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GENETIC DIVERSITY IN THE NATURAL POPULATIONS OF *Pinus dalatensis* Ferré (PINACEAE) ASSESSED BY SSR MARKERS

Dinh Thi Phong*, Vu Thi Thu Hien, Tran Thi Lieu, Nguyen Tien Hiep

Vietnam National Museum of Nature, Vietnam Academy of Science and Technology,
18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam

*Email: dinhthiphong@hotmail.com

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ABSTRACT

Pinus dalatensis Ferré (Family: *Pinaceae*) is an endemic plant with restricted habitats at higher altitudes in highland of Vietnam. The species is now near threatened by over-exploitation and habitat destruction. The genetic variation within and among populations of *P. dalatensis* was investigated by 41 microsatellite (single sequence repeat, SSR) primers, but only 11 SSR primers showed polymorphism bands. In all, 70 sampled trees collected from six populations located in different altitude (Xa Hieu, Dak Glei and Ngoc Linh in Kon Tum, Da Chais in Lam Đong, Hoa Son in Dak Lak and A Yun in Gia Lai) in highland of Vietnam were analyzed in this study. Among 40 putative genetic alleles were amplified, of which 39 were polymorphic (accounting for 97.5 %). The mean number of alleles per locus was 2.197. The SSR data showed a genetic diversity parameters within populations with an average of I = 0.524, Ho = 0.222, He = 0.317, Fis =0.333 and Ap = 0.106. The number private alleles (Ap) was only found in four populations, the Hoa Son population showed the highest (0.273), followed by Da Chais (0.182), and value of 0.091 for all Ngoc Linh and A Yun. Analysis of molecular variance analysis showed that most genetic variation was within populations of 57.504 % and among population of 42.496 %. The population pairwise differentiations indicated that most of the populations were significantly differentiated $p < 0.001$ with Fst values ranged from 0.195 to 0.418. This study highlights the importance of conserving the genetic resources of *P. dalatensis* species.

Keywords: genetic diversity, near threatened, *Pinus dalatensis*, species conservation, SSR markers.

1. INTRODUCTION

Pinus dalatensis, also known as Da Lat pine [1] is a species of pine endemic to Vietnam. In Vietnam the species grows in the mountains of the central and south-central parts of the country at elevations by 600 to 2,600 m [2, 3, 9] (Fig. 1).

Until recently, the species was regarded as one of the endemic trees of Vietnam, although several references have noted the possibility of its occurrence in the mountain ranges extending into Laos [2, 4, 5, 6]. The presence of this species in Laos was confirmed in 2006 by Thomas

(2007) due to the discovery of a population with over 200 trees at altitude between 800 – 1,100 m at the Nakai Nam Theun conservation area [7]. This new locality represents a significant extension of the northernmost limit of the species. The Checklist of the plant species in Vietnam recorded that *P. dalatensis* only exits in Kon Tum, Dak Lak, Lam Dong, Khanh Hoa and Ninh Thuan provinces [8, 9].

*Figure 1***.** Adult tree and twigs with cone of *P. dalatensis* in Lam Dong province, Vietnam. Photographs by Nguyen Tien Hiep.

P. dalatensis is a montane pine growing in tropical climate at 600 to 2,600 m above sea level and forms stands of a few to about 30 trees surrounded by the evergreen angiosperm forest dominated by members of the Fagaceae. Accoding to Hiep and Vidal (1996) *P. dalatensis* was found in altitude of 1,500 - 2,400 m, however, this was as high as 1,400 - 2,600 m by Loc and 1,500 - 2,200 m by Luu and Thomas [10, 11, 6]. Recently, Loc reported it can be found in 600-2600 m [9]. In most localities, the pines occupy rocky outcrops or ridges and adjacent slopes where competition from broadleaves is less intense. Some other conifers may also be present, among these are the rare pine *P. krempfii* and *Fokienia hodginsii*. Some detail reports on the morphology and taxonomy of *P. dalatensis* have been concerned [9, 12, 13, 14].

