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Full Length Research Paper

Genetic diversity on the tropical rare wood species of *Dalbergia* in Vietnam revealed by inter-simple sequence repeat (ISSR) markers

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Genetic diversities of three rare hardwood species of *Dalbergia* (*D. assamica*, *D. nigrescens* and *D. tonkinensis*) were evaluated for conservation based on inter-simple sequence repeat (ISSR) markers. A total of 47 ISSR primers were used for the analysis, but only 31 ISSR primers were successfully amplified for 25 samples from each species. There were 166 fragments across the 75 samples produced, in which 153 were polymorphic with an average of 4.94 polymorphic fragments per primer. The number of amplified fragments ranged from 1 (ISSR13, ISSR54 and ISSR59) to 11 (ISSR14) and their size varied from 200 to 1700 bp. The similarity coefficient ranged from 67.0 to 98.9% in *D. assamica*; from 71.2 to 98.5% in *D. nigrescens* and from 68.5 to 95.2% in *D. tonkinensis*. The estimated value of molecular diversity parameters within species such as the effective number of alleles, Shannon's information index, intralocus gene diversity and Nei's gene diversity were low among the individuals of the different *Dalbergia* species (1.227, 0.195, 0.662 and 0.146, respectively in *D. assamica*; 1.135, 0.111, 0.425 and 0.109, respectively in *D. nigrescens*; 1.198, 0.166, 0.526 and 0.123, respectively in *D. tonkinensis*). The analysis of molecular variance (AMOVA) of ISSR data indicated that the greater proportion of total genetic variation existed among species rather than within species. The correlation between genetic and geographic distance was also found in the three *Dalbergia* species.

Key words: *Dalbergia*, endemic species, genetic similarity, ISSR markers.

INTRODUCTION

Dalbergia, a large genus of small to medium-sized trees, shrubs and woody climbers, belongs to the family Fabaceae, subfamily Faboideae and distributed mainly in tropical and sub-tropical regions (Rout et al., 2003). Several species of *Dalbergia* are important timber trees and valuable for construction and ornamentation such as *D. nigrescens*, *D. tonkinensis* and *D. assamica*. The genus has 27 species distributed from the North to the South of Vietnam. Of those species, *D. tonkinensis* is on Vietnam's Red List (Dang and Nguyen, 2007) prohibiting exploitation, shipping and storage. The other species are also in danger of extinction. Therefore, it is necessary to understand patterns of genetic variation of these species in order to establish conservation strategies and protect them from further genetic erosion.

Over the years, the genetic diversity of forest trees has been detected and assessed extensively using both morphological and molecular methods. Several molecular marker techniques are now used in diversity studies. The most commonly used systems are restriction fragment length polymorphism (RFLP) (Garcia-Mas et al., 2000),

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random amplification of polymorphic DNA (RAPD) (Williams et al., 1990), amplified fragment length polymorphism (AFLP) (Vos et al., 1995) and inter simple sequence repeat (ISSR) (Lalhruaitluanga and Prasad, 2009). In higher plants or animals, ISSR markers are in more demand, because they are known to be abundant, very reproducible, highly polymorphic, highly informative and quick to use (Zietkiewicz et al., 1994, Bornet et al., 2002). ISSRs was proposed for genetic diversity by Lalhruaitluanga and Prasad (2009) and commonly used in population genetics, taxonomy and phylogeny of many plant species (Wolf and Randle, 2001). ISSR primers can also confirm specific amplified DNA polymorphic fragments within varieties (Li and Ge, 2001). Studies on biodiversity of valuable hardwood trees in *Dalbergia* using RADP, ISSR and AFLP markers have also been implemented in several countries. For instance, Olivarimbola et al. (2004) established genetic relationships of 122 individuals of *Dalbergia monticola* in Madagascar using 60 RAPD and 3 cpSSR markers. Results indicate that the population in the Central North originated from a region in the South. Similarly, French, Indian and Brazilian researchers have applied RAPD and ISSR markers, and specific genetic sequences to investigate genetic relationships among species and populations of *Dalbergia* (Rout et al., 2003; Subhash and Manojkumar 2004; Juchum et al., 2007; Andrianoelina et al. 2006).

This report is however, not on the phylogeny in the genus *Dalbergia*. In this study, we aimed to report the genetic relationships and genetic diversity among the individuals of three *Dalbergia* species revealed by ISSR markers.

MATERIALS AND METHODS

Leaf samples or wood pieces of *D. assamica*, *D. tonkinensis* and *D. nigrescens* were collected from a natural stand grown in the Yordon National Park (Dak Lak province), Cuc Phuong National Park (Ninh Binh province), Phong Nha - Ke Bang National Park (Quang Binh province), Copia Nature Reserve (Thuan Chau district, Son La province) and some streets of Ha Noi city in Vietnam.

Yordon National Park is the largest of Vietnam's nature preserves and one of seven internationally important Centers of Plant Diversity in Vietnam. This park encompasses over 1,000 km^2 and extends from Eastern Cambodia into Northern Dak Lak and Southern Gia Lai Provinces in Vietnam. The topography of most of this park is flat, with an elevation of approximately 200 m. Cuc Phuong is the oldest National Park in Vietnam, located only 120 km southwest of Hanoi and nestled between the provinces of Ninh Binh, Hoa Binh and Thanh Hoa. Cuc Phuong is situated in the foothills of the northern Annamite Mountains. The park consists of verdant karst mountains and lush valleys. Elevation varies from 150 to 656 m at the summit of May Bac Mountain or Silver Cloud Mountain. The area of this park is about 220,00 km^2. Phong Nha - Ke Bang National Park is located in the middle of the Annamite Mountain Range, 40 km from Dong Hoi, 500 km from Vietnam`s capital of Hanoi and close to the Vietnam - Laos border, just several kilometers to the west. Phong Nha-Ke Bang National Park is one of the world's two largest limestone regions. The park covers a total area of 857.54 km^2, which are divided into three zones, a "strictly protected zone" (648.94 km^2), an "ecological recovery zone" (174.49 km^2) and an "administrative service zone" (34.11 km^2). Copia proposed nature reserve is located in Thuan Chau district, Son La province. The proposed nature reserve is centered on Mount Copia, a 1,800 m peak. The area of this park is about 19,253 ha.

