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Molecular Analysis of Genetic Diversity of *Machilus odoratissima* Nees Species Collected from Central and Highland Areas of Vietnam

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Abstract

In this study, we analyzed the genetic diversity of 50 *Machilus odoratissima* Nees sample species collected from the central and highland areas of Vietnam by using 13 SSR markers. The results showed that that 54 different alleles (approximately 4.15 alleles/locus), in which 42 alleles were polymorphism (80.77%), 10 alleles were monomorphism (19.23%). The PIC value was varied from 0.63 to 0.84 (average value: 0.70), respectively. The rate of homogenates among the samples were ranged from 0.63 to 0.98 (average value 0.81). The 50 samples were divided into 5 different groups based on the genetic relationships: group I (10 samples) included the samples were collected in Quang Tri, Quang Nam and Gia Lai provinces; group II (5 samples) collected in Quang Nam and Quang Tri provinces; group III (05 samples) were collected in Thua Thien Hue and Kon Tum provinces; and the group IV (20 samples) were collected in Quang Tri, Gia Lai and Kon Tum provinces (the largest group (40%); the group V (10 samples) collected in Thua Thien Hue, Kon Tum and Gia Lai provinces. The results demonstrated that the rate of homogeneity was quite high in *M. odoratissima* Nees species collected in the areas of central and highland of Vietnam. This study may provide good information for further identification of high-quality genetic germ-

plasm of the medical plant resource for development and conservation in this country.

Keywords: *Machilus odoratissima*, Genetic diversity, SSR marker

Introduction

Machilus odoratissima Nees belongs to a genus in Lauraceae family. There are twelve species found in Vietnam. This species is adapted and widely grown in the wet tropical forests in Vietnam, especially in some provinces in the central and highland areas of Vietnam. *M. odoratissima* species grows rapidly in the high areas (300- 700m from sea level), the optimum temperature is from 23 to 25^{o}C and rainfall is from 2000 to 3000mm [1]. *M. odoratissima* Nees has been reported high medical property and economy value for human life [2]. The bark and leaves of this plant is traditionally used for snake bites, burn wounds and antiseptic, anti-inflammatory remedies [3].

In present, most studies have focused on analyzing bioactive compounds and pharmaceutical value in all parts of this species such as antioxidant, antibacterial [4-5]. Many compounds belonging to tannin, lignans, alkaloids and saponins, etc. involved in medical properties have been isolated and identified [6]. However, only few reports available on the genetic diversity of this species were reported. By sequencing ITS region and *matK* gene, the relationship of the species in Laureae family (complex Litsea, Lauraceae) was reported [7]. The polymorphism and the difference in the population of three species *L. coreana, L. lii, and L. acutivena* in Taiwan used 15 loci SSR was conducted [8]. Another reports showed the first complete chloroplast genome of *Litsea glutinosa* to determine polymorphism, distribution of these species on the islands of Pacific Ocean and Indian Ocean. The complete genome is 152.618bp, includes 127 genes (83 protein-coding genes, 36 tRNA genes and 08 rRNA genes) [9].

In Vietnam, due to overuse in nature and lack of much attention to directive development and reasonable protection, this species is being declined and damaged. There have been some studies on *M. odoratissima*. However, most researches have been focused on collections and selections of the dominant species. Therefore, the objective of this study was to evaluate the genetic diversity of 50 *M odoratissima* species samples collected in the central and highland areas of Vietnam by using SSR markers.

Materials and Methods

Material collection

A total of 50 *M.odoratissima* Nees samples used in this study was collected from the areas of the central and highland areas. Information of size of 13 SSR markers was provided by Bioneer company (Korea) were used as shown in Table 1, Table 2.

Table 1: List of *M.odoratissima* Nees leaf samples used in this study

No.	Name	Location	No.	Name	Location	No.	Name	Location
1	QT2	QT	18	QN19	QN	35	GL36	GL
2	QT3	QT	19	QN20	QN	36	GL37	GL
3	.QT4	QT	20	QN21	QN	37	GL38	GL
4	QT5	QT	21	KOT22	KT	38	GL39	GL
5	QT6	QT	22	KOT23	KT	39	GL40	GL
6	QT7	QT	23	KOT24	KT	40	GL41	GL
7	QT8	QT	24	KOT25	KT	41	GL42	GL
8	QT9	QT	25	KOT26	KT	42	GL43	GL
9	QT10	QT	26	KOT27	KT	43	GL44	GL
10	QT11	QT	27	KOT28	KT	44	GL45	GL
11	TTH12	TTH	28	KOT29	KT	45	GL46	GL
12	TTH13	TTH	29	KOT30	KT	46	GL47	GL
13	TTH14	TTH	30	KOT31	KT	47	GL48	GL
14	TTH15	TTH	31	GL32	GL	48	GL49	GL
15	TTH16	TTH	32	GL33	GL	49	GL50	GL
16	QN17	QN	33	GL34	GL	50	GL51	GL
17	QN18	QN	34	GL35	GL			

