

# Genetic structure in natural populations of *Dacrydium elatum* (Roxb.) Wall. (Podocarpaceae) in the Central Highlands of Vietnam inferred by Microsatellites

Dinh Duy Vu<sup>1\*</sup>, Quoc Khanh Nguyen<sup>1</sup> and Mai Phuong Pham<sup>1</sup>

Institute of Tropical Ecology, Vietnam - Russia Tropical Center, 63 Nguyen Van Huyen, Ha Noi, Vietnam

**Abstract:** To provide a reference for the conservation and application of breeding parentallines resource of *Dacrydium elatum* (Roxb.). Genetic diversity and population structure of eighty individuals from four populations (Kon Tum, Gia Lai, Dak Lak and Lam Dong) in Central Highlands were evaluated using eight SSR markers. Based on the SSR data, 21 alleles were detected by eight SSR with high polymorphism. The genetic diversity of levels within the populations were moderately high ( $H_o = 0.555$ ,  $H_e = 0.429$ ). The average number of shannon information index were 0.618 and genetic differentiation among populations was low ( $F_{st}=0.097$ ). The AMOVA revealed high genetic variation within individuals (87%) compared among populations (13%). The UPGMA phenogram showed that the results of molecular clustering largely agreed with the pedigree and geographic origin. Three populations (NL, KCR and BDNB) were clustered together and CYS population was separated. The maximum quantity  $\Delta K$  was observed for  $K=2$  in population structure analysis, indicating that the entire collection could be divided into two main groups of genes. This study can provide a theoretical basis for genetic resource management and varieties identification of *D. elatum* resources, and provide reference basis for breeding.

## 1 Introduction

*Dacrydium elatum* (Roxb.) Wall. genus *Dacrydium* (Podocarpaceae) is conifer species in Vietnam, grows in primary closed evergreen seasonal forest tropical mixed (elevations 700–2000m) they have been extensively used for furniture, handicrafts and construction [1-3]. In Vietnam it is found in Ha Giang, Tuyen Quang, Quang Ninh, Lai Chau, Ha Tinh, Quang Binh, Thua Thien Hue, Da Nang, Kon Tum, Gia Lai, Dak Lak, Lam Dong and Kien Giang provinces and other countries like China, Laos, Cambodia, Thailand, Malaysia and

---

\*Corresponding author: [dinhduyvu87@gmail.com](mailto:dinhduyvu87@gmail.com)

Indonesia [1-2]. *D. elatum* has been classified as an endangered species and put strictly on the conservation list as Vulnerable (VU) [1]. Up to now, many populations of *D. elatum* are endangered by over exploitation and deforestation and we have serious lack of information on ecological characteristics and genetic variation at population and species levels of *D. elatum*, especially the negative effects of human activities. This situation urged to undertake a study to address these problem and to provide additional information for the Forest Protection Department in order to stress the need for the conservation and sustainable use of the species of local communities.

Conservation and management of a species require information on the ecological and genetic diversity within and among it's populations [4-6]. To develop appropriate conservation strategies, microsatellite markers (simple sequence repeats, SSR) are commonly used to assess the levels of genetic diversity and genetic structure, both at population and species level [4, 7]. SSR has the advantages of high polymorphism, codominant inheritance, and widespread presence in the entire genome [8]. It is ideal marker to probe genetic diversity and population structure and has a broad application scope [9-10]. Many SSR markers were developed and have been widely used to probe genetic diversity within and among conifer populations [11-13]. Previous studies investigated the genetic variation and verified the taxonomic status of the *Dacrydium* species at the molecular level, such as *D. pectinatum* [14], *D. imbricatus* [15], *D. elatum* [16], however the genetic structure of *D. elatum* has not been explored in Vietnam. Meanwhile, knowing genetic diversity is important for the development of conservation strategies and the sustainable utilization of this species [6, 17]. Therefore, it is a dire need of the day to explore the diversity and population structure of *D. elatum* and set breeding and conservation strategies.