Due to the exploitation of *P. dalatensis* for its valuable timber by local people and forestry enterprises, its habitats are significantly affected by forest fragmentation, deforestation and unsustainable managements such as indiscriminate exploitation. Logging results in intense fragmented habitats and low density populations. This has threatened the long-term survival of the *P. dalatensis* resource. Conservation and management of a species are required information on the genetic diversity and ecology within and among populations. In order to obtain such information, especially a better understanding of genetic processes, powerful biological techniques are required. Organellar genomes (chloroplast and nuclear) are uniparentally inherited. Chloroplast is inherited via pollen whereas nuclear are inherited via seeds. Therefore, organellar DNA markers have been used for genetic differentiation between populations. Microsatellite markers (SSRs - single sequence repeats) are useful to analyze the effective pollen flow and seed dispersal within populations. Thus, these markers (high polymorphic) have been used for studies on gene flow, genetic parameters and mating systems in some pine species [15, 16, 17, 18, 19].

Evolutionary potential of a species depends on its genetic variation. Understanding the amount of genetic diversity provides information for the development of conservation strategies and sustainable utilization of a species. The objective of this study is to use SSRs as genetic markers to investigate the level of genetic variability within and between populations of *P.*

dalatensis species and to provide guidelines for the conservation, management and restoration of this species to the Protection Forestry Department, Vietnam.

2. MATERIAL AND METHODS

2.1. Materials

This research was carried out at six sites; 3 in Kon Tum and 01 each in Lam Dong, Dak Lak and Gia Lai (Fig. 2 and Table 1).

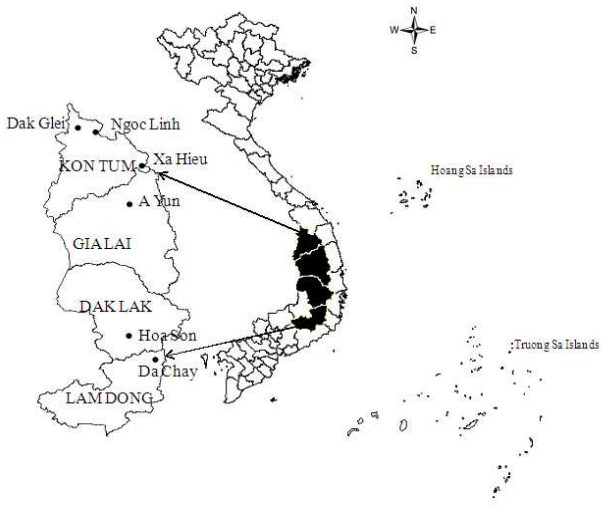

Figure 2. Map showing the studying sites of *P. dalatensis.*

Table 1. Collection locations of *P. dalatensis* samples.

Population code	Collection locality	Sample size	Sample code	Latitude (◦N)	Longitude (◦E)	Elevation (m)
Xa Hieu	Xa Hieu, Kon Plong, Kon Tum	6	Pd1 - Pd6	14°40’40”N	108°23’36”E	1159
Dak Glei	Dak Glei, Dak Glei, Kon Tum	9	Pd7 -Pd15	15°01’17”N	107°48’04”E	1553
Ngoc Linh	Ngoc Linh, Dak Glei, Kon Tum	5	Pd16 - Pd20	15°03’29”N	107°55’41”E	1935
Da Chais	Da Chais, Lac Duong, Lam Dong	20	Pd21-Pd40	12°11’02.7”N	108°41’24.3”E	1482
Hoa Son	Hoa Son, Krong Bong, Dak Lak	15	Pd41- Pd55	12°29’33.2”N	108°18’17.1”E	1116
A Yun	A Yun, Mang Yang, Gia Lai	15	Pd56- Pd70	14°12’40.7”N	108°16’48.9”E	895

In this study, the inner bark from 29 to 38 mature trees was randomly sampled from the 6 populations, representing the natural range of *P. dalatensis* in Vietnam, which were taxonomic identification. The samples were placed in Ziploc bags with silica gel in the field and then transferred to Laboratory of Molecular Biology, Vietnam National Museum of Nature and stored at 4 ^{o}C until DNA extraction.

Table 2. SSR loci, primer sequences, marker sizes, optimum annealing temperatures.