TTH: Thua Thien Hue province; QN: Quang Nam; QT: Quang Tri; KT: Kon Tum; GL: Gia Lai

Total DNA extraction

A total DNA of each *M.odoratissima* Nees leaf was extracted follow CTAB protocol with minor modifications [10].

PCR reaction

PCR reactions were conducted in a total volume of 10 µl, using 1.0 µl PCR buffer (10X), 0.4 µl dNTPs (10mM), 0.15 µl Taq DNA polymerase (5U/µl), 0.8 µl of each primer (10µM), 1µl ADN (30ng/µl) and 5.85 µl distilled water, in a thermocycler. PCR cycling conditions started with an initial denaturation at 94°C for 5 min, followed by 35-37 cycles of denaturation at 94°C for 50s, annealing at 55 - 60°C for 01 min and extension at 72°C for 01 min and 10s, and 72°C for 5 min, with a final extension.The PCR products were confirmed by gel electrophoresis in polyacrylamide 6% and visualized with ethidium bromide staining.

Data analysis

The statistics were based on the visualization or non-visualization of DNA fragments (alleles). The data were analyzed by using Exel version 5.0 programs and NTSYSpc 2.1 software.

IPC (Polymorphic Information Content) coefficient:

$$PIC = 1 - \sum_{i=1}^{n} P_i^2$$

(Where n is the number of alleles and Pi is the probability of the allele). Rate of primers which had no DNA fragment (M %):

$$M\% = \frac{Z}{P}$$

Where Z is total primers which had no DNA fragment. P is total primers used in this study.

Results and Discussion

PIC coefficient, allele numbers and total DNA fragments

In this study, we have found the different 54 alleles in total of 50 *M.odoratissima* Nees samples. The number alleles/locus fluctuated from 03 - 07, (4.15 alleles/locus in average). Specifically, three alleles were detected in five primers (NS040, L99, Nese10, NS12 and Nese6); four alleles yielded in four SSR primer pairs (NS001, Nese4, Nese8 and Nese1), and five alleles were in two primer pairs (NS021 and L61, respectively. The NS031 and L35 primers produced six alleles and seven alleles, respectively (Table 2, Figure 1).

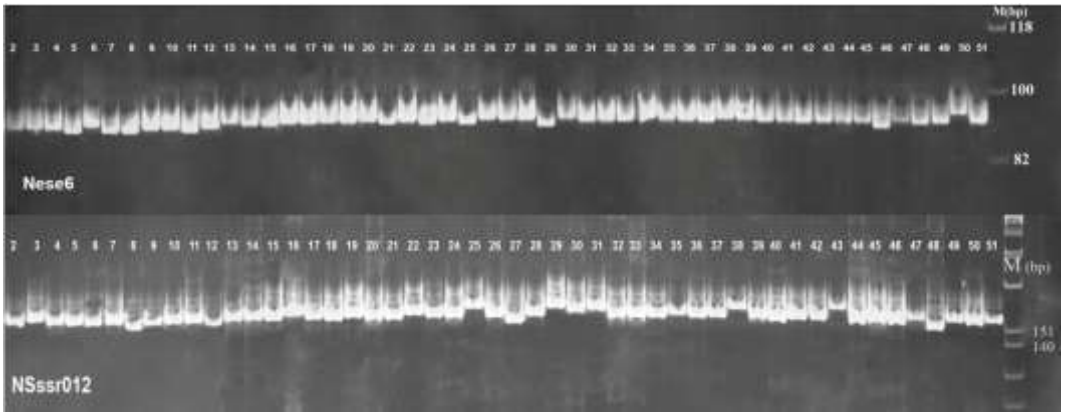

Fig. 1. PCR products of 50 *M.odoratissima* Nees samples used *Nese6* and *NSssr012* primers. Bands 2-51: *M.odoratissima* samples; M: Standard marker