The main goal of the current study was to determine level of genetic diversity and genetic structure of natural populations of *D. elatum* in the Central Highlands of Vietnam, and to provide guidelines for the conservation, management, and restoration of the species.

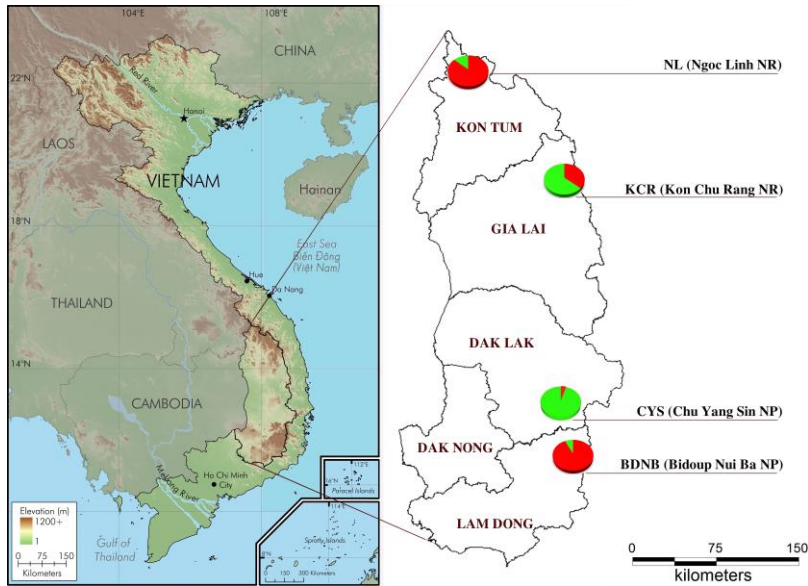
## 2 Materials and Methods

### 2.1 Sample collection

A total of 80 plant samples (leaf) were randomly collected from four populations in the Central Highlands of Vietnam (Table 1; Figure 1). The samples were immediately placed in liquid nitrogen, transferred to the molecular laboratory of Vietnam-Russia Tropical Centre and stored at -80°C until DNA extraction.

**Table 1.** Sampling location of *D. elatum* in the Central Highlands of Vietnam

Population code	Region	Sample size	Latitude (N)	Longitude (E)	Altitude (m)
NL	Ngoc Linh Nature Reserve, Kon Tum province	20	14°38'27''	108°24'35''	1211
CYS	Chur Yang Sin national park, Dak Lak province	20	12°24'47"	108°23'03"	1296
BDNB	Bidoup-Nui Ba national park, Lam Dong province	20	12°10'57"	108°41'32"	1471
KCR	Kon Chu Rang Nature Reserve, Gia Lai province	20	14°31'10"	108°32'46"	1001



**Fig 1.** Map showing the study location of *D. elatum* in the Central Highlands of Vietnam

## 2.2 DNA extraction

Total genomic DNA was extracted using the plant/fungi DNA isolation Kit (Norgenbiotek, Canada). The total DNA purity and integrity were tested by Nanodrop ND-2000 spectrophotometer (NanoDrop Technologies, DE, USA) and then diluted to a concentration of 10ng/ $\mu$ l

## 2.3 Microsatellite amplification

PCR-SSR was performed in a 25  $\mu$ L reaction mixture containing (20ng genomic DNA, Dreamtaq Master Mix (2X), 10 pmol of each primer, and H<sub>2</sub>O deionised). The PCR-SSR were performed in C1000 Touch Thermal Cycler as follows: an initial denaturing step at 94°C for 3 min, 35 cycles (94°C/60 s, 55°C/30 s and 72°C/60 s) and 10 min at 72°C for the final cycle to complete the extension of any remaining products before holding the samples at 4°C until they were analyzed. Eight pairs of primers (SSR) were selected (Table 2). The amplification products were separated using a Sequi-Gen<sup>®</sup>GT DNA electrophoresis system 8% polyacrylamide gels in 1 x TAE buffer and then visualized by GelRed 10.000X. The sizes of the PCR products were detected and analyzed using GelAnalyzer software of with a 20 bp DNA ladder (Invitrogen, USA).