There were 25 individuals of each species used in this study (Figure 1 and Table 1). The amount of population of each species was limited in Vietnam. The samples were stored at -20°C until DNA was extracted.

DNA isolation

Total genomic DNA was extracted from leaves and wood specimens using the cetyl trimethylammonium bromide (CTAB) method described by Doyle and Doyle (1990). The concentration of DNA was determined with a UV-visible light spectrophotometer (UVS 2700, Labomed, USA) and the DNA samples were diluted to 10 to 20 ng μL^{-1} and used as templates for polymerase chain reaction (PCR) amplification.

ISSR marker amplification

ISSR primers were obtained from Integrated DNA Technologies, USA (Table 2). The ISSR sequences were collected based on the publications of Arif et al. (2009); Bornet et al. (2002); Bhattacharya et al. (2010); Gupta et al. (2008); Lalhruaitluanga and Prasad (2009); Djè et al. (2006); Hou et al. (2005) and Goswami and Tripathi (2010). The reaction mixtures contained 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 10 to 20 ng genomic DNA, 10 pmol of primer, 2 to 4 mM MgCl2, 300 to 400 µM of each dNTP, and 0.8 to 1.2 U of Taq DNA polymerase (Amersham). The temperature profile consisted of an initial denaturation step at 94°C for 4 min, followed by 35 cycles: 94°C for 1 min, 38 to 55°C for 1 min and 72°C for 1 min. After the final cycle, samples were incubated for 10 min to ensure complete extension. The product was stored at 4°C. The PCR products were separated on 1.5% agarose gel in 0.5 x Tris-borate-ethylenediaminetetraacetic acid (TBE) buffer. The size of amplified DNA fragments was estimated with 1 kb ladders (Fermentas, USA). The gels were visualized under UV using gel documentation (CSL-MiCRODOC, Cleaver, England).

Data analysis

DNA fingerprints were scored for the presence (1) or absence (0) of bands of various molecular weight sizes in the form of binary matrix. The Simqual program was used to calculate Jaccard's coefficients (Jaccard, 1908); these were calculated as follows: $S_{ij} = a/(a + b + c)$, in which S*ij* is the coefficient of similarity between two individuals *i* and *j*; a is the number of fragments shared by samples; b represents amplified fragments in sample *i*; and c represents fragments in sample *j*. The UPGMA-based dendrogram was constructed using NTSYS 2.0 software, version 2.0 (Rohlf, 1992). Win-Boot software (Yap and Nelson, 1996) was used to compute bootstrap-based P-values to assess the strength of evidence for clustering; this data was bootstrapped with 1,000 replications, a long Jaccard's coefficient. The polymorphism information content (PIC) of each locus was determined as described by Weir (1990): $PIC = 1 - \sum P_i^2$, where P*i* is the frequency of the *i*th allele in the genotypes. Nei's gene diversity was calculated using the formula: H*i* = h*1* + h*2*/total number of loci where h*1* and h*2* [i.e., $h_j=(1-p^2-q^2)$] represent intralocus gene diversity. The analysis of AMOVA was calculated using GenAlEx 6.3 software (Peakall and Smouse, 2006) whereas, diversity in the frequency of fragment size of ISSR patterns was apportioned within and among species of *Dalbergia* using Shannon's information index (*i*) and gene diversity

Figure 1. Location of the three *Dalbergia* species in the Vietnam.

Table 1. Details of the three *Dalbergia* species employed for the study of genetic diversity from different location of Vietnam

Number	Scientific name	Code used in this study	Number of sample	Location	Conservation status*
1	*Dalbergia assamica*	Da	25	Ha Noi and Cuc Phuong National Park (Ninh Binh)	Endemic to Vietnam
2	*Dalbergia tonkinensis*	Dt	25	Ha Noi and Phong Nha - Ke Bang National Park (Quang Binh)	VU (A1acd) and IUCN Red List
3	*Dalbergia nigrescens*	Dn	25	Yordon National Park (Dak Lak) and Copia Nuture Reserve, Thuan Chau (Son La)	Endemic to Vietnam

**Vietnam Red List*; (Dang and Nguyen, 2007); (IUCN, 1998)

Table 2. Code and sequences of the 31 ISSR primers used for the study of genetic diversity of three *Dalbergia* species

