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GENETIC DIVERSITY AMONG NATURAL POPULATIONS OF *Keteleeria evelyniana* Mast. IN TAY NGUYEN OF VIET NAM USING SSR MARKERS

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Abstract. *Keteleeria evelyniana* Mast. is a big softwood species with high economic values. Therefore, the number of individuals are rapidly decreasing due to rampant exploitation as well as its habitat loss and recently, the species is considered vulnerable in Viet Nam. In this study, we assessed the genetic variation among seventy *K. evelyniana* samples of three natural populations in Lam Dong, Dak Lak and Kon Tum using 16 microsatellite markers. The results showed that thirteen markers were polymorphic. A total 39 DNA fragments were amplified, among them, thirty – five were polymorphic (accounting for 89.74 %). Among studied populations, the level of genetic diversity at Lam Dong (*Na* = 2.063; *Ne* = 1.730; *Ap* = 0.375; *I* = 0.558; *Ho* = 0.459 and *He* = 0.367) was the highest. Analysis of molecular variance (ANOVA) showed that the total level of molecular changes between populations was 34.65 % and between individuals in the same population was 65.35 %. Private alleles (*Ap*) and inbreeding values (*Fis*) of *K. evelyniana* species were founded of all three populations in Lam Dong, Dak Lak and Kon Tum (0.375 and - 0.234; 0.188 and - 0.065; 0.063 and - 0.047, respectively). The gene flow (*Nm*) also occurred among the *K. evelyniana* populations with the average of *Nm* = 5.423. A dendrogram (UPGMA) constructed based on the similarity matrix of 70 *K. evelyniana* samples divided into two main groups with their genetic similarity coefficient ranged from 76.5 % (Ke26 and Ke44) to 99 % (Ke23 and Ke25). The obtained results indicated the importance of conserving the genetic resources of *K. evelyniana* species in Tay Nguyen.

Keywords: *Keteleeria evelyniana*, population genetic diversity, species conservation, SSR, Tay Nguyen.

Classification numbers: 1.3.2; 3.1.2.

1. INTRODUCTION

Tay Nguyen is the central highlands of Viet Nam and covers two of six phytogeographic sub-regions of Viet Nam [1], including the sub-region South Truong Son and South Indochina in Kon Tum, Gia Lai, Dak Lak, Dak Nong and Lam Dong provinces. According to Nguyen Tien Hiep et al. [2], among the 34 known coniferous species of Viet Nam, there were 15 species of high economic and scientific value in Tay Nguyen, including *Keteleeria evelyniana* of the

Keteleeria genus in the Pinaceae family. It is an evergreen tree that can grow up to 25 - 30 (or 40) metres high. Its trunk is straight and can expand up to two metres in diameters. The mature trees have broad crown, grey bark and longitudinally fissured. The species is distributed mainly at altitudes ranging from 500 to 2000 metres above sea-level, in mixed conifer/broad-leaved forest, scattering from the North West Viet Nam to Tay Nguyen. In Tay Nguyen, *K. evelyniana* is currently found only in Lam Dong (at Suoi Vang, Da Chais and Hiep An regions of Lac Duong and Duc Trong districts, respectively), Dak Lak (Hoa Son, Krong Bong district) and Kon Tum (Dak Glei district). Beside Viet Nam, the species can also be found in China and Laos. However, due to over-exploitation and habitat loss, the species is becoming endangered. Currently, the number of mature trees in the wild is small, although the species had been described in the past and was harvested for timber, oil and resin. Based on data collection of surveys and ranking standards in the IUCN Red List, this species can be considered as vulnerable - VU A4acd, B1 + 2b (ii , iii, v), C [3]. Therefore, a study on genetic diversity of *K. evelyniana* should urgenlly be conducted in order to provide information for an effective conservation strategy in Tay Nguyen.

Genetic diversity analysis can be done based on morphological, biochemical, and molecular types of information. However, molecular markers have advantages over other kinds, where they show genetic differences on a more detailed level without interferences from environmental factors. Application of molecular markers in genetic diversity studies have been began from the 1980s and many techniques had developed. Among the molecular markers, SSR (Simple Sequence Repeats) are most popular because it is a codominant marker with high polymorphism and specificity. Therefore, it was considered highly effective in the study on genetic diversity in many species, including several species of conifer in the world as well as Viet Nam [4-7].

