https://doi.org/10.48047/AFJBS.6.14.2024.8064-8076

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Genetic Diversity and Phylogenetic Analyses of Ochna Species in Thua Thien Hue, Vietnam Revealed Nuclear Ribosomal DNA and Chloroplast *TrnL*-F Region

Dang Van Dong[1], Ngo Van Ky[1], Nguyen Van Tinh[1], Nguyen Van Tien[1], Bui Thi Hong[1], Mai Thi Ngoan[1], Nguyen Thanh Nhung[2], Tran Dang Khanh[3*],

[1] Fruit and Vegetable Research Institute, Trau Quy, Gia Lam, Hanoi, Vietnam
[2] Agricultural Genetics Institute, Pham Van Dong, Co Nhue, Hanoi, Vietnam
[3] Vietnam National University of Agriculture, Trau Quy, Gia Lam, Hanoi
* Corresponding author: khanhkonkuk@gmail.com

Volume 6, Issue 14, Aug 2024

Received: 15 June 2024

Accepted: 25 July 2024

Published: 15 Aug 2024

doi: 10.48047/AFJBS.6.14.2024.8064-8076

Abstract

Hue Yellow *Ochna*, also known as Hoang Mai Hue, is a precious ornamental plant in Vietnam. Its distinctive beauty has been celebrated in a long history, poetry, and in numerous paintings. However, there have been few studies on the population structure or phylogenetic relationships of Hue *Ochna*. In this study, we conducted genetic diversity and phylogenetic analysis using the *nrITS* DNA and *trnL-F* cpDNA sequences of Hue *Ochna*. The plant samples were collected from various areas of Thua Thien Hue province. We used *ITS1* and *ITS4* primers for the *ITS* region and *trnL* and *trnF* primers for the *trnL*-F region. The obtained DNA sequences were edited using BioEdit programs. The MEGA 11.0 program was used for phylogenetic analysis and to detect genetic distances. For the *trnL-F* region, 20 Hue *Ochna* samples had the same sequence and similarity to *Ochna integerrima* at the level of 99.75%. For the *ITS* region, the mean nucleotide composition was determined as 19.04% A, 34.6% C, 33.28% G, and 13.08% T. It was also found that divergence values differed between 0.000 and 0.014. The phylogenetic tree consisted of two large branches. According to the data obtained, phylogenetic trees based on the *ITS* region were found to be more useful than phylogenetic trees based on the *trnL-F* region. Estimates of genetic distance showed that the Hue *Ochna* samples were genetically homogeneous and were not correlated with geographic distance according to a Mantel test (r = 0.1842). This study is the first report on constructing a molecular database of Hue *Ochna* in Vietnam. Our findings have provided useful information for further identifying the genetic plant sources for propagation, development, and conservation of the high-valued economic materials of Hue *Ochna*.

Keywords: Hue Ochna, *ITS*, *TrnL-F*, genetic diversity, phylogeny

Introduction

The family Ochnaceae DC. comprises 36 genera and 638 species with a distribution primarily in tropical regions. Within Ochnaceae, the genus *Ochna*, the second-largest in Ochnaceae, consists of 79 species found in tropical regions of Africa, Madagascar, Mascarene Islands, and six native species in Asia (Shah et al., 2022). Prior investigations have examined

various aspects of Ochnaceae species, including their medicinal components, morphologies, biogeography, and divergence time (Makhafola et al., 2012; Schneider et al., 2022). In conducting phylogenetic analysis, various data sources, such as nuclear and chloroplast genomes, were employed. Notably, recent studies proposed novel infrageneric classifications and insights into the phylogenetic relationships within Ochnaceae, using nuclear genes and exon data. To date, these studies provide the most comprehensive classification of Ochnaceae (Nhat et al., 2023; Schneider et al., 2021; Trang et al., 2018). In Vietnam, *Ochna* flowers are regarded as a symbol of spring, vitality, and prosperity. *Ochna* species are frequently found in cultivated environments, used as individual plants in gardens or making hedgerows in residential and urban locales. In Thua Thien Hue province, a yellow apricot blossom variety with native genetic traits is called Hue *Ochna* or the "Hoang Mai Hue". Currently, there are many different varieties of *Ochna* flowers imported and bred with their own distinctive beauty, characteristic of many regions. However, research on Hue yellow *Ochna* has been scarce, and there has not been any in-depth research, and the development of *Ochna* varieties is spontaneous. Therefore, this precious flower has not been thoroughly studied on ecological characteristics, gene conservation, and solutions to develop yellow *Ochna* trees into a unique and distinctive product of the ancient capital. Therefore, identifying specifically the varieties of yellow *Ochna* with five petals existing in Thua Thien Hue is an urgent requirement, serving for conservation and exploiting valuable characteristics of this *Ochna* variety.

