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GENETIC DIVERSITY IN THE VIETNAMESE MEDICINAL PLANT *Celastrus hindsii* Benth.

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Abstract

This study reports on an investigation into the molecular biology *Celastrus hindsii*, a traditional medicinal plant. The genetic diversity of the species was investigated to understand distributions, the importance for conservation and prioritisation of efforts. The genetic diversity was determined using Randomly Amplified Polymorphic DNA (RAPD) and Randomly Amplified Microsatellite Polymorphism (RAMP) techniques. DNA-based molecular markers revealed low polymorphisms within the population, however, high genetic differentiation among populations of. *C. hindsii*. The underlying causes of the differences appeared to be the geographic distance among populations of *C. hindsii*. The latter molecular method proved more useful in distinguishing between individuals and populations. Recommendations on future conservation and management for the species are identified. Genetic conservation for *C. hindsiii* is necessary to promote cultivation and system development for possible medicines.

Keywords: *Celastrus hindsii, conservation, genetic diversity, molecular markers, medicinal plant.*

1. Introduction

The significant role of plant diversity in human survival and the environment has been recognised globally, not only because of increasing demand of human beings and food for everyone but also because of the diversity and fitness of flora which has become more and more threatened and extinct in the wild. Effective conservation of those wild plants and germplasms to ensure the provision of nutrients, food security, and traditional medicine, as well as a healthy ecosystem, is crucial to prevent the further depletion and extinction; particularly for rare and endangered plant species. Up to date, there have been many approaches to conserving those important plants adopting different strategies (*in situ* or *ex situ* conservation) from the most problematic concerns in the tropics to temperate areas of the globe [1]. Studies have mostly involved facilitating protected areas, controlling overexploitation and *ex situ* conservation activities, which often require time-consuming and costly inventory and conservation status assessment. However, the inconsistencies of these projects have been reported, and the full genetic diversity

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of a species has not been guaranteed to be conserved. Recently the perception that conserving a species may not merely grow enough number of individuals but more importantly to preserve the genetic diversity of that species. Therefore to formulate appropriate management strategies oriented towards conservation, the species' biological characteristics and their environmental vulnerability must be provided. In addition, information of genetic variation between and within populations needs to be examined and usually now by the assistance of molecular techniques [2].

C. hindsii (Celastraceae) is a scandent shrub widely distributed up to an altitude of 2,500m through most parts, but mainly in forests, thickets and mountainous regions of China, Northern Vietnam, India, Malaysia, and Myanmar. In Vietnam, *C. hindsii* was found to be distributed in different wild populations in Phu Tho, Ha Noi, Hoa Binh and Hai Phong, and have recently been planted in many other places (according to the study investigation). Previous studies on this species revealed a variety of bioactive chemical components including sesquiterpene, triterpenes, alkaloids, and flavonoids [3–5].

Plant material (stem, leaves, and seeds) from *C. hindsii* have been used for generations in Northern Vietnam, particularly by Muong people for treatment of ulcers, tumours, and inflammation, and for manufacturing of tea products [6]. Since the medicinal property and the traditional use were proven scientifically, the wild populations of *C. hindsii* have been overexploited, and plants are hardly found in the wild (according to our surveys). However, their cultivated forms were widely spread in home gardens and nurseries for commercial purposes because they are successful and easily regenerated by either vegetative propagation or sexual reproduction [7-8]. Genetic diversity of *C. hindsii* may be adversely affected due to their decrease numbers in the wild and unsystematic selection for cultivation which chiefly is based on morphological traits but not genetic diversity information. As a result, the maintenance of genetic diversity is one of the major objectives for conserving genetic variation among wild populations and beneficial in developing agricultural practices to circumvent genetic erosion and propose conservation strategies for the longstanding vigour of the species [9].