**Table 2.** Information of nine SSR markers for PCR amplification in *D. elatum*

Locus	Primer sequence (5'–3')	Repeat motif	PCR product (bp)	T <sub>m</sub> (°C)	Sources
SSR1	F: GAGACCAGACAAAGATGAAGA R: GAGTAAGAGCAAGACACCAAA	(AG) <sub>21</sub>	190-200	55	[11]
SSR2	F: GGCATTGGCTCAACAGA R: TCGTGGAGAGGTACTTCATT	(CT) <sub>9</sub>	160-180	55	[11]
SSR3	F: GCCAGGGAAAATCGTAGG R: AGAAGATTAGACATCCAACCC	(T) <sub>14</sub> (T) <sub>10</sub> C(T) <sub>5</sub>	140-160	55	[17]
SSR4	F: CCCGTATCCAGATATACTTCCA	(T) <sub>14</sub> (T) <sub>11</sub>	150-200	55	[17]

	R: TGGTTTGATTCATTCGTTTCAT				
SSR5	F: AATGAAAGGCAAGTGTCG R: GAGATGCAAGATAAAGGAAGTT	(GGT)10	200-220	55	[18]
SSR6	F: ATCCTGAGTCCCTGTATGTT R: CTACTATCTGAGCACGCCAC	(AG)18	220-240	55	[12]
SSR7	F: TCCAAGGATGCACATTCAAT R: AAACAAAACCTCACTCAATGAA	(AC)18	200-220	55	[12]
SSR8	F: AAACAAAACCTCACTCAATGAA R: CCCACTTCCTCCAGCAATAC	(AC)37(AG)22	120-130	55	[12]

## 2.4 Data analysis

The software GENALEX ver.6.5 [20] was used to calculate the characterization and polymorphism level of 8 SSR loci including the number of alleles per locus ( $N_a$ ), number of effective alleles ( $N_e$ ), Shannon-Weiner index ( $I$ ), the observed heterozygosities ( $H_o$ ), the expected heterozygosities ( $H_e$ ) and the genetic differentiation for all populations ( $F_{ST}$ ). Tests of deviation from the Hardy-Weinberg equilibrium per locus in each population were performed at a significance level of 0.05, using GENEPOP v4.6 [21]. The analysis of molecular variance (AMOVA) of data was calculated by Arlequin 3.1 [22]. The genetic association amongst populations was determined by Poptree2 using the UPGMA approach [23]. The population structure was explored by STRUCTUREv.2.3.4 [24]. The targeted population was separated into groups by Structure Harvester [25] based on the  $\Delta K$  by Evanno et al. [26].

## 3 Results

### 3.1 Genetic diversity

Eight SSR primer pairs amplified consistently under standard conditions, clear products and were used to assess the population genetic structure. All primers were submitted to amplification cycles with primer annealing at 55°C. All of SSR markers were polymorphic in four *D. elatum* populations. A total of 21 different alleles were recorded for *D. elatum*. Genetic diversity of each population was shown in Table 3. The number of observed alleles ( $N_a$ ) and the effective number of alleles ( $N_e$ ) varied from 2 to 3 and from 1.755 to 1.924, respectively. Shannon-Weiner index ( $I$ ) ranged from 0.57 in NL population to 0.684 in BDNB population with an average of 0.618. The average observed heterozygosity ( $H_o$ ) and expected heterozygosity ( $H_e$ ) were 0.555 (0.563-0.615) and 0.429 (0.398-0.467), respectively. Interestingly, there were also differences in the ratios of genetic diversity between four populations (NL, CYS, BDNB, and KCR). Among the four populations, population of BDNB ( $H_o = 0.684$  and  $H_e = 0.588$ ) was the highest genetic diversity. Three loci (SSR4, SSR5 and SSR7) showed significant deviations from the Hardy-Weinberg equilibrium in populations NL, CYS, BDNB, and KCR, respectively.