Number	Primers code	Primer sequence	Amplified product range (bp)	Number	Primers code	Primer sequence	Amplified product range (bp)
1	ISSR1	(CAG)5	450 - 1100	17	ISSR46	(AG)8T	300 - 1200
2	ISSR2	(CAA)5	500 - 1300	18	ISSR49	(GA)8T	200 - 1100
3	ISSR3	(GACA)4	300 - 1400	19	ISSR51	(GA)8A	300 - 950
4	ISSR5	(CCG)6	500 - 1700	20	ISSR52	(CT)8G	400 - 1400
5	ISSR6	(CTC)6	600 - 1200	21	ISSR54	(TC)8G	1400
6	ISSR7	(GGC)6	400 - 1200	22	ISSR55	(AC)8T	400 - 1200
7	ISSR8	(GAA)6	400 - 800	23	ISSR56	(AC)8G	450 - 1000
8	ISSR9	(TG)8GA	750 - 800	24	ISSR59	(GA)8CT	500
9	ISSR10	(CTC)8	450-1200	25	ISSR61	(AC)8TG	400 - 850
10	ISSR11	(CCA)5	300 - 900	26	ISSR62	CTC(AG)7	300 - 850
11	ISSR12	(CCCT)4	600 - 800	27	ISSR63	CTC(GA)7	250 - 800
12	ISSR13	(GT)8C	700	28	ISSR64	ACA(GT)7	300 - 950
13	ISSR14	(CTCT)4GTC	300 - 1400	29	ISSR65	CAC(TG)7	400 - 1200
14	ISSR15	(CA)8A	400 - 1400	30	ISSR67	(ATG)6	600 - 1200
15	ISSR17	(CT)8T	400 - 600	31	ISSR69	(GGGTG)3	450 - 1300
16	ISSR18	(CT)8A	500 - 1000				

index (H*i*) following Nei (1973) with the help of PopGen 32 software.

RESULTS

A total of 47 ISSR markers were used for this study, but only 31 ISSR primers were successfully amplified for 25 samples of each *Dalbergia* species. 29 primers showed polymorphism, 2 primers (ISSR7 and ISSR13) could not show distinguish within the species, while 6 primers showed monomorphic bands within the species. Most of the amplification reactions were duplicated and only bands that were consistently reproduced across amplifications were considered for the analysis. Bands with the same mobility were considered as identical fragments, receiving equal values, regardless of their staining intensity. When multiple bands in a region were difficult to resolve, data for that region of the gel was not included in the analysis.

PCR amplification of DNA using 31 ISSR primers produced 166 DNA fragments that were scored in all genotypes. The number of fragments varied from 1 (ISSR13, ISSR54 and ISSR59) to 11 (ISSR14) and their sizes varied from 200 to 1700 bp. Of the 166 amplified fragments, 153 were polymorphic with the average number of bands per primer and polymorphic bands per primer as 5.36 and 4.94, respectively (Table 7). The total number of polymorphic band, average number of band/primer and average numbers of polymorphic band/primer were 59, 3.74 and 2.19, respectively in *D. assamica*; 33, 3.08 and 1.32, respectively in *D. nigrescens* and 47, 3.58 and 1.81, respectively in *D. tonkinensis* (Table 7).

PIC value varied from 0 (ISSR 7 and ISSR13) to 0.564 (ISSR52), with an average of 0.352 (Table 3). The patterns of ISSR fragments produced by primers ISSR55 are shown in Figure 2. Further analysis of these ISSR profiles for band similarity indices could clearly differentiate all the species of *Dalbergia* (for instance at the size about 750 bp, all individuals of *D. tonkinensis* have amplified DNA fragment) (Figure 2C). More also, the results of the genetic similarity matrix obtained after multivariate analysis using Nei and Li's coefficient (Nei and Li, 1979) among the individuals of different *Dalbergia* species ranged from about 67.0 (Da10 and Da21) to 98.9% (Da2 and Da4) in *D. assamica*; from 71.2 (Dn2 and Dn25) to 98.5% (Dn10 and Dn12) in *D. nigrescens* and from 68.5 (Dt5 and Dt20) to 95.2% (Dt2 and Dt3) in *D. tonkinensis* (Tables 4, 5 and 6).

The dendrogram based on UPGMA analysis grouped 75 samples into three main clusters (I, II, and III) of which each main cluster contained one species. All the species shared more than 28% similarity among themselves. Genetic similarity within clusters ranged from about 28 to 72% with a bootstrap value of 70.5 (Figure 3). Genetic

Table 3. The statistical data for 31 ISSR primers used for the study of genetic diversity of three *Dalbergia* species

Number	Primers code	PIC	Poly band	Mono band	Total band	Nei's gene diversity among species	Nei's gene diversity		
							Da	Dn	Dt
1	ISSR1	0.305	4	1	5	0.201	0.271	0.000	0.000
2	ISSR2	0.318	6	0	6	0.431	0.134	0.000	0.000
3	ISSR3	0.431	10	0	10	0.369	0.128	0.196	0.370
4	ISSR5	0.243	2	3	5	0.106	0.000	0.067	0.080
5	ISSR6	0.320	5	0	5	0.379	0.026	0.115	0.330
6	ISSR7	0.000	0	5	5	0.000	0.000	0.000	0.000
7	ISSR8	0.211	1	1	2	0.222	0.000	0.000	0.000
8	ISSR9	0.428	2	0	2	0.440	0.077	0.000	0.000
9	ISSR10	0.227	5	1	6	0.340	0.064	0.142	0.000
10	ISSR11	0.485	6	0	6	0.343	0.246	0.134	0.000
11	ISSR12	0.283	2	0	2	0.418	0.160	0.000	0.000
12	ISSR13	0.000	0	1	1	0.000	0.000	0.000	0.000
13	ISSR14	0.419	11	0	11	0.383	0.342	0.106	0.235
14	ISSR15	0.471	10	0	10	0.363	0.305	0.408	0.280
15	ISSR17	0.347	2	0	2	0.405	0.202	0.000	0.000
16	ISSR18	0.495	2	0	2	0.374	0.000	0.000	0.246
17	ISSR46	0.334	6	0	6	0.432	0.154	0.000	0.093
18	ISSR49	0.505	7	0	7	0.365	0.266	0.000	0.000
19	ISSR51	0.401	8	0	8	0.340	0.134	0.341	0.206
20	ISSR52	0.564	4	0	4	0.288	0.421	0.000	0.038
21	ISSR54	0.423	1	0	1	0.444	0.000	0.000	0.000
22	ISSR55	0.344	9	0	9	0.432	0.096	0.090	0.117
23	ISSR56	0.330	5	0	5	0.236	0.243	0.125	0.203
24	ISSR59	0.423	1	0	1	0.444	0.000	0.000	0.000
25	ISSR61	0.315	4	1	5	0.342	0.093	0.157	0.000
26	ISSR62	0.470	6	0	6	0.318	0.000	0.145	0.229
27	ISSR63	0.286	7	0	7	0.385	0.000	0.109	0.215
28	ISSR64	0.386	9	0	9	0.355	0.254	0.218	0.308
29	ISSR65	0.483	8	0	8	0.388	0.000	0.263	0.000
30	ISSR67	0.324	4	0	4	0.461	0.245	0.000	0.182
31	ISSR69	0.346	6	0	6	0.265	0.074	0.098	0.054
	Total	10.917	153	13	166	10.269	3.935	2.714	3.186
	Average	0.352	4.935	0.419	5.354	0.331	0.146	0.109	0.123