The Internal Transcribed Spacer (*ITS*) region, located between nuclear small rDNA and nuclear large rDNA, is of paramount importance for phylogenetic relationship analysis inference at both generic and intrageneric levels in plants (Álvarez and Wendel, 2003). ITS-based phylogenetic analysis is widely used in plant molecular phylogenetic methodologies (Álvarez and Wendel, 2003). The *ITS* regions are particularly suitable for investigating closely related taxa because of the high genetic diversity resulting from the rapid evolution of these regions. Consisting of two variable non-coding regions (*ITS1* and *ITS2*), the *ITS* is located between the highly conserved small subunit 18S, the 5.8S, and the large subunit 28S of the rDNA gene cluster. The ITS is a molecular marker for fungal identification, with distinctive features: (i) The complete *ITS* region, spanning 600 to 800 bp, is easily amplified using universal primers matching rDNA sequences. (ii) The multicopy nature of the repeat regions of the rDNA enables *ITS* region amplification from small, diluted, or degraded DNA samples. (iii) Numerous studies have confirmed the high variability of the *ITS* regions among morphologically distinct fungal species (Nilsson et al., 2008). While chloroplast DNA (*cpDNA*) sequence variation is commonly used for exploring interspecific relationships among angiosperms and other plants, the low evolutionary rate of these molecules limits their utility at the interspecific level (Sevindik et al., 2016).

To date, research on Hue *Ochna* is limited. The plantation of Hue *Ochna* is mainly spontaneous. The significance of Hue *Ochna* genetic resources has not been fully explored. Many hundreds of years old trees are at risk of being sold outside the province or dying due to inappropriate care, thereby leading to the risk of depletion of genetic resources. In this study, a

molecular phylogenetic analysis using *nrITS* DNA and *trnL-F* cpDNA sequences was performed to elucidate the phylogenetic relationships of Hue *Ochna* samples.

Materials and Methods

Plant materials

Twenty samples of yellow *Ochna* from different locations in Thua Thien Hue province were collected and marked from M1 to M20. The morphological and taxonomical features of the plant flowers, leaves, and stems were used to identify and classify the plants. The leaves samples were labeled and stored in a freezer until processed. The list and sample code are shown in Table 1.

Table 1. List of the samples used in this study

No.	Code	Collection site	No.	Code	Collection site
1	M1	Hue	11	M11	Phu Vang
2	M2	Hue	12	M12	Phu Vang
3	M3	Hue	13	M13	Phong Dien
4	M4	Huong Thuy	14	M14	Phong Dien
5	M5	Huong Thuy	15	M15	Phong Dien
6	M6	Huong Thuy	16	M16	Phu Loc
7	M7	Huong Tra	17	M17	Phu Loc
8	M8	Huong Tra	18	M18	Phu Loc
9	M9	Huong Tra	19	M19	Quang Dien
10	M10	Phu Vang	20	M20	Quang Dien

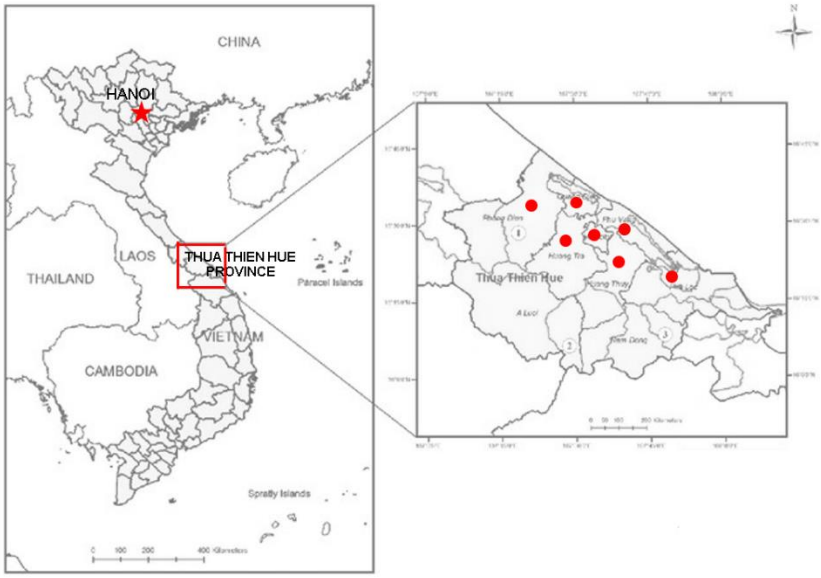

Figure 1. The sample collection map, the red dots indicate the locations of the samples from seven different communes in Thua Thien Hue province