**Table 3.** Polymorphism of eight SSR markers and the Hardy-Weinberg equilibrium testing for each population of *D. elatum*

	NL population (N= 20)						CYS population (N= 20)					
	Na	Ne	I	Ho	He	HWE P-value	Na	Ne	I	Ho	He	HWE P-value
SSR1	2.000	1.994	0.692	0.833	0.498	0.004**	3.000	1.590	0.639	0.200	0.371	0.159 <sup>ns</sup>
SSR2	2.000	1.800	0.637	0.111	0.444	0.001**	2.000	2.000	0.693	0.467	0.500	0.796 <sup>ns</sup>

SSR3	2.000	1.180	0.287	0.167	0.153	0.700 <sup>ns</sup>	2.000	2.000	0.693	1.000	0.500	0.000 <sup>***</sup>
SSR4	2.000	2.000	0.693	1.000	0.500	0.000 <sup>***</sup>	2.000	2.000	0.693	1.000	0.500	0.000 <sup>***</sup>
SSR5	2.000	2.000	0.693	1.000	0.500	0.000 <sup>***</sup>	2.000	2.000	0.693	1.000	0.500	0.000 <sup>***</sup>
SSR6	2.000	1.117	0.215	0.111	0.105	0.803 <sup>ns</sup>	2.000	1.965	0.684	0.200	0.491	0.022 <sup>*</sup>
SSR7	2.000	2.000	0.693	1.000	0.500	0.000 <sup>***</sup>	2.000	2.000	0.693	1.000	0.500	0.000 <sup>***</sup>
SSR8	2.000	1.946	0.679	0.278	0.486	0.069 <sup>ns</sup>	1.000	1.000	0.000	0.000	0.000	n.a
<b>Mean</b>	<b>2.000</b>	<b>1.755</b>	<b>0.574</b>	<b>0.563</b>	<b>0.398</b>		<b>2.000</b>	<b>1.819</b>	<b>0.599</b>	<b>0.608</b>	<b>0.420</b>	
<b>BDNB population (N=20)</b>						<b>KCR population (N=20)</b>						
	<b>Na</b>	<b>Ne</b>	<b>I</b>	<b>Ho</b>	<b>He</b>	<b>HWE P-value</b>	<b>Na</b>	<b>Ne</b>	<b>I</b>	<b>Ho</b>	<b>He</b>	<b>HWE P-value</b>
SSR1	2.000	1.940	0.677	0.471	0.484	0.906 <sup>ns</sup>	2.000	1.956	0.682	0.250	0.489	0.029 <sup>*</sup>
SSR2	2.000	1.778	0.630	0.294	0.438	0.176 <sup>ns</sup>	2.000	1.280	0.377	0.150	0.219	0.160 <sup>ns</sup>
SSR3	2.000	1.710	0.606	0.588	0.415	0.086 <sup>ns</sup>	2.000	2.000	0.693	1.000	0.500	0.000 <sup>***</sup>
SSR4	2.000	2.000	0.693	1.000	0.500	0.000 <sup>***</sup>	2.000	2.000	0.693	1.000	0.500	0.000 <sup>***</sup>
SSR5	2.000	2.000	0.693	1.000	0.500	0.000 <sup>***</sup>	2.000	2.000	0.693	1.000	0.500	0.000 <sup>***</sup>
SSR6	2.000	1.410	0.466	0.118	0.291	0.014 <sup>*</sup>	2.000	1.923	0.673	0.100	0.480	0.000 <sup>***</sup>
SSR7	2.000	2.000	0.693	1.000	0.500	0.000 <sup>***</sup>	2.000	1.724	0.611	0.100	0.420	0.001 <sup>***</sup>
SSR8	3.000	2.558	1.018	0.235	0.609	0.000 <sup>***</sup>	2.000	1.471	0.500	0.100	0.320	0.002 <sup>**</sup>
<b>Mean</b>	<b>2.125</b>	<b>1.924</b>	<b>0.684</b>	<b>0.588</b>	<b>0.467</b>		<b>2.000</b>	<b>1.794</b>	<b>0.615</b>	<b>0.463</b>	<b>0.428</b>	
<b>Genetic diversity of four <i>D. elatum</i> populations</b>												
<b>Total mean</b>	<b>2.031</b>	<b>1.823</b>	<b>0.618</b>	<b>0.555</b>	<b>0.429</b>							