Primers code	Nucleotide	Reference	Tm	Size (bp)
Pnh038	ACTCATTTCCGGATGGTGG	[20]	53	150-160
	TGGGGCTTGGACTTCAAGA			
Pnh188	GGCAATCATAGGGTTAGGTC	[20]	56	100-150
	GGCATGTATGTATTACTGTG			
Pnh277	ATGCATGTTGCCTAGTTCC	[20]	53	100-220
	ACAAGGATTTACCTTGGTTCACC			
NZPR6	GGAAGAAAAATTGGGCCTTA	[21]	51	110-410
	CTCTCTATCTCTGCCCCA			
Pt15169	CTTGGATGGAATAGCAGCC	[15]	53	150-200
	GGAAGGGCATTAAGGTCATTA			
Pt26081	CCCGTATCCAGATATACTTCCA	[15]	52	120-250
	TGGTTTGATTCATTCGTTCAT			
PtTX3030	AATGAAAGGCAAGTGTCG	[18]	53	200-225
	GAGATGCAAGATAAAGGAAGTT			
PtTX3034	TCAAAATGCAAAAGACG	[18]	55	240-420
	ATTAGGACTGGGGATGAT			
PRE13	GATGTGTCTTTAGGCTCGTTGC	[16]	52	120-480
	AGGGTTAGTAATCACGGCCTGT			
RPS60	ACGATAATGGCGGTGAGAACAA	[22]	52	165-240
	TCATTGTTCCCCAAATCAT			
RPS119	CCACCTGTCCTTCGTACATCCA	[22]	50	210-250
	TTGTGAGAAGATACTTCCTCCA			

2.2. Methods

DNA extraction: Seventy *P. dalatensis* genotypes were used for this study (coded from Pd1 to Pd70). Total genomic DNA was isolated from leaves using the method described by Porebski et al. (1997). Liquid nitrogen was added to about 100 mg of each sample which was then ground by hand [23]. The concentration of DNA was determined by a UV-visible light

spectrophotometer (UVS 2700, Labomed, USA), and the DNA samples were diluted to 20 ng/µL and used as templates for PCR amplification

SSR markers amplification: SSR primers were obtained from Integrated DNA Technologies, USA (Table 2). PCR amplifications were performed in a final volume of 25 µl in the presence of 50 ng DNA, 1 U of *Taq* polymerase (Platinum Taq DNA Polymerase, Invitrogen), 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 2.5 µl of 10 X PCR buffer (Invitrogen) and 0.5 mM of each primer. The PCR protocol was: 1 cycle at 94 °C for 3 min, 40 cycles at 94 °C for 30 s, 50 - 56 °C (see Table 2) for 1 min, and 72 °C for 1 min. SSR fragments were detected on 5% polyacrylamide gel in 0.5X TAE. The gels were visualized under UV using BioDocAnalyze (Biometra).

Data analysis: For each SSR locus, the allelic composition and number of total alleles were determined for each accession. Data were entered in the form of single-individual genotypes. Genetic polymorphism for each population was assessed using the program GENALEX version 6.3 [24], FSTAT [25] and POPGEN [26], including the percentage Shannon's information index (*I*), number private alleles (*Ap*), the expected heterozygosity (*He*), the observed heterozygosity (*Ho*) and Wright's inbreeding coefficient (*Fis*). The coefficient of gene differentiation (*Fst*) and between the SSR locus were also calculated using GENALEX 6.3 program with formula: *Fst* = (*Ht* - Mean *He*) / *Ht* and *Fis* = (mean *He* –mean *Ho*) / mean *He*, where *He* = 1 - $\sum(pi)^2$, *Ht* = total expected heterozygosity = 1 - $\sum(tpi)^2$ (*pi* is the frequency of the i^{th} allele, *tpi* is the frequency of the i^{th} allele for the total). Exact tests of deviation from the Hardy-Weinberg equilibrium for all loci and among populations were performed at the significance level (p) = 0.001.