The genetic diversity assessment at a population level and species level can be performed through a number of techniques such as morphological, biochemical and molecular analysis. However, molecular techniques have been preferred recently because of their speed, low cost and require a small amount of plant material [10]. Out of the molecular techniques available, RAPD-PCR and RAMP-PCR have necessary features and have been widely used to determine the genetic relationships within and between populations for various endangered plants species [9-13] and medicinal plant species [14-18]. In these techniques, short oligonucleotides of arbitrary sequence (in RAPD-PCR), and combination of RAPD and anchored microsatellites primers (in RAMP-PCR) are used to support the amplification of regions of selected plant genomes, and these PCR products are separated by size in

gel electrophoresis. The variation between genotypes is reflected as differences in the banding patterns.

Currently, there are no reports on the genetic variation of *C. hindsii* using any markers, although some studies have used molecular markers on related *Celastrus* species [19]. Therefore, the objectives of this study are to provide information on the genetic diversity of this species, using two different molecular markers for variations within and between populations. The study, therefore, contributes valuable information for conservation of *C. hindsii* – a medicinal plant.

2. Material and methods

Sampling strategy

Plants used in this project were collected and ecological parameters assessed on site in Vietnam; including population size and fragmentation. Healthy growing individual plants within larger populations were selected randomly (for *C. hindsii*). To ensure statistical reliability of the result, representative samples of the genetic variation were collected and named by using sufficient numbers where possible.

C. hindsii has been overexploited in the wild. However, they are widely cultivated in home gardens, nurseries and intensive farming for research and commercial purposes all over the provinces in the north of Vietnam. The reputation of the species has been highlighted in Hoa Binh province where folk medicine practices and commercial cultivations of this species have brought the residents significant economic benefits and an enviable reputation. Normally more leaf tissue was obtained than needed; however, the older counted leaves were omitted due to older plant tissue being generally unsuitable for DNA extraction. A total 16 individuals were used in the study, of which 12 were from cultivations in three provinces of Hoa Binh (HB), Phu Tho (PT), and Ha Noi (HN); and four were from natural forest in Cat Ba National Park in Hai Phong city (HP). Two out of 16 plants were classified as narrow leaf phenotype (both collected in PT) versus broad leaf phenotype of the rest of the material. To the author's best knowledge, the population found in the natural forest and studied in this research was the first time mentioned in the literature.

DNA extraction

A DNA extraction procedure based on CTAB using the modified version of the method described by [20] has proven to be successful over a number of species of plants and is well proven to maximise DNA amounts from woody samples [21]. CTBA is a positively-charged detergent that allows the extraction of DNA from plant material with a minimum of containing polysaccharides. The DNA was in most cases suitable to be used for RAMP-PCR and RAPD-PCR [22].

Before the DNA extraction process, all solutions (except heat labile or flammable compounds) and equipment used were sterilised by autoclaving (121^0C, 104kPa for 20 min) or by washing (the mortar and pestle only) in 10% sodium dodecyl sulfate (SDS). Fresh CTAB buffer including

2% (w/v) CTAB, 20mM EDTA, 100mM Tris-HCl, pH 8.0, 1.4M NaCl, 1% (v/v) 2-mercaptoethanol (2-ME) and 1% (w/v) polyvinylpyrrolidone PV-40 (PVP) and was prepared on the day of each extraction. The sequential DNA extraction protocol for the study species was implemented in the following steps:

DNA qualification

The quantity and quality of extracted DNA was estimated using a Pharmacia GeneQuant DNA/RNA calculator. Absorbance readings were taken at wavelengths of 230, 260 and 280nm. The purity of the DNA was then estimated by the ratio of readings taken at 260:280nm and 260:230nm (pure DNA having a 260:280 ratio of 1.8 - 2.0). The calculator automatically estimated DNA yield by multiplying the absorption at 260nm by 50 µg/ml (an OD_{260} of 1 corresponds to ~50 µg/ml of double-stranded DNA).