Note: N, number of individuals; Na, mean number of alleles per locus; Ne, mean number of effective alleles; I, Shannon's Information Index; Ho, observed heterozygosity, He, expected heterozygosity; HWE, Hardy-Weinberg equilibrium; n.s., not significant; n.a., Monomorphic locus. \* P<0.05, \*\* P<0.01, \*\*\* P<0.001

### 3.2 Population structure

It can be seen from the data in Table 4 that analysis of molecular variance (AMOVA) was performed based on 279.643 permutation. The AMOVA showed that total variation was highly significant (p<0.001) within individuals (87%), and among populations (13%) (Table 4).

**Table 4.** Analysis of molecular variance in *D. elatum* from 4 populations

Source of variation	df	Sum of squares	Variance components	Total variation (%)	P value
Among populations	3	39.769	0.343	13%	
Among individuals within populations	76	85.874	0.000	0%	<0.001
Within individuals	80	154.000	2.200	87%	
<b>Total</b>	<b>159</b>	<b>279.643</b>	<b>2.543</b>	<b>100%</b>	

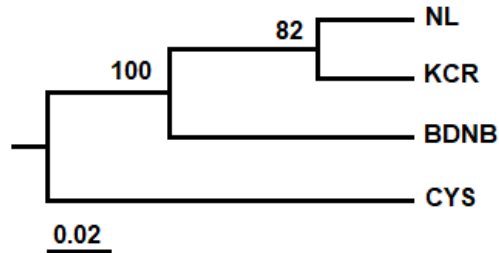
Genetic variation within populations was recorded as 0.097 (0.067-0.166) indicating low genetic differentiation. The highest differentiation value (F<sub>ST</sub> = 0.166) was between the two populations of NL/CYS and low (F<sub>ST</sub> = 0.067) between NL/BDNB (Table 5).

**Table 5.** Population pairwise F<sub>ST</sub> in *D. elatum*

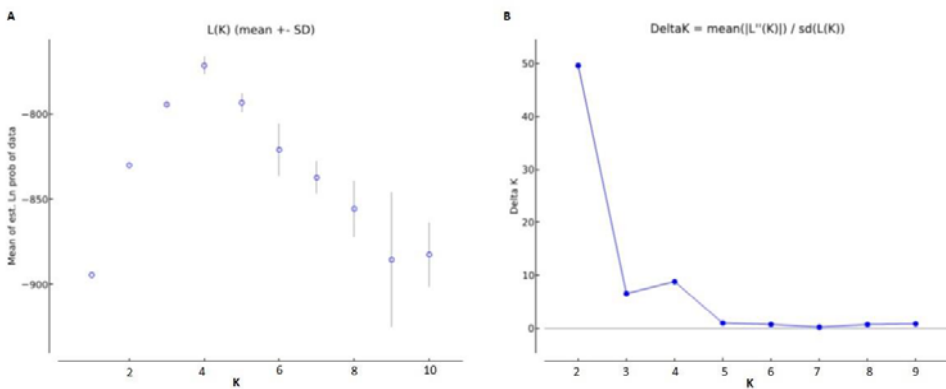
	NL	BDNB	CYS	KCR
<b>NL</b>	-			
<b>BDNB</b>	0.067	-		
<b>CYS</b>	0.166	0.109	-	
<b>KCR</b>	0.086	0.072	0.084	-