similarity among individuals of *D. tonkinensis* in cluster I ranged from 77 to 96% with a bootstrap value of 100. Clusters II of *D. nigrescens* had the genetic similarity about 80 to 98% with a bootstrap value of 100, while clusters III, that included *D. assamica*, had the genetic similarity of about 76 to 99% with a bootstrap value of 100.

Values of effective number of alleles, Shannon's information index, intralocus gene diversity (H*j*) and Nei's gene diversity (H*i*) among the individuals of different *Dalbergia* species using 31 ISSR markers were 1.227, 0.195, 0.662 and 0.146, respectively in *D. assamica*; 1.135, 0.111, 0.425 and 0.109, respectively in *D. nigrescens*; and 1.198, 0.166, 0.526 and 0.123 in *D.*

Table 4. Similarity matrix for Nei and Li's coefficient among the 25 individuals of *D. assamica*.

	Da1	Da2	Da3	Da4	Da5	Da6	Da7	Da8	Da9	Da10	Da11	Da12	Da13	Da14	Da15	Da16	Da17	Da18	Da19	Da20	Da21	Da22	Da23	Da24	Da25
Da1	100																								
Da2	90.0	100																							
Da3	89.8	95.5	100																						
Da4	91.1	98.9	94.4	100																					
Da5	85.2	91.0	86.5	90.0	100																				
Da6	78.3	80.0	79.6	79.2	81.1	100																			
Da7	79.3	81.1	80.6	82.1	80.2	89.7	100																		
Da8	77.2	80.9	80.4	80.0	80.0	91.8	95.2	100																	
Da9	77.8	81.5	81.1	80.6	80.7	79.8	83.0	82.8	100																
Da10	70.0	68.4	69.6	69.5	72.7	80.0	85.5	83.1	71.3	100															
Da11	82.2	85.9	83.5	84.9	85.2	82.2	81.3	83.1	81.8	75.9	100														
Da12	73.6	75.5	75.0	74.7	80.5	85.9	87.1	89.2	81.2	83.8	81.6	100													
Da13	78.0	81.7	81.3	80.9	83.0	84.1	83.1	85.1	88.1	75.6	86.2	85.7	100												
Da14	78.0	83.7	81.3	82.8	87.2	78.0	77.2	76.9	83.7	71.6	84.1	79.3	83.9	100.0											
Da15	71.1	73.1	72.5	72.3	75.9	77.0	78.2	77.9	74.4	78.8	81.2	82.7	74.7	78.8	100										
Da16	73.1	76.8	78.3	76.0	77.8	80.9	80.0	77.8	76.4	70.5	78.9	82.4	80.7	76.7	75.6	100									
Da17	74.5	80.0	77.7	79.2	81.1	82.2	81.3	79.1	77.8	73.9	82.2	83.7	82.0	82.0	81.2	87.2	100								
Da18	72.6	80.0	77.7	79.2	77.2	80.2	81.3	79.1	79.8	71.9	78.3	79.5	80.0	82.0	75.0	85.1	86.4	100							
Da19	72.5	76.3	79.8	75.5	77.3	76.4	77.5	75.3	75.9	69.8	80.5	75.6	80.2	82.4	77.1	85.5	84.7	84.7	100						
Da20	71.0	80.4	78.0	79.6	79.5	78.7	81.8	81.6	78.2	74.1	78.7	84.3	78.4	80.5	79.5	83.5	82.8	84.9	81.0	100					
Da21	71.7	75.5	75.0	74.7	76.4	73.6	74.7	72.5	73.0	67.0	77.5	74.7	77.3	79.3	76.2	86.7	90.4	81.6	84.1	75.9	100				
Da22	74.2	79.8	79.3	78.9	75.0	78.0	79.1	78.9	79.5	73.6	80.0	75.3	77.8	75.8	78.8	80.7	82.0	82.0	78.2	82.6	79.3	100			
Da23	70.8	72.8	74.2	72.0	73.6	70.8	73.9	73.6	72.1	69.9	74.7	73.8	76.5	76.5	73.2	84.0	81.0	78.8	79.0	72.9	87.2	76.5	100		
Da24	70.7	74.5	73.9	73.7	71.4	72.5	73.6	75.3	71.9	67.8	76.4	71.6	76.1	70.3	70.9	81.2	76.4	76.4	80.7	74.7	75.6	86.7	76.8	100	
Da25	73.4	80.9	80.4	80.0	78.0	77.2	80.2	80.0	78.7	72.7	79.1	80.5	83.0	76.9	73.9	83.9	85.2	81.1	79.3	81.6	80.5	87.2	79.8	85.7	100