Polymerase Chain Reaction

DNA polymorphisms in the genome of the selected plant species (*C. hindsii*) were assessed using the Polymerase Chain Reaction (PCR), and a variety of RAPD primers and microsatellite primers. Each PCR was performed in a final volume of 25µl containing 1 x Fisher Biotech Reaction Buffer (67mM Tris-HCl - pH 8.8, 16.6mM $[NH_4]_2SO_4$, 0.45% Triton X-100, 0.2mg/ml gelatin), 1M betaine, 2mM Fisher Biotech mixed dNTPs, 0.1µg/ml RNase A, and varying amounts of primer/s, $MgCl_2$, Fisher Biotech Taq DNA Polymerase, genomic DNA, and sterile double distilled water. PCR reactions were carried out using a Biometra Personal Cycler.

PCR preparation and Gel electrophoresis

To prevent contamination of the PCR reaction mixture with exogenous DNA, and to prevent cross-contamination of template DNA, the precautions were taken as recommended by [23]. Test reactions were performed in duplicate to ensure the reproducibility of the results.

The above PCR products were analysed by electrophoresis on agarose gels. Agarose gels were stained by GelRed™ by diluting the GelRed™ 10,000X stock reagent into the agarose TE buffer solution at 1: 10,000 (e.g, 5 µl of the GelRed™ 10,000X stock reagent added to 50ml of the gel solution). Images were printed in black and white using a Cannon-iR_{4251} laser printer.

Band scoring and Statistic analysis

The presence and absence of bands were determined by examining each gel photograph. Observed bands were marked in ink on one of two photographs taken of each gel to obtain a permanent record for later evaluation. Only those bands that were unambiguous were scored. The observed amplicons were scored '1' for the presence and '0' for the absence of bands, and the binary data used for statistical analysis. The band sizes were determined by comparison with a 100 bp DNA molecular weight ladder (Promega), and faint bands of doubtful reproducibility were ignored.

The binary data was analyzed with PopGen Version 1.31 [24], a Microsoft Windows-based freeware program for

population genetic analysis and PRIMER Version 5 [25] to determine genetic parameters such as Nei's genetic diversity (H), Shannon's diversity index (I), diversity within population (Hs), total gene diversity among populations (Ht), gene flow (Nm), coefficient of gene differentiation (G_{ST}), and genetic distance Jost (D). The G_{ST} estimates genetic differentiation and reduction in the number of heterozygote loci based on Nei's regular and unbiased genetic measures [24]. The Mantel test statistic (r) was used to determine the correlation between geographic and genetic distances using the program IBD Isolation by Distance Version 1.52 [26]. Principal components analysis (PCA) can be used as a simple visualisation tool to summarise dataset variance and show the dominant gradients in low-dimensional space. Multi-dimensional scaling (MDS) was used to understand patterns of variation within and amongst populations by converting a set of variables into a few dimensions so that individual variations are condensed into a set of limited axes. Cluster procedure was an average linking one, and all similarities used were Bray-Curtis to produce dendrograms [25].

ANOSIM and ANOVA are statistical tests of significance ANOSIM tests for the significant difference between two or more classes of objects based on any (dis)similarity measure [25]. It compares the ranks of distances between objects of different classes with ranks of object distances within classes. The basis of this approach is similar to the MDS ordination technique described above. As ANOSIM is based on ranks, it has fewer assumptions compared to regression techniques such as analysis of variance (ANOVA).

3. Results

Intra-population genetic diversity

Fifteen individuals of *C. hindsii* were sampled in four populations, and an average of four individuals per population, except HP which consisted of only three individuals. The criteria for selecting populations and individuals were based on the geographic distance between individuals and populations to estimate the genetic flow between populations. Moreover, vegetation situations and terrains condition were also included in to ensure that differentiation may lead to indications of genetic diversity of each population. Therefore, all individuals collected from the population in Hai Phong city (HP) were from natural forests of Cat Ba Island which is the first time mentioned in both local and international literature. The other two populations are close to their originally distributed limestone mountain habitats with Hoa Binh population (HB) collected in farms near Cuc Phuong National park, and Ha Noi population (HN) collected in the buffer zone of Ba Vi National Park and home garden. *C. hindsii* population in Phu Tho province (PT) was collected from home gardens which all specimens were previously unrooted and migrated from other unknown places.