The result of unweighted pair group method analysis (UPGMA) tree constructed on the basis of Nei's distance using POPTREE, which showed that other groups were separated

clearly at population level (Fig. 2). Two different groups were generated; the 1<sup>st</sup> group was contains 3 populations (NL, KCR and BDNB) with a bootstrap value of 100%. This main group divided into two subgroups. Sub-clusters 1 included two population (NL and KCR) were clustered together with a bootstrap value of 62% and sub-clusters 2 only have BDNB population were separated. And the second major was composed of CYS population.



**Figure 2.** UPGMA dendrogram based on Nei's chord distance of genetic relationship among four *D. elatum* populations

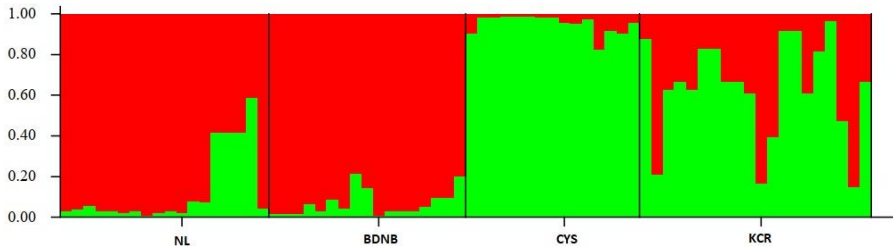


**Figure 3.** Plot of the Ln P(D)  $\pm$  SD and delta K (DK). **A.** The mean of Ln P(D) was based on 10 repetitions for each K value. **B.** Plot of  $\Delta K$  according to K.

We were performed population structure analysis, the highest  $\Delta K$  value (49.63), have a clear peak for 80 individuals when  $K=2$  to be the optimum number of genetic clusters and indicated that all the studied plants exhibited admixture from two clusters (Fig. 4). One group (red) was predominant in the two populations (NL and BDNB) with strong ancestry values 86.8% and 93%, respectively and the second group (green) was composed of two populations (CYS and KCR) with strong ancestry values 95.4% and 63.6%, respectively (Table 6; Fig. 1&4). In particular, 4 *D. elatum* populations in Central Highlands of Vietnam showed a rich a rich mixture of ancestral genes among individuals.

**Table 6.** Percentage of ancestry for four *D. elatum* populations was analyzed from Structure Harvester

Populations code	Genetic group	
	Cluster 1 (%) (Red)	Cluster 2 (%) (Green)
NL	86.8	13.2
BDNB	93.0	7.0
CYS	4.6	95.4
KCR	36.4	63.6



**Figure 4.** Bar plot of admixture assignment for 4 *D. elatum* populations to cluster (K=2, highest  $\Delta K$  value = 49.63) based on Bayesian analysis

## 4 Discussion

In the current study, all the 8 SSR loci were highly polymorphic in the 80 genotypes with a mean value of 2.031 alleles per locus, the mean number of effective alleles ( $N_e = 1.823$ ). The genetic diversity level was moderately high with  $H_o = 0.555$  and  $H_e = 0.429$ .  $H_o > H_e$  were detected of four populations in the present study, suggesting that these populations are predominantly allogamic, which might be a result of outcrossing and reflecting the population structures. Although, it has previously been studied on the genetic diversity of *D. elatum*. Tran et al. [16] had compared the effectiveness between ISSR and SSR markers in assessing genetic diversity of natural populations of *D. elatum* in Tay Nguyen, Vietnam and show that high level of genetic variation of *D. elatum* ( $H_e = 0.301$ ). This result was consistent with the study of Tran *et al.* [16]. Several reports have shown that high genetic diversity in other conifer using SSR markers [11, 12, 27-32] However, low of genetic diversity were found in prior studies [4, 33,34]. Our investigations confirm the suggestion that the genetic structure of natural populations of *D. elatum* was strongly affected by population sizes. Which a lot of individuals remaining in the natural forest from 100 individuals in KCR and NL populations to about 500 in BDNB and CYS populations. All of studied populations were found in the secondary forests at more than 1000 m elevations. Forests have been greatly fragmented by human activities and formed small forest patches. All populations of this species remain in such small patches. Such populations are likely to be the results of inbreeding and an effect of genetic drift in subsequent generations.