Extraction of genomic DNA

Genomic DNA was isolated from plant tissues using the CTAB extraction method with some minor modifications. (Huong et al., 2024; Huong et al., 2022). In brief, the leaf tissues (200 mg) were ground with CTAB buffer (2% CTAB, 100 mM Tris–HCl pH 8.0, 20 mM EDTA, and 1.4 M NaCl) at 60 °C and transferred to 1.5 ml Eppendorf tubes. The tubes were vortexed and then incubated at 60 °C for 30 min, followed by the addition of cold chloroform: isoamyl alcohol (24:1), mixing, and centrifugation at 13,000 rpm for 15 min. The supernatant was mixed with cold isopropanol in a new tube and incubated at -20 °C for 30 min to precipitate the DNA. The tubes were centrifuged at 13,000 rpm for 15 min and the DNA pellet was washed twice with 70% ethanol and air-dried. The DNA was resuspended in 50 µl of TE buffer (10 mM Tris–HCl pH 8.0 and 1 mM EDTA).

PCR amplification

Specific primer pairs are used to replicated targeted regions; For *ITS1-5.8S-ITS2* region *ITS1* (F) 5′ TCCGTAGGTGAACCTGCGG 3′, *ITS4* (R) 5′ TCCTCCGCTTATTGATATGC 3′, for *trn*L-F IGS region replicate *trnL* (F) 5′ GGT TCA AGT CCC TCT ATC CC 3′, *trnF* (R) 5′ ATT TGA ACT GGT GAC ACG AG 3′ primer pairs were used (Vijayan et al., 2010). The amplification process was performed in a 20 µL PCR reaction. Each PCR reaction contained 1 µL of forward and 1 µL of reverse primers, 10.0 µL of Master mix (MyTaq HS Mix, 2x, Bioline), 1.0 µL of genomic DNA template (approximately 50 ng), and water up to 20 µL. The PCR amplification was performed in a Mastercycler S. The thermocycling conditions for PCR were an initial denaturation step at 95 °C for 3 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 58 °C for 30 s, and extension at 72.0 °C for 60 s. The final extension was performed at 72.0 °C for 10 min. Subsequently, the PCR products were analyzed by electrophoresis on a 1% agarose gel containing ethidium bromide and visualized. Both the *ITS1/ITS4* and trnL/trnF primer pairs were used to amplify and sequence the DNA, and the amplification and sequencing were carried out at Apical Scientific (Malaysia). For each sample, both forward and reverse sequencing reactions were performed, and the resulting sequences were compared with GenBank (NCBI) using BLASTn. Subsequently, the resulting DNA sequences were manually edited and processed with BioEdit (Hall, 1999).

Analysis of sequencing data

BLASTN at the NCBI site compared the sequences obtained from the samples with sequences in the GenBank database. The BLAST tool assessed the similarity and coverage of the DNA sequences. Gene sequences were aligned using ClustalW, and the regions with gaps were cut using BioEdit. For the genetic analyses using the ITS gene region sequences, MEGA 11 constructed the phylogenetic tree using the neighbor-joining method with a bootstrap value of 1000 to evaluate the robustness of the inferred tree (Tamura & Kumar, 2021). Sequences of *O. mossambicensis* (KF263218.1) and *O. natalia* (KF263189.1) taxa used as reference species in the tree were retrieved from NCBI. A Mantel test was performed to estimate the correlation between genetic distance and geographical distances using TFPGA (Miller, 1997; Wang et al., 2016).

Population structure analysis

The structure software is used to identify the grouping of individuals with genetic similarity

into groups (subpopulations). The algorithm of this software is based on the simultaneous analysis of the genotype of multiple loci. The algorithm starts with the assumption that the observed groups are randomly drawn from a parameter model. The model assumes that individuals can be assigned to a group based on the genotype basis of multiple loci or the allele frequency estimated for each group, the number of genetic clusters (K) is determined by using the prior probability lnPr(X|K), and the groups of individuals are inferred through the iterative process at each K value (Pham et al., 2008).