Table 1. DNA polymorphism between four populations of *C. hindsii* detected by RAPD-PCR. Six RAPD primers were used. (H) is Nei's (1973) gene diversity. (I) is Shannon's Information index [27]

Parameter	PT	HN	HB	HP
Number of individuals	4	4	4	4
H	0.20	0.14	0.15	0.18
I	0.29	0.22	0.22	0.27
Number of polymorphic bands	63	46	45	54
Percentage of polymorphism	52.50	38.33	37.50	45.00

C. hindsii was investigated with six RAPD primers (OPA-2, OPA-7, OPC-2, OPD-2, OPD-7, and OPD-10). All primers tested in this study produces various patterns of bands within populations with more bands produced several primers or populations, while fewer bands generated by the others (Figure 1). The data from Table 1 shows that high polymorphisms (about 53%) were detected within the population PT whereas much lower polymorphic bands as well as (H) and (I) were calculated in population HB. The isolated geographical condition of Cat Ba Island probably did not cause low gene flow and diversity of *C. hindsii* population in HP. This result not only reflected the furthest distance from this population to the rest but also may indicate that the primers used in this experiment are not necessarily useful primers to differentiate among between populations.

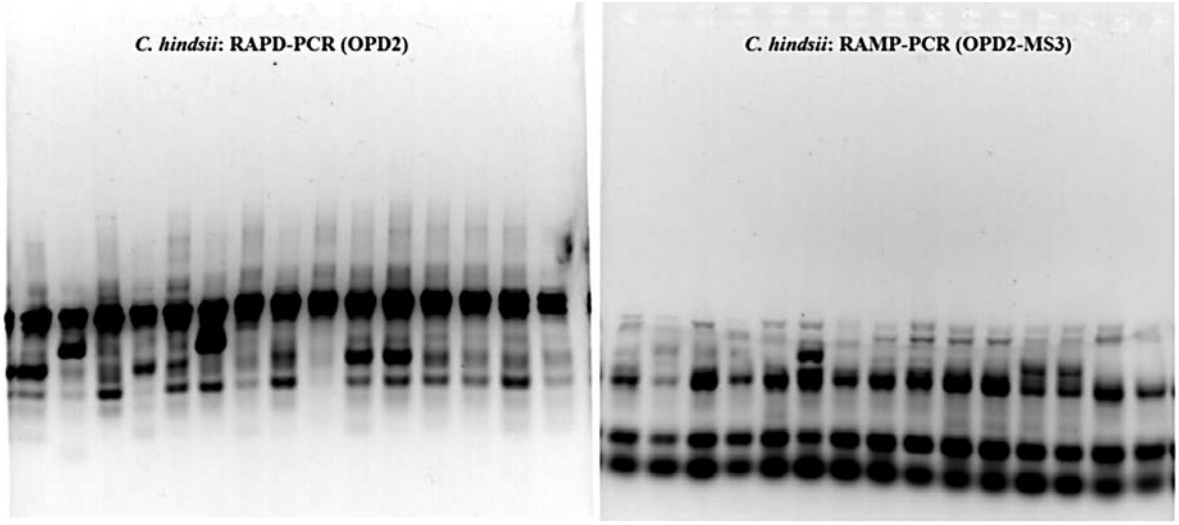

Figure 1. Example of a RAPD-PCR and RAMP=PCR profile among *C. hindsii* individuals

While OPD-2 and its combination with MS_3 produced a roughly equal number of bands per fingerprint for this species (11 and 12 bands, respectively), the other three RAMP primer combinations produced fewer bands than the respective RAPD primers alone

(32 bands over 16 bands between OPA-7 and OPA-7 with either MS_1 or MS_2; 17 bands over 11 band between OPC-2 and OPC-2 with MS_3). It probably due to the RAMP primers, which are not useful to detect the polymorphism difference between populations of *C. hindsii*. Therefore, the number of polymorphic bands of all population was much lower than that produced by respective RAPD primers, from the lowest of 19 bands (in HP) to the highest of 30 bands (in PT). Furthermore, there was the difference between the number of bands produced by MS_1 and MS_2 as they combined with RAPD primer OPA-7.