Population structure and genetic relationships are important for establishing the appropriate scale and subunits for conservation management [35]. It is affected by mutation, gene flow, natural selection and genetic drift [36-37]. The STRUCTURE analysis suggested two different clusters among 80 studied individuals. Similarly, UPGMA analysis based on Nei's chord distance using POPTREE2 identified two clusters. This indicates that geography has effects on genetic structure of *D. elatum*. Based on our results, effective management strategies for *D. elatum* should include both in-situ and ex-situ conservations. Ex-situ conservation of *D. elatum* could be immediately implemented. It will be a protective and managed site to grown this species and prevent potential genetic erosion of wild *D. elatum* populations. Establishment of seed orchards from all the populations should secure genetic sources of *D. elatum*.

## 5 Conclusions

The present study shows that the current situation of *D. elatum* maintains moderately high levels of genetic diversity and low levels of genetic differentiation among populations. The Bayesian analysis confirmed two main genetic groups with UPGMA dendrogram. The high genetic variation within individuals. Therefore, conservators should focus on maintaining the genetic variation within populations. These strategies should also be implemented with ex-situ conservation activities to avoid inbreeding in the future.

This research was financially supported by Russian-Vietnamese Tropical Center basis project, 2020-2022 and project E 1.2. We thank E. Sterling (New York) and K. Koy (Berkeley) for providing the map.

## References

1. T.H. Nguyen, K.L. Phan, D.T.L. Nguyen, P.I. Thomas, A. Farjon, L. Averyanov, *Viet Nam Conifers: Conservation Status Review 2004, Fauna & Flora International*, 92-93 (Vietnam Programme, Hanoi, 2004) (Vietnamese)
2. K.L. Phan, L. Phan, J. Regalado, L. Averyanov, B. Maslin, *Pak J Bot*, **49**(5), 2037-2068 (2017)
3. M.P. Pham, V.H. Tran, D.D. Vu, Q.K. Nguyen, S.N.M. Shah, *Czech J Genet Plant Breed*, (2021) <https://doi.org/10.17221/88/2020-CJGPB>
4. D.D. Vu, T.T.X. Bui, M.T. Nguyen, D.G. Vu, M.D. Nguyen, X. Huang, Y. Zhang Y, *J For Res*, **28**(2), 265-272 (2017)
5. D.D. Vu, T.T.X. Bui, M.D. Nguyen, A.N.M. Shah, D.G. Vu, Y. Zhang, M.T. Nguyen, X.H. Huang, *J For Res*, **30**(5), 1821-1831 (2019)
6. M.T. Nguyen, D.D. Vu, P.H. Dang, T.T.X. Bui, P.L.H. Nguyen, M.D. Nguyen, *Planta*, **253**, 66 (2021)
7. M.T. Nguyen, D.D. Vu, M.D. Nguyen, P.H. Dang, K.L. Phan, X.P. Bui, *J Forest Res*, **15**(3), 198-201 (2020)
8. R.K. Varshney, A. Graner, M.E. Sorrells, *TRENDS Biotechnol*, **23**, 48-55 (2005)
9. A.Y. Larionova, A.K. Ekart, A.N. Kravchenko, *Eurasian J For Res*, **10**, 185–192 (2007)
10. R.K. Kalia, M.K. Rai, S. Kalia, R. Singh, A. Dhawan, *Euphytica*, **177**, 309-334 (2011)
11. R. Mellick, C. Porter, M. Rossetto, *Mol Ecol Res*, **9**(6), 1460-1466 (2009)
12. Y. Miao, X. Lang, S. Li, J. Su, Y. Wang, *Int J Mol Sci*, **13**, 11165-1117 (2012)
13. K.H. Hung, C.Y. Lin, C.C. Huang, C.C. Hwang, T.W. Hsu, Y.L. Ku, W.K. Wang, C.Y. Hung, T.Y. Chiang, *Botanical Studies*, **53**, 191-196 (2012)
14. Y.J. Su, T. Wang, F. Deng, *Biochem Syst Ecol*, **38**(4), 576-584 (2010a)
15. Y.J. Su, T. Wang, F. Deng, *Aust J Bot*, **58**(4), 318-326 (2010b)
16. T. L. Tran, T.T.H. Vu, T.L. Nguyen, T.P. Dinh, *Vietnam J Biotechnol*: **15**(2), 293-305 (2017) (Vietnamese)