Results

Morphological description of the samples

Hue *Ochna* has green buds, thick branches, flowers with short stalks; 5 dark yellow petals with wavy borders and flat surfaces, closely aligned wings; and a mild fragrance. These are the sensory characteristics of Hue *Ochna* that are used to distinguish it from other types of apricots. Hue *Ochna* leaves do not have red leaves like apricots in the Central Central region or many petals like apricots in the South, but only 5 petals and green leaves. The petals are thick and bright yellow, carrying a light and pure fragrance. The purity and serenity of Hue *Ochna* are very distinct. Hue *Ochna* leaves are elongated and less rounded than other apricot varieties.

Figure 2. Morphological characteristics of Hue *Ochna*
Left side: Flower morphology; Right side: hundred-year-old Hue Ochna tree in the Imperial city of Hue

Identification of Hue Ochna samples

The comparison of the sequences of the Hue *Ochna* samples showed that polymorphisms were found after amplification of the *ITS* sequences. In contrast, the Trn sequences were highly conserved, so no polymorphisms were recorded in this sequence when comparing 20 samples. The sequencing results of the *Trn* region showed that 100% of the Hue *Ochna* samples had the same sequence. The annotated sequences of the *ITS* spacer region ranged in length from 556 to 628 bp. The average nucleotide composition of the *ITS* region was 19.04% A, 34.6% C, 33.28% G, and

13.08% T. The number of nucleotides in each sequence fragment varied among the samples, possibly due to insertions or deletions (InDels) at some gene positions (Ha et al., 2020). Twenty-five sites with nucleotide variation were detected, comparing the nucleotides in the *ITS* region between 20 Hue *Ochna* samples and two reference samples in GenBank, and 13 sites with nucleotide variations were identified, comparing the nucleotide in the *ITS* region between 20 Hue *Ochna* samples, primarily involving substitution or insertion of 1-3 nucleotides (Table 2). The polymorphisms of the 20 Hue *Ochna* samples were determined by using the *ITS1/ITS4* primer. However, this indicated low genetic diversity in the Hue *Ochna* population. This result was in agreement with the study that assessed the genetic diversity of 21 *Disporopsis* species by amplifying the *trnL-trnF* locus in the chloroplast and of 11 *Coptis* sp. samples collected from Hoang Lien National Park using nuclear DNA sequence analyses (Hoang et al., 2023; Hoang et al., 2023).

Table 2. The position of SNPs in the nucleotide sequence of Hue *Ochna* samples

	Nucleotide position																								
Genotype	8	21	26	62	63	64	65	71	75	77	80	88	90	91	104	106	144	173	192	202	333	381	504	508	509
Ochna mossambicensis	-	T	-	-	-	-	-	G	C	A	C	C	C	C	A	A	T	C	C	C	T	-	G	C	A
Ochna natalitia	-	C	-	-	-	-	-	G	A	G	T	M	C	C	A	G	A	C	C	C	T	-	G	C	A
M1	-	C	G	-	-	-	-	G	C	G	C	C	-	-	G	G	A	T	A	A	C	C	A	A	T
M2	-	C	-	-	-	-	-	G	C	G	C	C	-	-	G	G	A	T	A	A	C	C	A	A	T
M3	-	C	G	-	-	-	-	G	C	G	C	C	-	-	G	G	A	T	A	A	C	C	A	A	T
M4	-	C	-	-	-	-	-	G	C	G	C	C	-	-	G	G	A	T	A	A	C	C	A	A	T
M5	-	C	G	-	-	-	-	G	C	G	C	C	-	-	G	G	A	T	A	A	C	C	A	A	T
M6	A	C	G	-	-	-	-	G	C	G	C	C	-	-	G	G	A	T	A	A	C	C	A	A	T
M7	A	C	-	-	-	-	-	G	C	G	C	C	-	-	G	G	A	T	A	A	C	C	A	A	T
M8	A	C	-	-	-	-	-	G	C	G	C	C	-	-	G	G	A	T	A	A	C	C	A	A	T
M9	A	C	G	-	-	-	-	G	C	G	C	C	-	-	G	G	A	T	A	A	C	C	A	A	T
M10	A	C	G	-	-	-	-	G	C	G	C	C	-	-	G	G	A	T	A	A	C	C	A	A	T
M11	A	C	G	-	-	-	-	G	C	G	C	C	-	-	G	G	A	T	A	A	C	C	A	A	T
M12	A	C	G	-	-	-	-	G	C	G	C	C	-	-	G	G	A	T	A	A	C	C	A	A	T
M13	A	C	G	-	-	-	-	G	C	G	C	C	-	-	G	G	A	T	A	A	C	C	A	A	T
M14	A	C	G	-	-	-	-	G	C	G	C	C	-	-	G	G	A	T	A	A	C	C	A	A	T
M15	A	C	G	-	-	-	-	G	C	G	C	C	-	-	G	G	A	T	A	A	C	C	A	A	T
M16	-	C	-	T	G	G	G	C	C	G	C	C	C	C	A	G	A	A	C	A	T	-	A	A	T
M17	-	C	-	T	G	G	G	C	C	G	C	C	C	C	A	G	A	A	C	A	T	-	A	A	T
M18	-	C	-	-	-	-	-	G	C	G	C	C	-	-	G	G	A	T	A	A	C	C	A	A	T
M19	-	C	-	T	G	G	G	C	C	G	C	C	C	C	A	G	A	A	C	A	T	-	A	A	T
M20	A	C	G	-	-	-	-	G	C	G	C	C	-	-	G	G	A	T	A	A	C	C	A	A	T