Analysis of molecular variance (AMOVA) was conducted to calculate levels of significant variation among the six distribution regions, among populations within a region, and within populations using GENALEX 6.3. Genetic distances, identities and UPGMA cluster analysis were generated to determine the genetic association among population based on Nei's 1972 using POPGEN.

3. RESULTS

3.1. Genetic diversity

The eleven SSR markers produced a total of 40 alleles ranging in size from 100 bp to 480 bp, across all 70 trees of the 6 natural populations of *P. dalatensis*. The mean number of alleles observed over all markers in six populations was 2.197. Shannon's information index (*I*) among population, ranging from 0.395 to 0.611. The value of *He* and *Ho* varied from 0.253 to 0.377 and from 0.182 to 0.305, respectively, in six populations. Among them, *N*a, *N*e, *I, Ho* and *H*e of locus Pt15169 were polymorphic in six populations. Allelic diversity (*Na* and N*e*) and gene diversity (*I, He* and *Ho*) of each locus in every population are shown in Table 3. Due to the important parameters for measuring allele polymorphism, we used *I, He* and *Ho* to compare different population. In the six populations, the Da Chais population showed the highest *Ho* (0.305), followed by Xa Hieu (0.227), A Yun (0.212), Hoa Son (0.208), Ngoc Linh (0.200), and Dak Glei (0.182) (Table 3).

In order to exam genetic diversity of studied subpopulations, genetic parameters such as *Na, Ne, I, Ho, He, Ap* was calculated and shown in Table 3. The highest estimates of *He* were obtained from Dak Glei (0.377), followed by Hoa Son (0.346), Ngoc Linh (0.320), Da Chais (0.311), A Yun (0.294), and Xa Hieu (0.253), while *I* was the highest Hoa Son (0.611), followed by Dak Glei (0.607), A Yun (0.523), Da Chais (0.516), Ngoc Linh (0.493), and Xa Hieu (0.395).

In all the studied populations, there were positive fixation index values (*Fis*> 0), indicating an excess of homozygotes and inbreeding. However, among six populations only Da Chais had the lowest levels of positive value (*Fis*= 0.045) with p value < 0.05 and suggested a least decrease in hetezygogotes within these populations. The results in Table 3 showed the number private alleles was only found in four populations (Xa Hieu and Dak Glei populations were not founded private alleles), the Hon Son population showed the highest *Ap* (0.273), followed by Da Chais (0.182), and value of 0.091 for all Ngoc Linh and A Yun.

Table 3. Genetic diversity of the six *P. dalatensis* populations at eleven microsatellite loci.

Population	***N***	***Na***	***Ne***	***I***	***Ho***	***He***	***Ap***	***Fis***
Xa Hieu	6	1.727	1.556	0.395	0.277	0.253	0.000	0.189
Dak Glei	9	2.182	1.954	0.607	0.182	0.377	0.000	0.559
Ngoc Linh	5	1.909	1.696	0.493	0.200	0.320	0.091	0.467
Da Chais	20	2.364	1.620	0.516	0.305	0.311	0.182	0.045
Hoa Son	15	2.636	1.871	0.611	0.208	0.346	0.273	0.428
A Yun	15	2.640	1.852	0.523	0.212	0.294	0.091	0.309
Mean	11.67	2.197	1.758	0.524	0.222	0.317	0.106	0.333
Species	70	3.636	2.524	0.952	0.234	0.524	-	-

Note: N: population size; *Na*: mean number of alleles per locus; *Ne*: mean number of effective alleles per locus; *I*: Shannon's information index; *Ho* and *He:* Mean of observed and expected heterozygosity; *Ap*: mean number of private alleles per locus; *Fis*: Wright's inbreeding coefficient with $p < 0.05$.

3.2. Genetic structure

The results of AMOVA revealed that 57.504% of the total variation was due to the difference within populations. This was higher than the proportion among populations (Table 4). The variation between populations was significant in *P. dalatensis* (42.496 % with $p < 0.001$).

Table 4. Analysis of molecular variance (AMOVA) among/within *P. dalatensis* populations.