In this study, genetic diversity of natural *K. evelyniana* populations in Tay Nguyen, Viet Nam was determined. The obtained results will provide the information for conservation, management and restoration of biological diversity of this species in Tay Nguyen in particular and in Viet Nam in general.

2. MATERIALS AND METHODS

2.1. Materials

Table 1. Details of *K. evelyniana* genotypes and populations used in this study.

Population code	Collection locality	Sample number	Sample code	Latitude (°N)	Longitude (°E)	Elevation (m)
Lam Dong	Suoi Vang, Lac Duong, Lam Dong	10	Ke1 – Ke10	11° 59’ 58.8"	108° 21’ 59.3”	1464
	Hiep An, Duc Trong, Lam Dong	12	Ke11 – Ke22	11° 50’ 14.0”	108° 26’ 35.5”	1390
	Da Chais, Lac Duong, Lam Dong	4	Ke23 – Ke26	12°12’04.5”	108°40’06.2”	1485
Dak Lak	Hoa Son, Krong Bong, Dak Lak	21	Ke27 – Ke47	12° 25’ 05.2"	108° 22’ 17.1”	1116
Kon Tum	Dak Glei, Dak Glei, Kon Tum	23	Ke48 – Ke70	15^0 01’ 17’’	107^o 48’ 04”	1553

Seventy leaf and bark samples of 70 individuals randomly selected from 219 *K. evelyniana* trees in Lam Dong, Dak Lak and Kon Tum were used in this study. The fresh samples were kept in a Ziplock bag including silica gel, and then stored at room temperature until use. Information of the samples was showed in Table 1 and Figure 1.

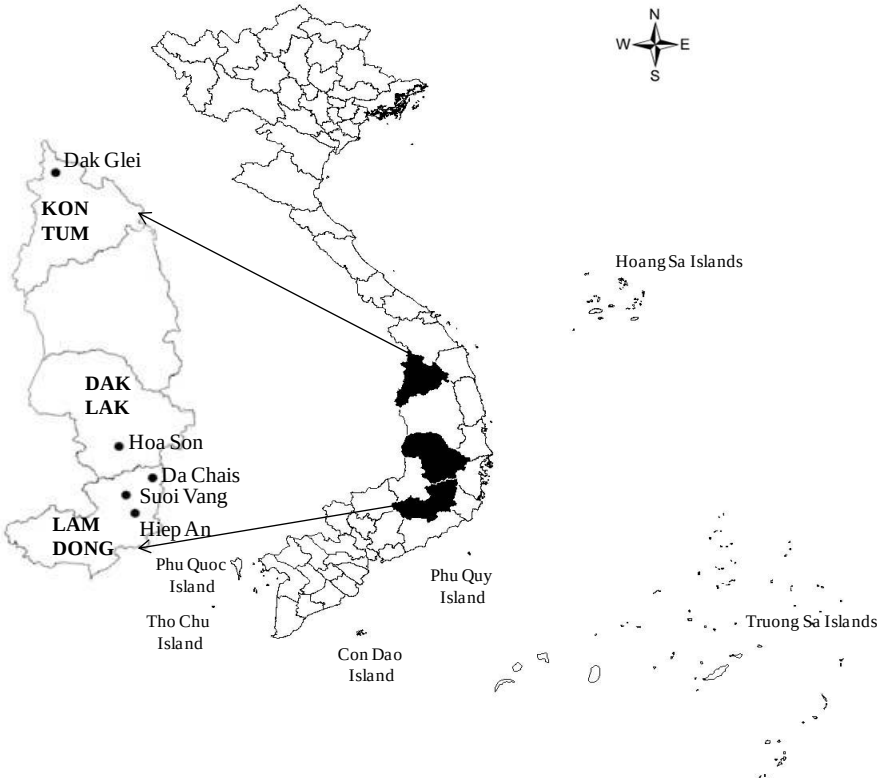

Figure 1. Map showing the studying sites of *K. evelyniana.*

The primers in this study were synthesized by the IDT, (Intergrated DNA Technology, USA). The nucleotide sequences of these primers referred from previously studies are listed in Table 2.

2.2. Methods

DNA extraction: Total DNA was extracted and purified using the method described by Porebski et al. [8]. 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.

PCR_SSR reaction: PCR reactions were performed on the PCR system 9700 (USA) with a total volume of 25 µl. The composition of the reaction and the thermo cycle followed the ones described in Dinh Thi Phong et al. [7]. The SSR fragments were detected on 5 % polyacrylamide gel 1X TAE, then were visualized under UV using BioDocAnalyze (Biometra).