17. D.D. Vu, S.N.M. Shah, M.P. Pham, V.T. Bui, M.T. Nguyen, T.P.T. Nguyen, *BMC Plant Biology*, **20**, 358 (2020)
18. G.G. Vendramin, L. Lelli, P. Rossi, M. Morgante M, *Mol Ecol* **5**, 595-598 (1996)
19. C.G. Elsik, V.T. Minihan, S.E. Hall, A.M. Scarpa, C.G. Williams, *Genome*, **43**, 550-555 (2000)
20. R. Peakall, P.E. Smouse, *Bioinformatics*, **28**, 2537-39 (2012)
21. F. Rousset, *Genepop'007: a complete re-implementation of the genepop software for Windows and Linux* (2008).
22. L. Excoffier, G. Laval, S. Schneider, *Evol Bioinform Online*, **1**, 47-50 (2005)
23. N. Takezaki, M. Nei, K. Tamura, *Molecular Evolution*, **27**, 747-752 (2010)
24. J.K. Pritchard, M. Stephens, P. Donnelly, *Genetics*. **155**(2), 945-959 (2000)
25. D.A. Earl B.M. von-Holdt, *Conservation Genet Resour*, **4**, 359-361 (2012)
26. G. Evanno, S. Regnaut, J. Goudet, *Mol Ecol*, **14**, 2611-2620 (2005)
27. A. Terrab, O. Paun, S. Talavera, K. Tremetsberger, M. Arista, T.F. Stuessy, *Am J Bot* **9**, 1274-1280 (2006)
28. M. Dubreuil, F. Sebastiani, M. Mayol, S.C. Gonza'lez-Martinez, M. Riba, G.G. Vendramin, *Conserv Genet* **9**, 1665-1668 (2008)
29. J.B. Yang, H.T. Li, D.Z. Li, J. Liu, L.M. Gao, *Hort Science*, **44**(7), 2043-2045 (2009)
30. H. Xue, C.H. Lu, X.B. Wu, *Genet Mol Res*, **11**(2), 1296-1299 (2012)
31. T.P. Dinh, T.L. Tran, T.T.H. Vu, T.H. Nguyen, *Genet Mol Res*, **14**(3), 7727-7739 (2015)
32. K.C. Rai, H.S. Ginwal, *Silvae Genetica*, **67**, 99-105 (2018)
33. Y.C. Miao, J.R. Su, Z.J. Zhang, H. Li, J. Luo, Y.P. Zhang, *Conserv Genet*, **9**, 1683-1685 (2008)
34. M.T. Nguyen, D.D. Vu, T.T.X. Bui, M.D. Nguyen, *Indian J Biotechnol*, **12**(4), 499-503 (2013)
35. R. Ferrière, U. Dieckmann, D. Couvet, *Evolutionary Conservation Biology* (2004)
36. M.D. Loveless, J.L. Hamrick, *Annu Rev Ecol Syst*, **15**, 65-95 (1984)
37. J. Schneller, B. Liebst, *Am J Bot*, **94**(6), 965-71 (2007)