Source of variance	**Degree of freedom**	**Sum of squares**	**Variance components**	**Total variation (%)**	**P value**
Among population	5	92.884	1.483	42.496	<0.001
Within population	64	128.456	2.007	57.504	

The population pairwise differentiations (generated from AMOVA) indicated that most of the populations were significantly differentiated ($p < 0.001$). *Fst* values ranged from 0.195 to 0.418 (Table 5).

Table 5. Pairwise population *Fst* values.

	Xa Hieu	Dak Glei	Ngoc Linh	Da Chais	Hoa Son	A Yun
Xa Hieu						
Dak Glei	0.256					
Ngoc Linh	0.278	0.199				
Da Chais	0.326	0.316	0.418			
Hoa Son	0.299	0.288	0.318	0.214		
A Yun	0.326	0.228	0.366	0.195	0.308	

3.3. Genetic distances and cluster analyses

The results in Table 6 showed the pairwise Nei's genetic distances and genetic identities between populations. The largest genetic distance (1.127) was found between populations of Da Chais (Lam Dong province) and Ngoc Linh (Kon Tum province) and the smallest (0.262) between populations of A Yun (Gia Lai province) and Da Chais (Lam Dong province). Similar to the results of genetic distances, the largest identity (0.770) was also recorded between populations of Da Chais and A Yun and the smallest (0.324) between populations of Ngoc Linh and Da Chais. The mean values of genetic distance between the studied populations ranged from 0.262 to 1.127 with an average of 0.529. The mean value of genetic identity between the populations ranged from 0.324 to 0.770 with an average of 0.603.

Table 6. Pairwise population matrix of Nei genetic distance (below diagonal) and genetic identity (above diagonal).

	Xa Hieu	Dak Glei	Ngoc Linh	Da Chais	Hoa Son	A Yun
Xa Hieu		0.667	0.659	0.556	0.605	0.644
Dak Glei	0.406		0.714	0.518	0.568	0.709
Ngoc Linh	0.417	0.337		0.324	0.476	0.435
Da Chais	0.587	0.657	1.127		0.703	0.770
Hoa Son	0.502	0.566	0.743	0.352		0.611
A Yun	0.440	0.343	0.832	0.262	0.492	

An UPGMA dendrogram (Fig. 3) revealed genetic relationships among all the populations investigated on the basis of the Nei's matrix of genetic distances among populations. A total of 6 populations were divided into 2 major groups, the first one corresponding to 3 populations in provinces of Lam Dong (Da Chais), Dak Lak (Hoa Son) and Gia Lai (A Yun). The second corresponding to 3 populations belong to Kon Tum province (Xa Hieu, Dak Glei and Ngoc Linh). According to Mantel tests, the correlation between genetic distance and geographic distance between provinces was not much significant ($r = 0.285$, $p < 0.05$). Grouped results according to the three-dimensional chart of 70 *P. dalatensis* samples was also reflects the same results as tree diagram (Fig. 4).

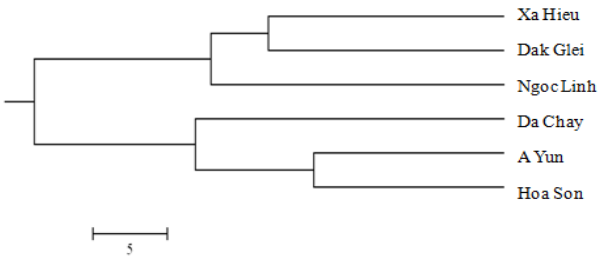

Figure 3. UPGMA dendrogram based on Nei's genetic distance among the six populations.

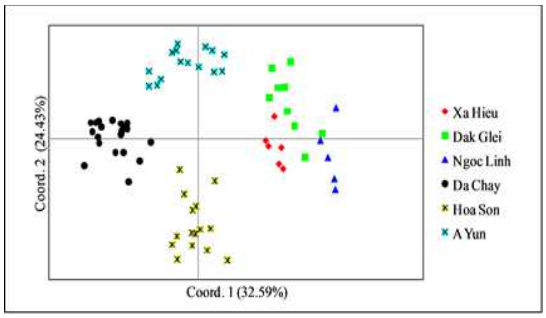

Figure 4. Principal coordinates of 70 *P. dalatensis* samples.