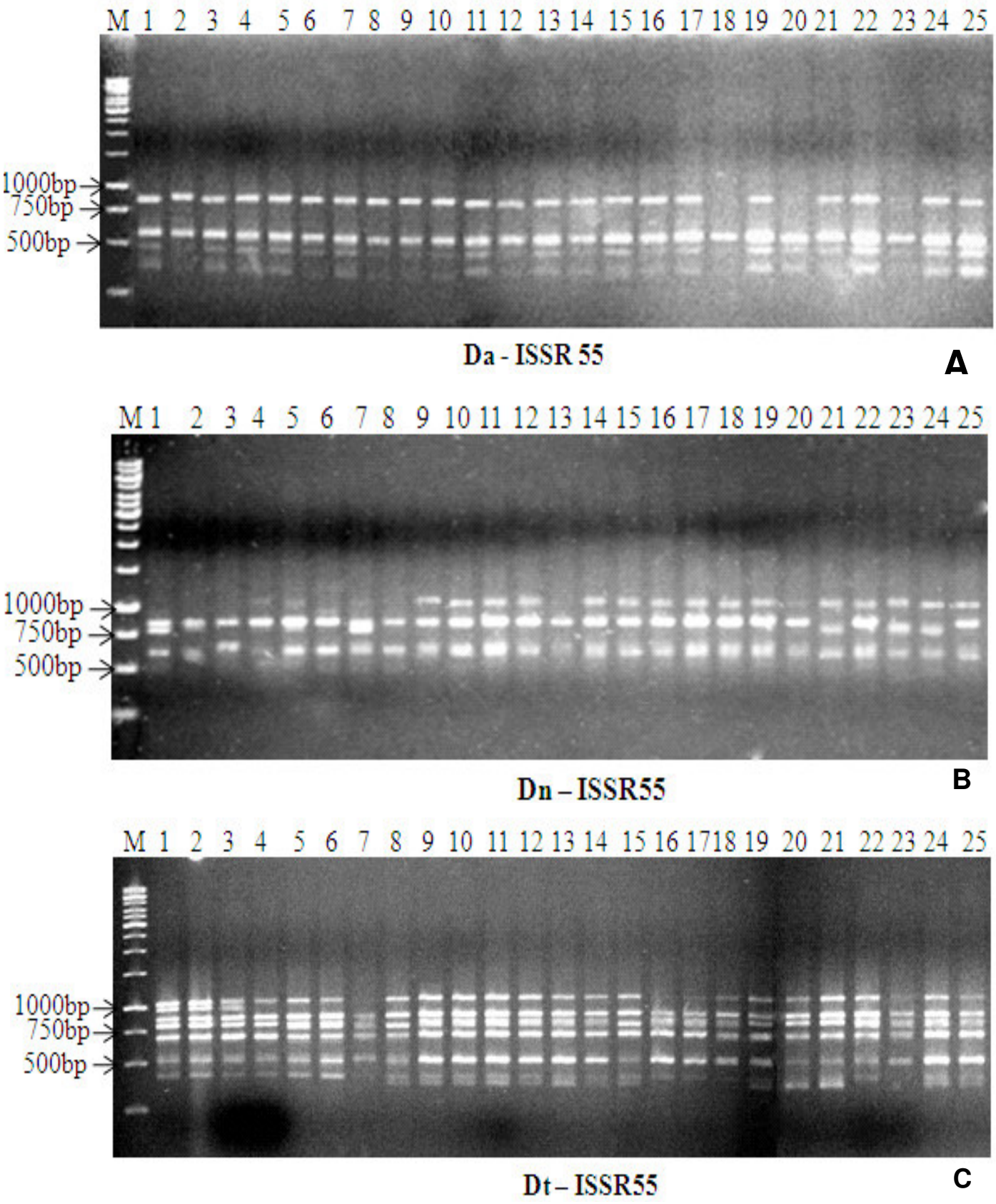

Figure 2. ISSR patterns of the three species of *Dalbergia* generated by primer ISSR55 (A, *D. assamica;* B, *D. nigrescens;* C, *D. tonkinensis*).