From the data produced by RAMP primers as can be seen in Table 2 that PT was dominant from the rest with the highest percentage of polymorphism (43.48%) as well as H and I indexes (0.15 and 0.22, respectively), and HP remained the second highest diversity indices as produced by RAPD primers. On the other hand, HN and HB showed almost equal results of all genetic diversity indices.

Table 2. DNA polymorphism between four populations of *C. hindsii* detected by RAMP-PCR. Four combinations of two RAPD primers (OPA-7, OPC-2 and OPD-2) and three microsatellite primers (MS_1, MS_2, MS_3) were used. (H) is Nei's (1973) gene diversity. (I) is Shannon's Information index

Parameter	PT	HN	HB	HP
Number of individuals	4	4	4	4
H	0.15	0.11	0.11	0.12
I	0.22	0.16	0.17	0.18
Number of polymorphic bands	30	20	20	21
Percentage of polymorphism	43.48	28.99	28.99	30.54

Inter – population genetic diversity

Genetic diversity

Genetic diversity parameter such as percentage polymorphism (P%), Nei's gene diversity (H), Shannon's diversity index (I), diversity within a population (Hs), and total gene diversity among populations (Ht) was shown in Table 3. These five parameters (P%, H, I, Hs, Ht) predicted by RAPD data were found to be higher (81.67%, 0.27, 0.41, 0.27, 0.18) than RAMP data (66.67%, 0.19, 0.29, 0.19, 0.12), respectively, at species level. The P%, H, and I indices were found lower at the population level, and represent low genetic diversity at the species level but high population differentiation. Population PT of *C. hindsii* showed the highest average level of genetic diversity indices (47.99% polymorphisms; I = 0.26; H = 0.18), while the lowest was recorded in HB (33.25% polymorphisms; I = 0.18; H = 0.13).

Table 3. DNA polymorphism and population statistics and Mantel test

Parameter	*C. hindsii*	
	RAPD	**RAMP**
Number of individuals	16	16
Number of population	4	4
Number of polymorphic bands	98	46
Percentage of polymorphism (%)	81.7	66.7
Nei genetic diversity (H)	0.28	0.20
Shannon information index (I)	0.42	0.31
Total gene diversity among populations (H_T)	0.27	0.19
Diversity within population (H_S)	0.18	0.12
Estimation of gene flow (N_m)	0.95	0.89
Genetic differentiation (G_{ST})	0.32	0.36
Jost genetic differentiation (D)	0.15	0.10
Mantel r test (probability)	0.55 (P=0.03)	0.51 (P=0.05)

Genetic differentiation and structure

There was significant differentiation among the populations of *C. hindsii*. The coefficient of genetic differentiation between populations (GST) which was estimated by partitioning of the total gene diversity based on RAPD data and RAMP data was 0.32 and 0.36 in *C. hindsii*, respectively. Also, the moderate level (not low) of gene flow between populations (Nm) and Jost genetic differentiation (D) were estimated between populations by RAPD data and RAMP data, indicated that significant genetic differences were present in individuals. In contrast, both RAPD and RAMP data predicted a significant correlation between genetic variation and geographic distance between four populations of *C. hindsii*.