4. DISCUSSION

The obtained results in the present study indicated the levels of moderate genetic diversity within populations of *P. dalatensis* in natural tropical mixed broadleaf forest in highland of Vietnam, mean *Ho* = 0.222 (0.200 - 0.305) and *He* = 0.317 (0.253 - 0.377) reported here are higher than that of other *Pinus* species using microsatellite markers, for example red pine with *Ho* value ranged from 0.067 to 0.317 and a mean of 0.185 [16], but the lower *P. strobes* with *Ho* ranged from 0.125 to 0.812 and a mean of 0.515 [22], or *P. radiate* with *Ho* values ranged from 0 to 0.85 and a mean of 0.625 [27].

Although the temperate levels of *Ho* and relatively polymorphic loci used, the average of 3.636 alleles per locus (Table 3) across the eleven polymorphic loci for *P. dalatensis* was comparable to genetic studies of other pines based on the microsatellite, such as the analysis 6 alleles per locus observed in *P. radiate* by Smith and Devey [27], 5.4 alleles per locus in *P. strobus* by Echt [22], and 6.7 alleles per locus in *P. sylvestris* by Soranzo [28].

The expected heterozygosity (*He*) values for *P. dalatensis* are much higher than *Ho* values. The highest expected heterozygosity (*He*) revealed in Dak Glei population (*He* = 0.377) and the lowest in Xa Hieu population (*He* = 0. 253) (Table 3). Compared with some other coniferous species, *He* value for *P. dalatensis* in this study (*He*= 0.317) was higher than *P. nigra* (*He* = 0.175) and *P. squamata* (*He* = 0.029) [29, 30] and lower than other *Pinus* species which were similarly analyzed by ISSR markers, such as *P. sibirica* (*He* = 0.2699) [31], *P. sylvestris* (*He* = 0.2620) [32], *P. tabulaeformis* (*He* = 0.4152) [33] and *P. koraiensis* (*He* = 0.3477) [34] (Table 5).

At the populations level, in all studied populations have positive *Fis* value (Table 3). Such small populations are the results of inbreeding and an effect of genetic drift within the

populations with the *Fis* value averaged 0.333, ranging from 0.045 for Da Chais (Lam Dong) to 0.559 for Dak Glei (Kon Tum). The obtained results suggested the excess of homozygosity and inbreeding in six *P. dalatensis* populations, inspite of efficiency of heterozygosity and breeding for this population. In this study, the shortage of private alleles (*Ap*) was also found in two Xa Hieu and Dak Glei populations. This also means that the decline in genetic diversity in two populations.

In comparison with some level of genetic diversity among these species, it may be related to geographic distribution, population size of the species, number of tested populations, and the effect of climate changes during the last glacial maximum. Forests having such populations have been greatly degraded and fragmented by human activities and formed small forest patches. All populations of *P. dalatensis* remained in such small patches. The number of observed individuals was less than 50 individuals in each population. In addition, the high level of genetic variability in *P. dalatensis* might be caused by founder related to altered climatic conditions. The creation of gaps in the forests by logging activity causes changes in the original vegetation structure. There are differences in the spatial distribution and the age class structure of trees and the invasion of exotic species in small forest patches of this *Pinus* species. The analysis of molecular variance (AMOVA) revealed that most of the genetic diversity resided within *P. dalatensis* populations. The genetic diversity partitioned among populations was significant ($p < 0.001$).

The overall degree of population differentiation was high in *P. dalatensis* with *Fst* value average 0.287, ranging from 0.195 to 0.418 (Table 5) compared with some other *Pinus* species studied such as *P. cembra* (*Fst* = 0.02), *P. resinosa* (*Fst* = 0.280) using cpSSR analysis [16, 35]. Overall, *Fst* data was shown a measure of population differentiation not due to genetic structure in *P. dalatensis.*

Our research showed that 57.504% of the observed genetic variability was contained within populations and 42.496% was due to difference among populations. These results could be seen to reflect with the expectation of high variability among populations for long-lived and outcrossing species. The gene flow via limited pollen and seed dispersal plays the important role for these results. These conditions have contributed to the differentiation between local populations. In spite of *P. dalatensis* distributed in a wet rainforest environment, which could preclude efficient wind pollination [36]. In summary, despite relative proximity of individual populations, the high population density of *P. dalatensis* and humid environment have no prevented gene flow and led to certain population differentiation level. These findings indicated that even species with a very limited distribution still can be maintained genetic differentiation in populations.