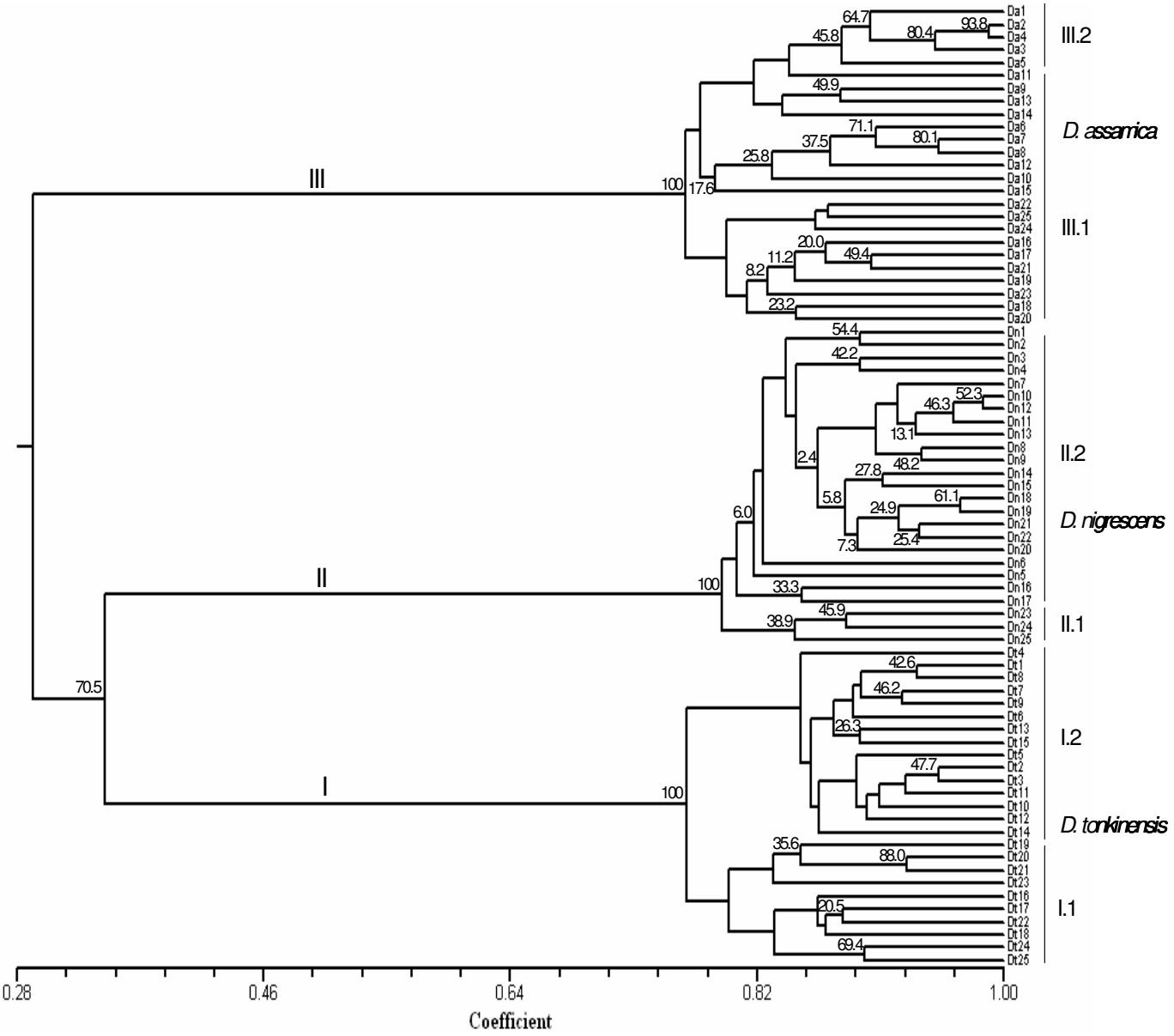

Figure 3. Dendrogram of cluster analysis of ISSR markers, illustrating the genetic among three species of *Dalbergia*.

tonkinensis (Tables 8). AMOVA of ISSR data revealed that a greater proportion of total genetic variation existed among species (80%) rather than within species (20%) (Table 9).

DISCUSSION

In our studies, ISSR markers were effective tools for understanding the genetic diversity of *Dalbergia*. This result was not surprising because these techniques have been used successfully in population genetic studies and in detecting genetic diversity in many species (Arif et al., 2009; Li and Ge, 2001; Djè et al., 2006; Blair et al., 1999). The polymorphism of the three tropical hardwood species of *Dalbergia* genus was compared (Table 7). The total number of polymorphic bands (59) and average number of polymorphic bands/primer (2.19) detected by ISSR primers were much higher in *D. assamica*. This method is obviously advantageous in differentiating closely related cultivars and has been used for cultivar identification in numerous plant species, including rice (Joshi et al., 2000), apple (Goulaõ and Oliveira, 2001), mulberry (Zhao et al., 2006) and strawberry (Arnau et al., 2003). The results indicate that the mean levels of genetic variation were low among the individuals of different *Dalbergia* species. Molecular markers have become common for assessing diversity within plant

Table 5. Similarity matrix for Nei and Li's coefficient among the 25 individuals of *D. nigrescens.*