Table 2. The nucleotides sequences, PCR production sizes and optimum annealing temperatures of SSR markers in this study.

No.	Primers code	Nucleotides	Reference	Ta (^oC)	Size (bp)
1	FRPP91	5‘ GTACTCCCACATAAAATGAGACTT 3’ 3’ CCGAAATACATTGCAGGTTA 5’	[9]	53	100 – 180
2	Cm3	5’ TGGGTTGACCAGTGCTTCT 3’ 3’ ATGCCCAACACCTCATTAGA 5’	[4]	53	120 – 200
3	PeB31BGT	5’ GGCATTGGCTCAACAGA 3’ 3’ TCGTGGAGAGGTACTTCATT 5’	[10]	54	190 – 500
4	Pinus10	5’ CGGGCTGGTATCTCAAGAGT 3’ 3’ ACACACACACACAGAGAGAGAG 5’	[5]	55	285 – 305
5	PRE10	5’ CTGGTCTTGGCCTAAGAATATGAAG 3’ 3’ CATTGGGACGTAAACAACAATACCA 5’	[11]	52	120 – 210
6	PRE13	5’GATGTGTCTTTAGGCTCGTTGC 3’ 3’ AGGGTTAGTAATCACGGCCTGT 5’	[11]	54	170 – 180
7	PRE16	5’ TCCTGCGATGAGTCTCTTTGT 3’ 3’ TCCATTTTTTACTTTTGATAACTTTAC 5’	[11]	52	195 – 490
8	PRE24	5’ GTTTTTTAAATTGGGAAGGCG 3’ 3’ CGTGGGGGAGATAGTGATAGAGT 5’	[11]	54	380 – 400
9	P1	5’ CTCCCTCTATGTGTTTCTCC 3’ 3’ GAAAATCTTTCTACCCTTCCAG 5'	[12]	55	300 - 400
10	P5	5’ GTTCGCTAGTTTGTTTGATCCC 3’ 3’ TCCCAGCAAATCCTTGACTC 5’	[12]	53	145 – 160
11	Pt36480	5’TTTTGGCTTACAAAATAAAAGAGG 3’ 3’ AAATTCCTAAAGAAGGAAGAGCA 5’	[13]	52	160 – 160
12	Pt87268	5’ GCCAGGGAAAATCGTAGG 3’ 3’ AGAAGATTAGACATCCAACCC 5’	[14]	56	160 – 160
13	PtTX3026	5’ AATACTTGGGAGGGATAC 3’ 3’AATAGCCAGTTTTGTTTG 5’	[15]	53	130 – 255
14	PtTX3034	5’ TCAAAATGCAAAAGACG 3’ 3’ ATTAGGACTGGGGATGAT 5’	[15]	53	200 – 210
15	RPS1b	5’ GCCCACTATTCAAGATGTCA 3’ 3’ GATGTTAGCAGAAACATGAGG 5’	[16]	54	100 – 100
16	RPS2	5’ CATGGTGTTGGTCATTGTTCCA 3’ 3’ TGGAGGCTATCACGTATGCACC 5’	[16]	54	185 – 210

Data analysis: The parameters including genetic diversity of each population as the average of number of observed alleles (*Na*), effective alleles (*Ne*) and private alleles (*Ap*) per locus, percentage of polymorphic bands (*PPB*), the Shannon’s genetic diversity index (*I*) [17], the expected (*He*) and observed heterozygosity (*Ho* = number of heterozygous individuals/ total individuals), and the Wright’s inbreeding coefficient (*Fis*) were analyzed and obtained using the GENALEX 6.3 [18] and FSTAT software [19]. The genetic differences coefficient (*Fst*) and gene flow (*Nm*) for each locus were calculated using the formula: *Fst* = (*Ht* - Mean *He*)/ *Ht* and *Nm* = [(1/*Fst*)-1]/ 4, in where *He* = 1 - $\sum$(pi), *Ht* (total expected heterozygosity) = 1 - $\sum(tpi)^2$, (p*i* is the frequency of the i^{th} allele, tp*i* 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) of 0.001. Analysis of molecular variance (ANOVA) was also conducted to calculate level of significant variation among and within populations using the GENALEX 6.3 program. We also constructed a dendrogram base on similarity matrix of 70 *K. evelyniana* samples using the method of Nei and Li [20] in NTSYS software version 2.0. The bootstrap value was repeated 1000 times and supported by Win-Boot software [21].