5. CONCLUSIONS

P. dalatensis maintained the relatively high levels of of genetic diversity. The high value of the heterozygosity obtained for all six populations recommends this metapopulation as a valuable center for dynamic conservation of genetic resources in Vietnam. On the contrary, positive *Fis* value (*Fis* > 0.1), indicates an excess of homozygotes and inbreeding for five population (Xa Hieu, Dak Glei, Ngoc Linh, Hoa son and A Yun). Only Da Chais population had the lowest level of positive value (*Fis* = 0.045) with p value <0.05 and suggested efficiency in hetezygogotes in this population. The level of genetic variation in populations of different altitudinal levels did not suggest a significant in fluency of altitude.

From conservation of these species, effective management strategies should be considered for both *in situ* and *ex situ* activities. For example, logging activities should be controlled. The establishment of seed orchards from all the populations should secure genetic sources of this conifer species.

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TÓM TẮT

ĐÁNH GIÁ TÍNH ĐA DẠNG DI TRUYỀN QUẦN THỂ TỰ NHIÊN LOÀI THÔNG ĐÀ LẠT *Pinus dalatensis Ferré* (PINACEAE) BẰNG CHỈ THỊ SSR

Đinh Thị Phòng*, Vũ Thị Thu Hiền, Trần Thị Liễu, Nguyễn Tiến Hiệp

Bảo tàng Thiên nhiên Việt Nam, Viện Hàn lâm Khoa học và Công nghệ Việt Nam

18 Hoàng Quốc Việt, Cầu Giấy, Hà Nội, Việt Nam

*Email: dinhthiphong@hotmail.com

Thông Đà lạt (*Pinus dalatensis* Ferré (họ: Pinaceae) là loài đặc hữu hẹp ở Tây Nguyên của Việt Nam. Loài hiện đang đứng trước nguy cơ bị đe dọa tuyệt chủng do khai thác và tàn phá môi trường sống. Trong nghiên cứu này, 41 chỉ thị SSR (single sequence repeat) đã được sử dụng để phân tích sự thay đổi di truyền trong và giữa các tiểu quần thể Thông Đà lạt. Kết quả phân tích đã chỉ ra 11/41 chỉ thị SSR có tính đa hình khi nghiên cứu 70 cá thể lấy ngẫu nhiên từ 6 tiểu quần thể thu được ở các độ cao khác nhau (Xã Hiếu, Dak Glei và Ngọc Linh tỉnh Kon Tum, Đa Chais tỉnh Lâm Đồng, Hòa Sơn tỉnh Đắc Lắc và A Yun tỉnh Gia Lai).Tổng số đã nhân bản được 40 alen, trong đó 39 alen đa hình (chiếm 97,5 %). Trung bình số alen/locus là 2,197. Thông số tính đa dạng di truyền của quần thể cũng đã được chỉ ra (*I* = 0,524, *Ho* = 0,222, *He* = 0,317, *Fis* = 0,333 và *Ap* = 0,106). Số alen hiếm (*Ap*) chỉ tìm thấy trong bốn quần thể, cao nhất là quần thể Hòa Sơn (0,273), tiếp theo là Đa Chais (0,182), thấp nhất là hai quần thể Ngọc Linh và A Yun (0,091). Tổng sự thay đổi phân tử giữa các quần thể là 42,496 % và giữa các cá thể trong quần thể là 57,504 % ($p < 0,001$) với giá trị *Fst* dao động từ 0,195 đến 0,418. Kết quả phân tích cho thấy việc bảo tồn nguồn gen loài Thông Đà lạt cần phải được quan tâm ngay.

Từ khóa: bảo tồn loài, chỉ thị SSR, đa dạng di truyền, gần tuyệt chủng, *Pinus dalatensis*.