	Dn1	Dn2	Dn3	Dn4	Dn5	Dn6	Dn7	Dn8	Dn9	Dn10	Dn11	Dn12	Dn13	Dn14	Dn15	Dn16	Dn17	Dn18	Dn19	Dn20	Dn21	Dn22	Dn23	Dn24	Dn25
Dn1	100																								
Dn2	89.6	100																							
Dn3	82.4	84.5	100																						
Dn4	86.4	85.7	89.6	100																					
Dn5	80.3	77.5	80.9	84.8	100																				
Dn6	80.6	80.3	78.6	79.7	79.1	100																			
Dn7	79.7	81.9	82.9	89.6	83.6	83.8	100																		
Dn8	82.6	84.7	83.1	84.3	81.2	81.4	91.2	100																	
Dn9	82.4	84.5	80.3	78.9	75.7	81.2	85.5	94.0	100																
Dn10	85.3	84.7	83.1	87.0	83.8	86.8	94.0	94.1	91.2	100															
Dn11	84.3	88.9	84.7	85.9	82.9	83.1	90.0	92.9	90.0	95.7	100														
Dn12	84.1	86.1	84.5	85.7	85.3	85.5	92.6	92.8	89.9	98.5	97.1	100													
Dn13	86.4	83.1	81.4	88.1	84.8	87.9	92.4	92.5	86.8	95.5	91.3	94.0	100												
Dn14	83.8	83.3	84.3	82.9	79.7	85.3	89.7	84.5	81.7	89.9	86.1	88.6	88.2	100											
Dn15	83.8	85.9	84.3	85.5	77.1	85.3	87.0	84.5	81.7	89.9	88.7	88.6	88.2	91.2	100										
Dn16	83.3	80.3	81.2	85.1	79.1	82.1	83.8	81.4	81.2	81.4	80.6	80.3	79.7	82.6	80.0	100									
Dn17	81.2	80.8	79.2	80.3	72.2	75.0	81.7	79.5	84.3	79.5	78.7	78.4	75.3	83.1	80.6	85.3	100								
Dn18	86.2	80.3	83.8	87.9	84.6	82.1	86.6	84.1	78.6	86.8	83.1	85.5	90.8	88.1	85.3	82.1	80.0	100							
Dn19	86.4	83.1	84.1	88.1	84.8	82.4	89.6	87.0	81.4	89.7	85.9	88.4	90.9	91.0	88.2	85.1	82.9	96.8	100						
Dn20	82.1	84.3	85.3	83.8	80.6	80.9	85.3	85.5	82.6	88.2	87.1	89.7	89.4	86.8	89.6	78.3	76.4	86.4	89.4	100					
Dn21	83.6	85.7	86.8	88.1	84.8	79.7	89.6	84.3	78.9	87.0	85.9	88.4	88.1	91.0	88.2	82.4	80.3	90.8	93.8	89.4	100				
Dn22	80.9	83.1	86.8	85.3	82.1	79.7	86.8	84.3	81.4	87.0	85.9	88.4	88.1	88.2	88.2	82.4	80.3	90.8	93.8	92.3	93.8	100			
Dn23	75.8	73.2	79.1	77.6	74.2	74.6	81.8	82.1	81.8	82.1	78.6	80.9	80.3	77.9	77.9	80.0	75.4	80.0	80.3	78.8	77.6	83.1	100		
Dn24	80.3	75.0	78.3	79.4	78.8	76.5	83.6	83.8	83.6	83.8	80.3	82.6	82.1	79.7	79.7	84.6	82.4	87.5	87.7	80.6	82.1	84.8	88.5	100	
Dn25	73.5	71.2	74.3	77.9	80.0	83.1	79.4	79.7	82.1	82.4	78.9	81.2	83.3	75.7	75.7	75.0	73.2	80.3	77.9	79.1	72.9	77.9	83.9	85.7	100

populations (Smith and Wayne, 1996; Lalhruaitluanga and Prasad, 2009).

The dendrogram analysis of all the three clusters considering them as three populations generated an over-view of population distribution (Figure 3). It is interesting to note that three populations comprising of three distinct *Dalbergia* species are seen as genetically distinct groups. Analysis of molecular variance of ISSR markers data (Table 9) revealed that the genetic variation within species (20%) was lower than those among species (80%). And the analysis of intralocus gene diversity (H*j*) and Nei's gen diversity (H*i*) (0.662 and 0.146, respectively) in *D. assamica* also were higher than those of two species; *D. nigrescens* (0.425 and 0.109, respectively) and *D. tonkinensis* (0.526 and 0.123, respectively) (Table 8). Compared to the other species in *Dalbergia* at the population level of genetic diversity, Shannon's diversity index and Nei's genetic diversity were 0.239 and 0.358 for *D. sissoo* (Wang et al., 2011), 0.223 and 0.150 for *D. monticola* (Andrianoelina et al., 2006) and 0.205 and 0.135 for *D. odorifera* (Yang et al., 2007). While in our present study, they were 0.195 and 0.146 for *D. assamica*, 0.111 and 0.109 for *D. nigrescens* and0.166 and 0.123 for *D. tonkinensis* (Table 8). The analysis of Shannon's information index and Nei's gene diversity values within species showed the existence of higher genetic diversity in *D.*

Table 6. Similarity matrix for Nei and Li's coefficient among the 25 individuals of *D. tonkinensis*.