3. RESULTS

3.1. Genetic diversity

The sixteen SSR primer pairs were used to assess the genetic diversity for 70 *K. evelyniana* individuals of three populations in Lam Dong, Dak Lak and Kon Tum. There were 13/16 primer pairs showing polymorphism with an average of polymorphism information content (PIC) and the intra locus gene diversity (*Hj*) of 0.182 and 0.210, respectively. A total of 39 DNA fragments were amplified with their sizes ranging from 100 bp to 500 bp, of which 35 fragments were polymorphic (accounting for 89.74 %), an average of 2.44 fragments per marker (Table 3). Figure 2 showed the representative results of PCR products from the 70 samples using the Pinus10 marker.

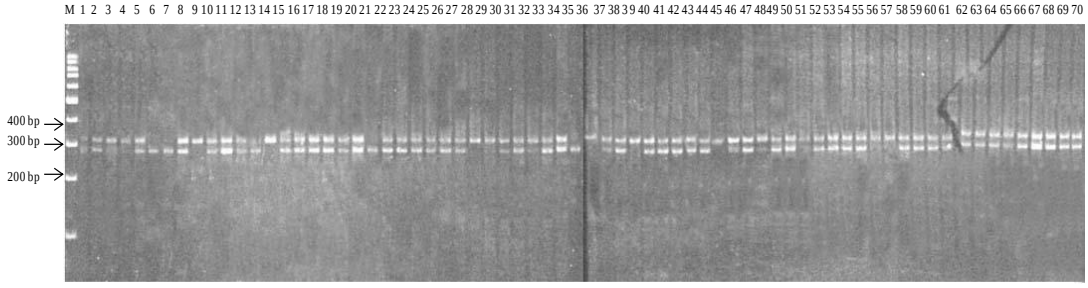

Figure 2. The PCR-SSR products of the 70 specimens using Pinus10 on 6 % polyacrylamide gels (numbers 1 –70: the samples from Ke1 to Ke70, M: marker 100 bp).

The analyzed parameters such as the average of number of alleles observed (*Na*), effective alleles (*Ne*) and private alleles (*Ap*) per locus, the Shannon's genetic diversity index (*I*), the expected (*He*) and observed heterozygosity (*Ho*) of each population were showed in Table 4. The results showed that genetic diversity in Lam Dong population (2.063; 1.730; 0.375; 0.558; 0.459 and 0.367, respectively) was the highest and there was in significant difference between Dak Lak and Kon Tum populations. The number of effective alleles per locus, observed and expected heterozygosity in Kon Tum population (*Ne* = 1.651; *Ho* = 0.370 and *He* = 0.346) were higher than those of Dak Lak (*Ne* = 1.637; *Ho* = 0.363 and *He* = 0.333), while the average number of observed alleles and private alleles per locus, Shannon's genetic diversity index in Dak Lak population (*Na* = 1.875, *Ap* = 0.188 and *I* = 0.495) were higher than those of Kon Tum (*Na* = 1.750; *Ap* = 0.063 and *I* = 0.489, respectively) (Table 4). At the population level, the mean of Shannon's genetic diversity index of *K. evelyniana* population was 0.514. This genetic diversity level was higher than that of *P. krempfii* population (*I* = 0.377) [6], but lower than that of *P. dalatensis* population in Tay Nguyen (*I* = 0.524) [7].

Table 3. Value PIC, intralocus genetic diversity and the percentage of polymorphic bands of SSR markers.

No.	Primers	Size (bp)	PIC	Polymorphic bands	Monomorphic bands	Total bands	Percentage of polymorphic bands	Intralocus genetic diversity (H*j*)
1	FRPP91	100-180	0.113	1	1	2	50	0.240
2	Cm3	120-200	0.482	4	0	4	100	0.359
3	PeB31BGT	190-500	0.174	3	0	3	100	0.118
4	Pinus10	285-305	0.146	2	0	2	100	0.250
5	PRE10	120-210	0.418	5	0	5	100	0.226
6	PRE13	170-180	0.145	2	0	2	100	0.359
7	PRE16	195-490	0.386	4	0	4	100	0.455
8	PRE24	380-400	0.014	2	0	2	100	0.054
9	P1	300- 400	0.237	3	0	3	100	0.234
10	P5	145- 160	0.246	2	0	2	100	0.191
11	Pt36480	160-160	0.000	0	1	1	0	0.000
12	Pt87268	160-160	0.000	0	1	1	0	0.000
13	PtTX3026	130-255	0.118	2	0	2	100	0.345
14	PtTX3034	200- 210	0.146	2	0	2	100	0.250
15	RPS1b	100- 100	0.000	0	1	1	0	0.000
16	RPS2	185- 210	0.277	3	0	3	100	0.278
Sum		**100-500**	**2.902**	**35**	**4**	**39**	**-**	**3.359**
Mean			**0.182**	**2.188**	**0.250**	**2.438**	**89.74**	**0.210**