The sequencing results obtained demonstrated that 20 samples collected from different gardens in Thua Thien Hue were accurately the *Ochna* species. BLAST analysis of the *trnL-trnF* gene fragment of 20 *Ochna* samples collected in Hue showed 99.75% similarity with the sequence of *O. integerrima* (NC_072724.1). This result was consistent with the description of Huynh (2004), who stated that the main *Ochna* species with five petals in Vietnam was *O. integerrima*. However, for the *ITS1–5.8S rDNA–ITS2* region, BLAST comparison with the GenBank database revealed that 15 Hue *Ochna* samples had 96.3-98.69% similarity with the reference sample KF263218.1 *O.mossambicensis*, while 5 samples had 96.32-97.2% similarity with the reference sample KF263189.1 *O.natalia* (table 3). *O.mossambicensis* and *O. natalia* are particularly difficult to differentiate from one another. Chloroplasts contain genes that are highly conserved for more variable regions that are informative for essential plant life and evolutionary history (Filiz et al. 2018), while the available databases on the *ITS* region are very useful and facilitate the identification of phylogenetic relationships of many plant species at interfamilial levels (Estrada-Bárcenas et al., 2014; Freeman et al., 2001; Vaez-Sarvari et al., 2022). This study shows that the *ITS* region is not suitable for identifying Hue *Ochna* species, but its sequence found a solvent in

the phylogenetic relationship between Hue *Ochna* populations, while the phylogenetic relationship between the *trnL-F* sequence and Hue *Ochna* populations was not found. This is similar to the research of Vaez-Sarvari et al. (2022) on the genetic diversity of cantaloupe (*Cucumis melo* L.) landraces. To enhance the reliability of phylogenetic outcomes, it is recommended to sequence various gene regions, such as *nrDNA ITS*, or use diverse marker techniques to reveal genetic diversity. Moreover, the data obtained in the current study will inform subsequent phylogenetic investigations of both Hue Ochna populations and other plant species.

Table 3. Comparison of *ITS* sequences of 20 Hue *Ochna* samples

Sample	Taxonomy	Identify	Reference
M1	*Ochna natalia*	96.83	KF263189.1
M2	*Ochna natalia*	96.86	KF263189.1
M3	*Ochna mossambicensis*	96.83	KF263218.1
M4	*Ochna mossambicensis*	96.99	KF263218.1
M5	*Ochna natalia*	96.83	KF263189.1
M6	*Ochna mossambicensis*	96.36	KF263218.1
M7	*Ochna natalia*	96.32	KF263189.1
M8	*Ochna natalia*	96.83	KF263189.1
M9	*Ochna mossambicensis*	96.05	KF263218.1
M10	*Ochna mossambicensis*	96.49	KF263218.1
M11	*Ochna natalia*	97.2	KF263189.1
M12	*Ochna mossambicensis*	96.42	KF263218.1
M13	*Ochna mossambicensis*	96.5	KF263218.1
M14	*Ochna mossambicensis*	96.5	KF263218.1
M15	*Ochna mossambicensis*	96.51	KF263218.1
M16	*Ochna mossambicensis*	94.49	KF263218.1
M17	*Ochna mossambicensis*	98.68	KF263218.1
M18	*Ochna mossambicensis*	96.3	KF263218.1
M19	*Ochna mossambicensis*	97.01	KF263218.1
M20	*Ochna mossambicensis*	96.5	KF263218.1

