.00000	0.00000	0.00000																	
A7	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000																
A8	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000															
A9	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000														
A10	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000													
B1	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000												
B2	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000											
B3	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000										
B4	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000									
B5	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000								
B6	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000							
B7	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000						
B8	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000					
B9	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000				
B10	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000			
A. delavayi	0.00102	0.00102	0.00102	0.00102	0.00102	0.00102	0.00102	0.00102	0.00102	0.00102	0.00102	0.00102	0.00102	0.00102	0.00102	0.00102	0.00102	0.00102	0.00102	0.00102		
A. concolor	0.00307	0.00307	0.00307	0.00307	0.00307	0.00307	0.00307	0.00307	0.00307	0.00307	0.00307	0.00307	0.00307	0.00307	0.00307	0.00307	0.00307	0.00307	0.00307	0.00307	0.00204	

(b) Base on rps18-rpl20



	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	A. delavayi	A. concolor
A1																						
A2	0.00000																					
A3	0.00000	0.00000																				
A4	0.00000	0.00000	0.00000																			
A5	0.00000	0.00000	0.00000	0.00000																		
A6	0.00223	0.00223	0.00223	0.00223	0.00223																	
A7	0.00000	0.00000	0.00000	0.00000	0.00000	0.00223																
A8	0.00000	0.00000	0.00000	0.00000	0.00000	0.00223	0.00000															
A9	0.00000	0.00000	0.00000	0.00000	0.00000	0.00223	0.00000	0.00000														
A10	0.00000	0.00000	0.00000	0.00000	0.00000	0.00223	0.00000	0.00000	0.00000													
B1	0.00000	0.00000	0.00000	0.00000	0.00000	0.00223	0.00000	0.00000	0.00000	0.00000												
B2	0.00000	0.00000	0.00000	0.00000	0.00000	0.00223	0.00000	0.00000	0.00000	0.00000	0.00000											
B3	0.00000	0.00000	0.00000	0.00000	0.00000	0.00223	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000										
B4	0.00000	0.00000	0.00000	0.00000	0.00000	0.00223	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000									
B5	0.00223	0.00223	0.00223	0.00223	0.00223	0.00000	0.00223	0.00223	0.00223	0.00223	0.00223	0.00223	0.00223	0.00223								
B6	0.00223	0.00223	0.00223	0.00223	0.00223	0.00000	0.00223	0.00223	0.00223	0.00223	0.00223	0.00223	0.00223	0.00223	0.00000							
B7	0.00000	0.00000	0.00000	0.00000	0.00000	0.00223	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000						
B8	0.00000	0.00000	0.00000	0.00000	0.00000	0.00223	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000					
B9	0.00223	0.00223	0.00223	0.00223	0.00223	0.00000	0.00223	0.00223	0.00223	0.00223	0.00223	0.00223	0.00223	0.00223	0.00000	0.00000	0.00223	0.00223				
B10	0.00223	0.00223	0.00223	0.00223	0.00223	0.00000	0.00223	0.00223	0.00223	0.00223	0.00223	0.00223	0.00223	0.00223	0.00000	0.00000	0.00223	0.00223	0.00000			
A. delavayi	0.00000	0.00000	0.00000	0.00000	0.00000	0.00223	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00223	0.00223	0.00000	0.00000	0.00223	0.00223		
A. concolor	0.01576	0.01576	0.01576	0.01576	0.01576	0.01803	0.01576	0.01576	0.01576	0.01576	0.01576	0.01576	0.01576	0.01576	0.01803	0.01803	0.01576	0.01576	0.01803	0.01803	0.01576	

(c) Base on trnL-trn Fregion



	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	A. delavayi	A. concolor
A1																						
A2	0.00000																					
A3	0.00000	0.00000																				
A4	0.00000	0.00000	0.00000																			
A5	0.00000	0.00000	0.00000	0.00000																		
A6	0.00000	0.00000	0.00000	0.00000	0.00000																	
A7	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000																
A8	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000															
A9	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000														
A10	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000													
B1	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000												
B2	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000											
B3	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000										
B4	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000									
B5	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000								
B6	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000							
B7	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000						
B8	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000					
B9	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000				
B10	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000			
A. delavayi	0.00248	0.00248	0.00248	0.00248	0.00248	0.00248	0.00248	0.00248	0.00248	0.00248	0.00248	0.00248	0.00248	0.00248	0.00248	0.00248	0.00248	0.00248	0.00248	0.00248		
A. concolor	0.00746	0.00746	0.00746	0.00746	0.00746	0.00746	0.00746	0.00746	0.00746	0.00746	0.00746	0.00746	0.00746	0.00746	0.00746	0.00746	0.00746	0.00746	0.00746	0.00746	0.00947	