Table 4. Genetic diversity of three *K. evelyniana* populations at 16 SSR markers.

Populations	*Na*	*Ne*	*I*	*Ho*	*He*	*Ap*	*Fis*	*PPB (%)*
Lam Dong	2.063	1.730	0.558	0.459	0.367	0.375	- 0.234	81.25
Dak Lak	1.875	1.637	0.495	0.363	0.333	0.188	- 0.065	81.25
Kon Tum	1.750	1.651	0.489	0.370	0.346	0.063	- 0.047	75.00
Mean	1.896	1.673	0.514	0.397	0.349	0.208	- 0.115	79.17
At the species level	2.438	2.027	0.677	0.401	0.418	-	-	81.25

Notes: *Na*: the average number of alleles per locus; *Ne*: number of effective alleles per locus; *I*: Shannon's genetic diversity index; *Ho* and *He*: the observed and expected heterozygosity; *Ap*: number of private alleles per locus; *Fis*: Wright's inbreeding coefficient with $p < 0.05$; PPB%: percentage of polymorphic bands.

The results in Table 4 also showed that among the private alleles (*Ap*) found in all 3 populations, the highest number was in Lam Dong population (0.375) and the lowest was in Kon Tum population (0.063). The fixation index values of all studied populations were negative (*Fis* < 0) with ranging from - 0.047 of Kon Tum population to - 0.234 of Lam Dong population. The observed heterozygosity of *K. evelyniana* populations were higher than the expected heterozygosity (*Ho* > *He*). This result indicated that the excess heterozygosity of *K. evelyniana* species in Tay Nguyen could be attributed to the phenomenon of cross-pollinating between populations.

At the species level, genetic diversity of *K. evelyniana* was expressed by their effective allele number per locus *Ne* = 2.027, Shannon's genetic diversity index *I* = 0.677 and expected heterozygosity *He* = 0.418 (Table 4). Comparison to some other conifer species in Tay Nguyen showed that the expected (*He*) and observed heterozygosity (*Ho*) values of *K. evelyniana* (0.418

and 0.401) were the higher than those of *P. krempfii* (0.234 and 0.310) [6]. It's expected heterozygosity value found the lower than that of *P. dalatensis* (*He* = 0.418 vs 0.524), however it's observed heterozygosity value was the higher (*Ho* = 0.401 vs 0.234) [7].

In order to further evaluate genetic diversity degree of *K. evelyniana*, genetic parameters for each SSR marker are also analyzed. The analysis indicated that the average of observed (*Ho*) and expected heterozygosity (*He*) values of *K. evelyniana* population were 0.423 and 0.324, respectively (Table 5). Comparison of heterozygosity level of species in the genus *Keteleeria* showed that *K. evelyniana* species in Tay Nguyen (*Ho* = 0.423, *He* = 0.324) were lower than those of *K. davidiana* var. *formosana* species in Taiwan (*Ho* = 0.68, *He* = 0.82) and (*Ho* = 0.63, *He* = 0.78) in study of Ho et al. [22]. The results in Table 5 also showed that the average of gene flow (*Nm*) of *K. evelyniana* was 5.423. The marker PtTX3026 gave the highest *Nm* (*Nm* = 34.882 and *Fst* = 0.007) and the marker Cm3 was the lowest (*Nm* = 0.312 and *Fst* = 0.445). Compared with *P. krempfii* species in Tay Nguyen, gene flow level of *K. evelyniana* species was the higher (2.315 vs 5.423, respectively) [6].

Table 5. The genetic parameters of all *K.evelyniana* populations for 16 SSR markers.