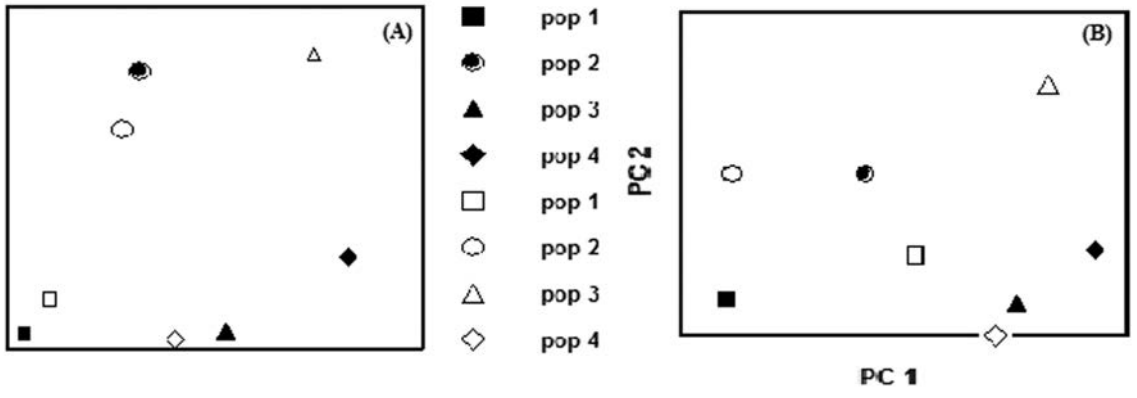

Figure 2. Multi-dimensional scaling (MDS) – (Figure A) and Principle component analysis (PCA) - (Figure B) of *C. hindsii* using RAPD-PCR data (closed symbols) and RAMP-PCR data (open symbols). Where Pop1 = PT, pop2 = HN, pop 3 = HB, and pop4 = HP.

In *C. hindsii*, MDS and PCA were carried out for further population differentiation of this species and showed significant differentiation between populations. It can be seen in Figure 2 that MDS and PCA are consistent amongst RAPD and RAMP data, and clearly show the further genetic distance between population 1,2 and 3,4.

Cluster analysis (dendrograms Figure 3) tended to support the structure and genetic diversity between the populations and showed a strong consistency between RAPD and RAMP data. The first group includes HP, and the second group includes HN and HB in one subgroup, whereas PT is separated into another subgroup. HN, HB, and PT are three closer populations, about 30 km (between HN and PT or HN and HB) to 60 km (between HB and PT), while HP is situated in Cat Ba island which is isolated with three other populations (from 150 km to 200 km). These geographic distances are relatively consistent with their relationship in the dendrogram.

The dendrogram based on Nei (1972) genetic distance produced by RAPD and RAMP data showed a similar grouping, indicating the similar effectiveness of the two markers in differentiating populations of this species. It is consistent with geographic distance between populations the distance HP to other populations (from 150km to 200km) and close relationship between HN and HB.

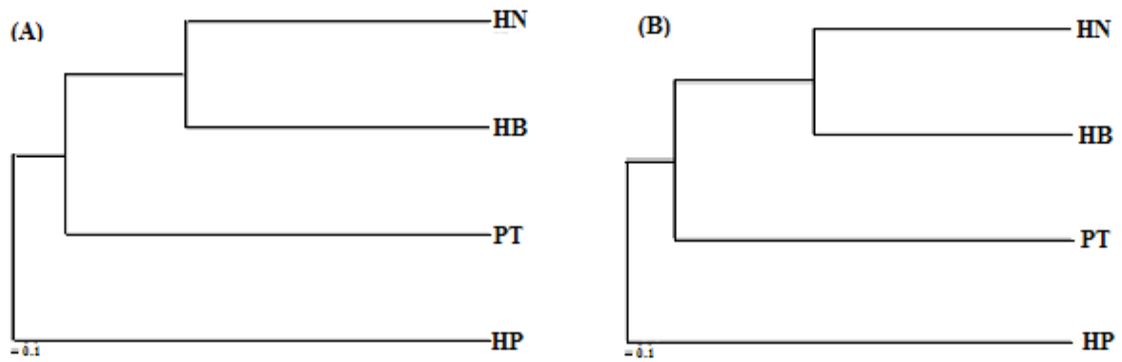

Figure 3. Dendrograms (cluster analysis using Nei genetic diversity) in RAPD-PCR (A) and RAMP-PCR (B) of the medicinal plant *C. hindsii*. The result was analysed by Popgen and Treeview program.