The value of PIC (Polymorphic Information Content) is the rule of the allele polymorphism in each SSR locus (the genetic diversity of the gene) and was frequently used to assess genetic diversity accessions [11]. SSR markers have been widely used as one of the most potential genetic markers for a wide range of applications in population genetics, conservation biology and evolutionary biology [12]. Moreover, they are not only useful in determining heterozygosity and estimating genetic distances among closely related species [13]. The data in Table 2 illustrated that the *M.odoratissima* Nees samples were diverse in alleles at the loci. The PIC coefficient varied from 0.63 to 0.84 (Nese4 primer (04 alleles) and L35 primer (07 alleles), respectively). The average PIC of 13 SSR primer pairs was 0.70. The primers Nese1 and Nese6 (15.38%) appeared 02 rare alleles (the probability of the allele < 5%).

Table 2: PIC coefficient, number of alleles and number of DNA fragments of each primer pair.

Marker name	Forward primer (5'-3')	Reverse primer (5'-3')	Size ranger (bp)	Na	PIC	FIS
NS001	AGGACTGAGCAAGTTG TGGA	GTTTGATGCAAGCCTC CGATGGTA	239-245	4	0.73	0
NS021	AGGCAAGGTTAATTGG TAGGGA	GTTTAGCCTCCAACCTT CTCTCCT	223-264	5	0.71	0
NS031	AAAGCTCCACACAACC CGAA	GTTTACATACGGCAGA GAGAGCAC	231-264	6	0.77	0
NS040	GCACAGCTCTCGGAGA AGTC	GTTTCGCCAACTTCCTG TGAAACC	267-316	3	0.64	0
L35	GATTTGCAGCAAGGTG GGTC	TCTGGATCTTCTCAAG CGTG	68-121	7	0.84	0
L61	CTCCATTTTTATTCCAG GTG	AACCCGTTGCTTCTAA CTGC	120-290	5	0.73	0
L99	CGTACAGTACCCCTTG GACG	AGTGTGTACACAAACA TGCC	112-180	3	0.65	0
Nese1	ACACACACACACAGAG AGAGAG	GTTTAGATGGGTTGGA CTT	185-245	4	0.68	1
Nese4	ACACACACACACAGAG AGAGAG	GTTATTCAGTTCGTTTG GGATG	193-221	4	0.63	0
Nese6	ACACACACACACAGAG AGAGAG	GCCTTGATGAGGGTCT TGATTT	86-122	3	0.66	1
Nese8	ACACACACACACAGAG AGAGAG	GAGGAAAAGAGAAGT CCAAGGT	205-241	4	0.74	0
Nese10	ACACACACACACAGAG AGAGAG	CTGACCCAAAGGTCCA GAATAT	138-144	3	0.64	0
NSssr012	CTTGCTCAGAGGAGGC AGTG	GTTTTGAGGCACAGAA CATGCATTC	169-216	3	0.64	0

*Na: number of alleles per locus; PIC: polymorphic information contents; FIS: fixation index (*P<0.05)*

Genetic relationship of 50 *M.odoratissima* Nees samples

The obtained results showed the homogenetic rate and genetic relationship among the 50 *M.odoratissima* samples (Figure 2). It had a high homogenetic rate (0.78 to 1.00), the average rate was 0.89. The homogenetic level of these samples were 91%, they were distributed 05 different genetic groups:

Group I: included 10 *M.odoratissima* Nees samples: QT2, QT11, QT4, GL42, QT3, QN20, QT7, QT8, QT9 and GL34. The samples of this group had the homogenetic rate fluctuated between 0.93 (QT9 and GL34) and 1.00 (QT2 and QT11), respectively;

Group II: comprised of 05 *M.odoratissima* Nees samples collected in Quang Tri and Quang Nam provinces: QT5, QN18, QN17, QN21 and QN19. The samples of this group had the homogenetic rate fluctuated between 0.96 (QT5 - QN19; QN18 - QN19; QN17 - QN19) and 0.99 (QN17 and QN21);

Group III: contained 05 *M.odoratissima* Nees samples collected in Thua Thien Hue and Kon Tum provinces: TTH12, TTH15, TTH16, TTH14 and KOT26. The samples of this group had the homogenetic rate ranged between 0.94 (TTH12 - TTH14 and TTH14 - KOT26) and 0.98 (TTH15 - TTH16 and TTH16 - KOT26);

Group IV: this group was the largest group within 20 samples (40%): QT6, KOT23, GL47, GL38, GL43, GL51, GL36, GL48, GL39, GL33, GL49, GL46, GL40, GL35, GL45, KOT30, QT10, GL32, GL41 and GL37. The samples of this group had the homogenetic rate varied between 0.90 (GL37 and GL40) and 1,00 (GL47 - GL51; GL47 - GL38; GL47 - GL43; GL47 - GL36);
Group V: clustered 10 *M.odoratissima* Nees samples: TTH13, KOT22, GL50, KOT29, GL44, KOT31, KOT24, KOT25, KOT28 and KOT27. The samples of this group had the homogenetic rate alternated between 0.94 (KOT24 - KOT25; KOT25 - KOT27; KOT27 - KOT28) and 1,00 (KOT25 and KOT28).