Primers	***Na***	***Ne***	***I***	***Ho***	***He***	***Fis***	***Fst***	***Nm***
FRPP91	2.00	1.688	0.564	0.647	0.384	-0.686	0.123	1.776
Cm3	1.80	1.669	0.514	0.134	0.361	0.629	0.445	0.312
PeB31BGT	2.20	1.769	0.625	0.505	0.405	-0.249	0.104	2.153
Pinus10	2.00	1.973	0.686	0.819	0.493	-0.661	0.008	30.856
PRE10	2.20	2.132	0.761	0.519	0.523	0.007	0.235	0.814
PRE13	2.00	1.847	0.647	0.695	0.455	-0.527	0.030	8.096
PRE16	1.80	1.333	0.365	0.214	0.229	-0.433	0.376	0.416
PRE24	2.00	2.000	0.693	1.000	0.500	0.064	0.065	3.620
P1	1.60	1.346	0.318	0.198	0.211	-1.000	0.138	1.563
P5	1.00	1.000	0.000	0.000	0.000	0.062	0.158	1.337
Pt36480	1.00	1.000	0.000	0.000	0.000	-	-	-
Pt87268	2.00	1.981	0.688	0.763	0.495	-	-	-
PtTX3026	2.00	1.566	0.499	0.313	0.330	-0.542	0.007	34.882
PtTX3034	2.00	1.872	0.654	0.662	0.462	0.052	0.322	0.526
RPS1b	1.80	1.579	0.486	0.302	0.334	-	-	-
RPS2	1.00	1.000	0.000	0.000	0.000	0.095	0.377	0.412
Mean	**1.775**	**1.610**	**0.469**	**0.423**	**0.324**	**-0.245**	**0.184**	**5.423**

Notes: *Na*: average of number of alleles per locus; *Ne*: number of effective alleles per locus; *I*: Shannon diversity index; *Ho* and *He*: the observed and expected heterozygosity; *Fis*: Wright's inbreeding coefficient with $p < 0.05$; *Fst*: coefficient of genetic differences; *Nm*: gene flow.

3.2. Population structure

Molecular variance (ANOVA) analysis among *K. evelyniana* populations using SSR markers indicated that 65.35 % of the total genetic diversity was distributed within groups and only 34.65 % was attributed to differences between regions (with p value < 0.001) (Table 6). The low variability between populations was also reported by Tam et al., 2013, in which the genetic variation was found in the populations of *Glyptostrobus pensilis* of Viet Nam [23].

Table 6. Analysis of molecular variance among/within *K. evelyniana* populations.

Source of variance	Degree of freedom	Sum of squares	Variance components	Total variation (%)	p value
Among population	2	65.259	1.298	34.65	< 0.001
Within population	67	164.112	2.449	65.35	

Genetic differences between populations of *K. evelyniana* were calculated based on alleles frequency comparison of markers between pairwise populations and showed in Table 7. The average genetic difference level between studied populations was low with *Fst* = 0.118. The lowest one (*Fst* = 0.092) was between Lam Dong and Dak Lak population and the highest (*Fst* = 0.137) was between Dak Lak and Kon Tum populations (Table 7). When comparison with some other coniferous species, this value of *K. evelyniana* in Tay Nguyen (*Fst* = 0.118) was found to be lower than *P. dalatensis* (*Fst* = 0.287) [7], *P. resinosa* (*Fst* = 0.280) and *P. radiata* (*Fst* = 0.14) [10, 24], and higher than *P. cembra* (*Fst* = 0.02) [25].

Table 7. Value of genetic differences between pairwise of populations *K. evelyniana*.

	Lam Dong	Dak Lak	Kon Tum
Lam Dong			
Dak Lak	0.092		
Kon Tum	0.105	0.137	

The low in both molecular variance (34.65 %) and genetic difference (*Fst* = 0.118) between the populations of *K. evelyniana* in Tay Nguyen indicated the studied species is relatively conservative in its genome and only slight variation can be occurred depending on geographical features.

3.3. Genetic relationships among 70 *K. evelyniana* samples

A UPGMA dendrogram constructed based on similarity matrix with SSR markers (Figure 3) divided 70 *K. evelyniana* samples into two main groups (I and II) with their genetic similarity coefficient ranged from 76.5 % (Ke26 and Ke44) to 99 % (Ke23 and Ke25). Each main group also divided into two subgroups. Subgroup I.1 included 23 samples collected in Kon Tum (Ke48 – Ke70) with their genetic similarity coefficient ranged from 84 % to 98.6 %. The second subgroup I.2 included 21 samples originated from Dak Lak (Ke27 – Ke47) with their genetic similarity coefficient ranged from 81.4 % to 98.3 %. The subgroup II.1 and II.2 included 12 samples of Hiep An (Lam Dong) (Ke11 – Ke22) and 14 samples of Suoi Vang and Da Chais (Lam Dong), respectivelly. The obtained results obviously showed that the samples in the same geographic regions were clustered into the some subgroups. The genetic similarities between samples in the dendrogram (from 76.5 % to 99 %) were also consistent with population structure analysis above (genetic variation among populations 34.65 % and genetic difference *Fst* = 0.118).