4. Discussion

Intra-population genetic diversity

Genetic diversity

C. hindsii has a much larger range of distribution with high geographic distance and genetic distance between populations. PT of *C. hindsii* showed the highest averaged level of genetic diversity indices of RAPD and RAMP data, while the lowest was recorded in HB. PT includes multiple collections from diverse unknown populations which are probably either the wild or cultivated ones. Moreover, two out of four sampled individuals from PT were morphologically distinguished from others as their small leaf characteristics.

Whereas, population HB showed the lowest genetic diversity as it was situated on the buffer zone of Ba Vi National Park which might indicate the genetic migration from vegetative introduction between home gardens within a restricted area.

Low genetic diversity within populations

The results of the present study using RAPD and RAMP markers revealed a low level of genetic diversity within the population and remarkable genetic differentiation among populations of *C. hindsii*. According to Hamrick & Godt [29], genetic diversity among populations in a species is influenced by several evolutionary factors such as mating system, gene flow and seed dispersal, geographic range as well as natural selection. One of the most influenced factors contributing to the levels of genetic diversity within a population is likely the geographic range.

C. hindsii which is rarely distributed in the wild, but widely cultivated in home gardens and nurseries for commercial and research purposes, has a broad range of distribution. The only wild population was found in the isolated Cat Ba National Park situated in Cat Ba Island, Hai Phong City (HP), about from 150 km to 200 km away from the other three populations investigated in this study. From the best of our knowledge, the occurrence of this species in HP has not ever been reported in the literature. Based on RAPD and RAMP data, genetic polymorphisms of this species also existed more between populations than that within populations. Genetic diversity levels represented considerably different between at species level and at population level. The lower genetic diversity within the population of *C. hindsii*, particularly estimated by RAMP data was probably due to inbreeding of isolated populations or the effect of vegetative propagation methods. Also, *C. hindsii* is an easily regenerated species by cuttings or clonal propagation which produces multiple individuals with high genetic similarity in the absence of genetic variation events [30]. Cultivated populations of PT, HB, and HN are built mostly from home gardens, organised nurseries farms for biomass trades. All these reasons are likely to make the genetic diversity low within four population of *C. hindsii*. The result is in contrast to Celastrus paniculatus species in the Celastraceae family, which revealed high genetic diversity within a population due to a high level of outcrossing rates contributed by cross-pollination [19]. However, this low level of genetic diversity is in line with Ismail *et al.* [9] who studied on the medicinal plant of Clinacanthus nutans and reported that plant populations cultivated in the farm that practised intensive harvesting practices preventing the production of flowers which is essential for cross-pollination.

Inter-population genetic diversity

High population differentiation

Genetic diversity of plant species diverges noticeably by the mean of genetic variation distribution between populations. The present study determined that distribution through estimating Nei's genetic diversity and Shannon's diversity index which predicted the equivalent results about the genetic structure of the species. The coefficient of gene differentiation (GST) among populations

demonstrated the species tested contained high gene differentiation as these values ranged from 0.32 to 0.36 for *C. hindsii* (higher GST was obtained from RAMP-PCR data). The high GST indicated that high degree of genetic variability was distributed among populations of these two species. The high population differentiation of *C. hindsii* is due to a recently rapid reduction of population size with insufficient time to isolate and spread gene flow (Nm) [31]. Moreover, geographic distance is also a major factor that contributes to high genetic diversity at the species level. *C. hindsii* has been widely distributed in Vietnam, occurring in wild and cultivated habitat with diverse climatic, geographical and edaphic conditions [8,32]. These differences have an impact on genetic diversity of a species. Genetic drift over thousands of generations would lead to significant divergence among populations. The results from RAPD and RAMP analyses indicate that genetic drift might have occurred among the studied populations of *C. hindsii*, additional to increasing vegetative propagation activities, thereby producing differentiation. Furthermore, *C. hindsii* is a high cross-pollinated plant species, an introduction of a different variant, especially with the occurrence of genomic mutations, certainly, increase population differentiation. Pollen flow and local selection may also facilitate this trend. The higher genetic diversity of *C. hindsii* populations confirmed by the UPGMA dendrogram, (which were consistent with RAPD and RAMP data) where four populations were classified into three different groups and sub-groups, and well-separated to each by MDS and PCA analyses.