	Dt1	Dt2	Dt3	Dt4	Dt5	Dt6	Dt7	Dt8	Dt9	Dt10	Dt11	Dt12	Dt13	Dt14	Dt15	Dt16	Dt17	Dt18	Dt19	Dt20	Dt21	Dt22	Dt23	Dt24	Dt25
Dt1	100																								
Dt2	90.5	100																							
Dt3	92.8	95.2	100																						
Dt4	87.8	88.1	85.9	100																					
Dt5	89.4	89.7	89.7	84.9	100																				
Dt6	91.1	84.5	84.5	86.3	85.7	100																			
Dt7	89.0	84.9	84.9	84.3	86.0	89.9	100																		
Dt8	93.7	84.7	86.9	84.1	85.9	87.3	90.0	100																	
Dt9	89.2	87.2	87.2	84.5	90.6	87.7	92.6	90.1	100																
Dt10	90.4	92.9	90.6	90.2	89.5	84.3	82.6	84.5	84.9	100															
Dt11	91.5	91.7	94.0	82.4	90.6	85.4	83.5	85.5	88.1	89.3	100														
Dt12	87.8	90.4	88.1	83.1	87.1	86.3	84.3	86.4	86.7	90.2	91.4	100													
Dt13	87.3	85.4	85.4	80.2	82.1	83.3	88.5	85.9	86.3	82.9	86.3	84.8	100												
Dt14	85.5	90.4	92.7	83.1	82.8	79.5	82.1	81.9	84.5	83.5	86.7	83.1	84.8	100											
Dt15	90.0	85.7	88.0	87.5	89.2	88.5	88.8	88.6	88.9	85.5	88.9	85.2	89.5	85.2	100										
Dt16	82.2	82.6	84.6	82.0	81.7	80.7	81.1	76.9	81.3	80.4	81.3	78.0	75.3	82.0	79.8	100									
Dt17	85.9	82.0	82.0	83.5	81.1	84.3	82.6	80.2	80.7	83.9	80.7	79.3	80.7	77.3	83.3	86.5	100								
Dt18	79.3	79.8	79.8	81.2	75.0	77.6	76.1	73.9	74.4	81.6	74.4	75.0	74.1	77.0	76.7	86.4	85.9	100							
Dt19	77.9	78.4	78.4	77.6	75.6	78.3	78.8	76.5	79.1	78.2	77.0	75.6	72.6	77.6	77.4	78.9	82.4	82.1	100						
Dt20	74.7	75.3	73.3	78.8	68.5	75.0	69.4	71.1	69.8	77.1	71.8	70.2	69.1	74.4	72.0	71.9	75.0	83.5	84.4	100					
Dt21	76.2	74.7	74.7	78.0	70.0	74.4	72.9	72.6	71.3	78.6	71.3	69.8	68.7	73.8	73.5	75.3	78.6	85.0	85.9	93.0	100				
Dt22	79.5	78.0	80.0	79.3	77.2	77.9	78.4	76.1	76.7	79.8	74.7	71.4	72.4	75.3	77.0	86.5	88.2	88.1	86.7	79.3	85.2	100			
Dt23	75.3	75.9	75.9	77.1	73.0	77.8	78.3	75.9	74.4	77.6	72.4	75.0	76.3	75.0	76.8	76.4	81.9	86.3	84.8	81.6	83.1	84.1	100		
Dt24	77.0	75.6	77.5	74.7	76.7	77.4	77.9	73.6	76.1	77.3	74.2	72.7	75.9	74.7	76.5	82.0	85.7	81.2	79.8	74.4	78.0	88.0	81.5	100	
Dt25	78.8	79.3	79.3	76.5	78.4	77.1	75.6	75.3	75.9	77.0	75.9	72.4	77.8	78.6	78.3	79.8	85.5	81.0	79.5	76.3	75.6	83.3	81.3	89.9	100

assamica population compared to *D. nigrescens* and *D. tonkinensis*.

In our study, the relative genetic distances within species showed the separation of samples from the geographical distances. For instance, for the species *D. tonkinensis,* 15 genotypes; Dt1-Dt15 (collected from Ha Noi province) were grouped into subgroup I.2 and 15 genotypes; Dt16-Dt25 (collected from Quang Binh province) were grouped into subgroup I.1 with a bootstrap value of 100; similar three genotypes, Dn23, Dn24 and Dn25 of *D. nigrescens* species population (collected from Son La province) were grouped into subgroup II.1 to a clade of 22 rest genotypes (Dn1-Dn22) with the bootstrap value of 100 and five genotypes (Da1, Da2, Da3, Da4 and Da5) of *D. assamica* species population (collected from Hanoi province) were grouped into a minor subgroup III.2 to a lade of 20 rest genotypes (Da6-Da25) with the bootstrap value of 45.8 (Figure 3).

The genetic structure of any species are normally affected by a number of evolutionary factors including mating system, gene flow, seed dispersal, mode of reproduction, as well as natural selection (Hamrick et al., 1992). Infact, little occurred within and between the individual populations of *Dalbergia* species, which is more concordant with the hypothesis of a highly clonal population resulting from reproduction by seeds. Mohana et al. (2001) reported that the pairs of seeds developing within a pod of *D. sisso* are genetically more similar than any random pairs of

Table 7. A comparative list of genetic diversity of three *Dalbergia* species ISSR primers.

Primer	Among three species	Da	Dn	Dt
Total number of polymorphic bands	153	59	33	47
Total number of monomorphic bands	13	42	44	46
Total number of bands	166	101	77	93
Average number of bands/primer	5.36	3.74	3.08	3.58
Average number of polymorphic bands/primer	4.94	2.19	1.32	1.81

Table 8. Genetic diversity parameters characterizing of three *Dalbergia* species using ISSR primers.

Primer	Among *Dalbergia* species	Da	Dn	Dt
Sum of effective number of alleles (SENA)	1.187	1.227	1.135	1.198
Shannon's Information index (I)	0.157	0.195	0.111	0.166
Polymorphic information content (PIC)	0.352	0.103	0.091	0.095
Intralocus gene diversity (H*j*)	1.806	0.662	0.425	0.526
Nei's gene diversity (H*i*)	0.331	0.146	0.109	0.123

Table 9. Summary of AMOVA analysis based ISSR primers of three *Dalbergia* species.

Source	df	SS	MS	Est. Var.	Percentage
Among species	2	1566.720	783.360	31.030	80
Within species	72	547.520	7.604	7.604	20
Total	74	2114.240		38.635	100

SS, Sums of squares; MS, mean sums of squares; Est. Var, estimated variance.

seeds in a tree. Thus, formation of two-seeded pods appears to be associated with increased genetic relatedness among the developing seeds. The lowest levels of genetic similarity among the populations of *D. sisso*, *D. assamica*, *D. latifolia*, *D. paniculata* and *D. spinosa* were 82.8, 87.3, 84.0, 87.5 and 84.5, respectively. It could be the reason for the observed genetic differentiation between the individuals of the *D. assamica, D. nigrescens* and *D. tonkinensis* populations growing from 120 to 1600 km apart.

This is the first study on the DNA diversity of some species of *Dalbergia* genus in Vietnam. Besides the high level of genetic diversity between species, a significant variation within a species was also found. This may be the result of a long-term of adaptation in diversity climatic and geographical conditions of Vietnam. Nevertheless, a further assignment should be established in more natural conditions to study the relationship between them and provide precise information for the protection of diversity.

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