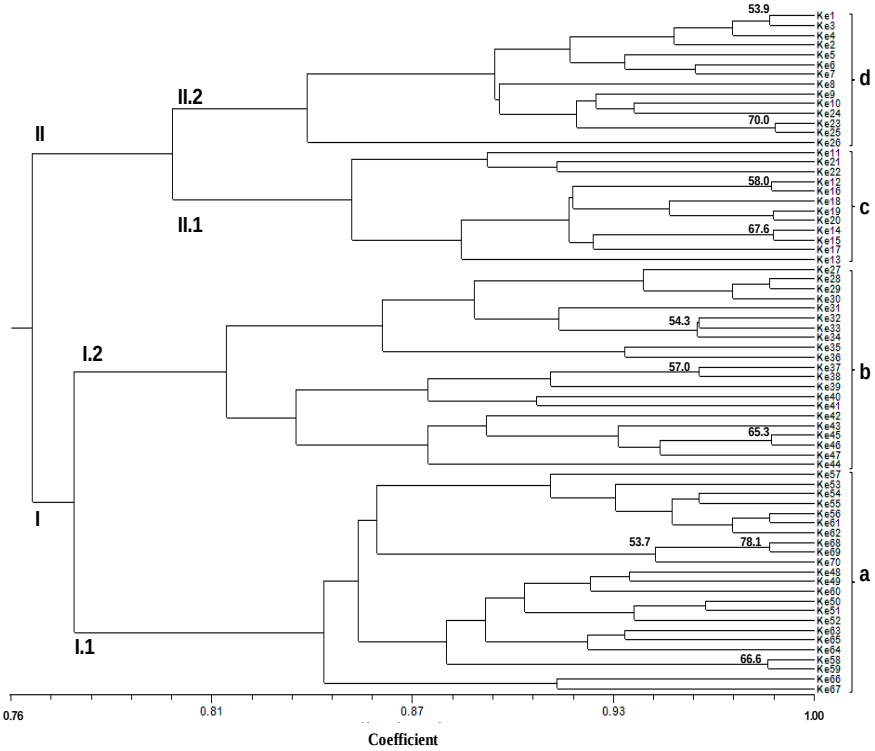

Figure 3. UPGMA dendrogram based on similarity genetic coefficient of 70 *K. evelyniana* samples analysis SSR markers with the bootstrap values repeat 1000 times (Note: a: samples in Kon Tum; b: samples in Dak Lak; c: samples in Hiep An (Lam Dong); d: samples in Da Chais and Suoi Vang (Lam Dong).

4. CONCLUSION

In this study, genetic diversity indicators of *K. evelyniana* within and between popullations were analysed using SSR markers. They were the highest in Lam Dong (*Na* = 2.063; *Ne* = 1.730; *Ap* = 0.375; *I* = 0.558; *Ho* = 0.459 and *He* = 0.367) and not significant different between Dak Lak and Kon Tum populations. The rate of cross-pollination among the three populations was high (*Fis* < 0), indicating a very low rate of inbreeding in each population occurred. The number of private alleles (*Ap*) were found in all Lam Dong (0.375), Dak Lak (0.188) and Kon Tum (0.063) populations using SSR markers. The gene flow (*Nm*) also occurred in the populations of *K. evelyniana* with *Nm* = 5.423. The total level of molecular changes between populations was relatively low (4.65 %) and high within populations (65.35 %). The genetic similarity coefficient of *K. evelyniana* in Tay Nguyen ranged from 76.5 to 99 %. Based on *Fis* value, gene flow rate and total level of molecular variance analysis, it can conclude that the genetic diversity of *K. evelyniana*is in Tay Nguyen is alarming, and conservation and management strategies for this species are urgently needed.

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Tum province), Bidoup Nui Ba National Park (Lam Dong province), Chu Yang Sin National Park (Dak Lak province) and other organizations in Tay Nguyen for species survey and sample collections.

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