High population differentiation was not only consistently predicted by Nei's genetic diversity and Shannon's diversity index but also the coefficient of gene differentiation and Jost genetic differentiation (D). The GST value is 0.32 and 0.36 (by RAPD and RAMP, respectively), indicating a high level of gene differentiation, while the average Nm value of 0.95 by RAPD and 0.89 by RAMP were only from low to moderate levels. These results are similar to a study on a medicinal plant species Justicia adhatoda with GST = 0.30 and Nm = 1.28 (by RAPD) also indicating moderate Nm and high level of GST values [33]. High GST values mean high variations within populations, whereas moderate Nm values estimate that one species in every generation can link the gene pools among populations. In summary, results from the molecular data showed that a considerable amount of genetic variation between populations was present at the fragmented sites sampled.

Populations relationship

Geographic distance between populations of *C. hindsii* were reflected how they were grouped in dendrogram as predicted my Mantel Test ($r = 0.55$, $P = 0.03$ by RAPD data, and $r = 0.51$, $P = 0.05$ by RAMP data). This population differentiation is not only due to geographic distribution but also induced by inbreeding occurrence and the expanding of vegetative production via cutting and clonal propagation in most of the tested populations.

5. Conclusion

In this study, DNA-based molecular markers revealed low polymorphisms within the population, however, high

genetic differentiation among populations of. *C. hindsii*. The underlying causes of the differences appeared to be the geographic distance among populations of *C. hindsii*. The study results serve as guidance for the delineation of *in situ* and *ex situ* conservation approaches, in which all four population of the species should be selected for preservation. Natural populations of the species should be paid more attention in conservation program as they contain high genetic diversity, compared to other cultivated populations. The generated baseline data are valuable for follow-up research (e.g., re-assessment of genetic diversity of rehabilitation programs) and for future decision-making processes associated with management and conservation of genetic resources.

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ĐA DẠNG DI TRUYỀN CỦA CÂY DƯỢC LIỆU XẠ ĐEN *Celastrus hindsii* Benth. TẠI VIỆT NAM

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Tóm tắt

Bài báo nghiên cứu đặc điểm sinh học phân tử *Celastrus hindsii*, một cây thuốc truyền thống của Việt Nam. Sự đa dạng di truyền của loài đã được nghiên cứu để phục vụ và xác định các ưu tiên trong công tác bảo tồn. Đa dạng di truyền của loài được xác định bằng cách sử dụng các kỹ thuật phân tích DNA thông qua chỉ thị phân tử RAPD-PCR và RAMP-PCR. Các chỉ thị phân tử dựa trên DNA cho thấy tính đa hình thấp trong quần thể, tuy nhiên, có sự khác biệt di truyền cao giữa các quần thể của *C. hindsii*. Nguyên nhân cơ bản của sự khác biệt được xác định có thể là do khoảng cách địa lý giữa các quần thể. Phương pháp chỉ thị phân tử RAMP-PCR phân biệt giữa các cá thể và quần thể tốt hơn. Kết quả nghiên cứu khuyến nghị công tác bảo tồn di truyền cho *C. hindsiii* là cần thiết để tạo cơ sở di truyền phục vụ phát triển nguồn dược liệu chất lượng cao cho thị trường.

Từ khóa: *Bảo tồn, cây dược liệu, chỉ thị phân tử, đa dạng di truyền, Xạ đen.*