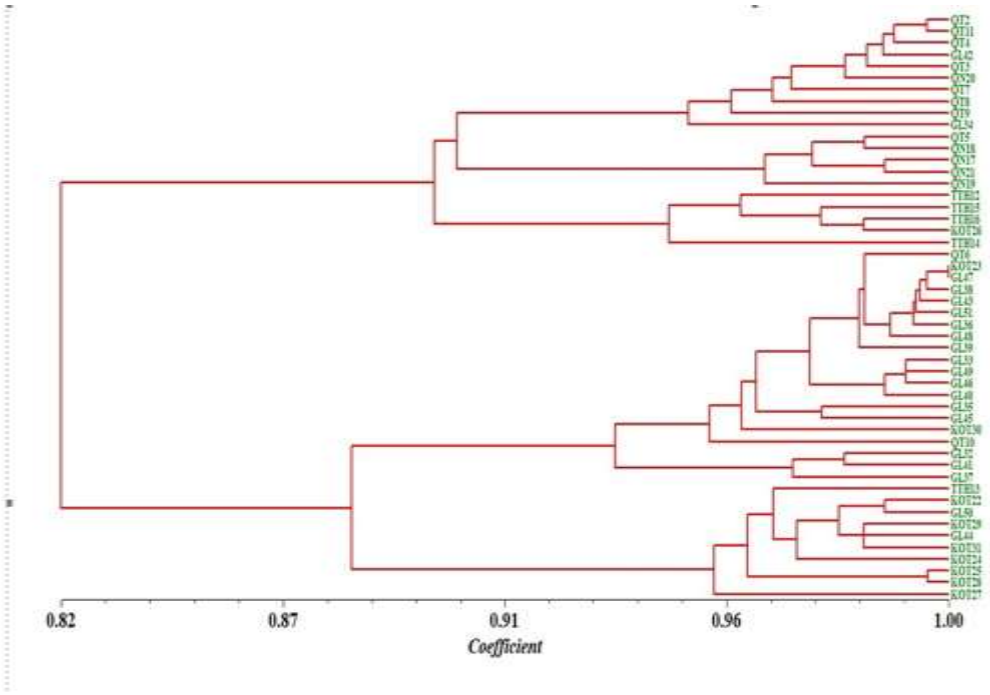

Fig. 2. The genetic relationship of 50 *M. odoratissima* Nees samples

In this study, total of 50 *M.odoratissima* Nees samples collected in several provinces of the central and highland areas of Vietnam had a high homogenetic rate. The *M.odoratissima* Nees samples in this study had not enough regional distribution but they had interference among locations. The number of samples collected in Gia Lai province was the largest (20 samples), distributed to group I, group IV and group V (the most was group IV: 16 samples). The number of samples that were collected in Quang Tri and Kon Tum provinces was equal, 10 samples. The Quang Tri samples clustered in 03 groups: I, II, IV. The Kon Tum samples were concentrated in group V (07 samples). Thua Thien Hue and Quang Nam were 02 provinces had the smallest numbers, 05 samples of each province, distributed to 2 groups (III, V) and (I, II), respectively.

The result of genetic relationship of five *M.odoratissima* Nees groups showed that the samples had concentrated contributively in the same location, such as: group I had 07 samples that were collected in Quang Tri (total 10 samples).

Similarly, group II contained almost samples that collected in Quang Nam (4/5), group III contained almost samples that collected in Thua Thien Hue (4/5), group IV contained almost samples that collected in Gia Lai (16/20), and group V contained almost samples that collected in Kon Tum (7/10). The reason of this contribution may be the samples had the same origin or ecological area. In addition, these samples may be asexual propagation or the seed moved to other locations by the wind.

Our study is the first report on assessing the genetic diversity of *M.odoratissima* species collected in the central and highland areas of Vietnam by using molecular SSR markers. This study may provide useful information to distinguish and select individuals of *M.odoratissima* Nees for further research.

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