Genetic relationship analyses of 20 samples of Hue Ochna

The genetic distance method based on the *ITS* sequence was performed with MEGA 11.0 software. Tajma's Neutrality Test was calculated on the basis of the *ITS* sequences of all 20 Hue *Ochna* samples. The 20 sequences (m) gave 9 segregation sites (S) and a nucleotide diversity (π) of 0.00274053, revealing very low nucleotide diversity (Table 4). The substitution pattern and rates were assessed using the Tamura and Nei (1993) model, considering the relative values of instantaneous r during evaluation. For simplicity, the r values were standardized to 100. Notably, the transition rates were higher than the transversion rates, in bold and italics, respectively. The nucleotide frequencies were distributed as follows: A = 25.00%, T/U = 25.00%, C = 25.00%, and G = 25.00% (table 5).

A tree topology was automatically generated to estimate maximum-likelihood values, yielding a maximum log-likelihood of -941.944 (Table 5). Genetic relationship analysis of 20 Hue *Ochna* samples using the Kimura-2 parameter model showed a low genetic distance (0.00 - 0.014) between the samples from Thua Thien Hue, indicating minimal or very slight differences in the population. This indicated a high level of genetic similarity in the population. Notably, this genetic distance was lower than the genetic distance found among 21 *O. integerrima* varieties from different regions of northern and southern Vietnam (Trang et al., 2018).

However, the genetic distance between these samples and two reference samples from GenBank was relatively high (0.00 – 0.023), indicating greater nucleotide diversity between them (Table 6). Further investigation using other molecular markers may offer insights into clarifying the taxonomic status and phylogenetic relationships of these samples.

Table 4. Tajima's Neutrality Test Values based on ITS sequence of 20 Hue *Ochna* samples

m	S	Ps	Θ	π	D
20	9	0.01433121	0.004039533	0.00274053	-1.10586774

(m = number of sequences, n = total number of sites, S = Number of segregating sites, $p_s = S/n$, $\Theta = p_s/a_1$, π = nucleotide diversity, and D is the Tajima test statistic).

Table 5. Maximum Likelihood Estimate of Substitution Matrix

	A	T/U	C	G
A	-	*6.24*	*6.24*	**12.51**
T/U	*6.24*	-	**12.51**	*6.24*
C	*6.24*	**12.51**	-	*6.24*
G	**12.51**	*6.24*	*6.24*	

Table 6. Pairwise distance between aligned sequences using Kimura-2 parameter model of nucleotide substitution.

	Ochna mossambicensis	Ochna natalitia	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12	M13	M14	M15	M16	M17	M18	M19	M20
Ochna mossambicensis																						
Ochna natalitia	0.015																					
M1	0.023	0.023																				
M2	0.023	0.023	0.000																			
M3	0.023	0.023	0.000	0.000																		
M4	0.023	0.023	0.000	0.000	0.000																	
M5	0.023	0.023	0.000	0.000	0.000	0.000																
M6	0.023	0.023	0.000	0.000	0.000	0.000	0.000															
M7	0.023	0.023	0.000	0.000	0.000	0.000	0.000	0.000														
M8	0.023	0.023	0.000	0.000	0.000	0.000	0.000	0.000	0.000													
M9	0.019	0.019	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000												
M10	0.023	0.023	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000											
M11	0.023	0.023	0.002	0.002	0.002	0.002	0.002	0.000	0.002	0.000	0.000	0.000										
M12	0.020	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000									
M13	0.023	0.023	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.000								
M14	0.023	0.023	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.000							
M15	0.023	0.023	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.000						
M16	0.017	0.017	0.013	0.013	0.013	0.013	0.013	0.010	0.014	0.010	0.011	0.010	0.013	0.011	0.013	0.013	0.013					
M17	0.017	0.017	0.012	0.012	0.012	0.012	0.012	0.010	0.011	0.010	0.011	0.010	0.011	0.011	0.012	0.012	0.012	0.005				
M18	0.020	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.011			
M19	0.017	0.017	0.010	0.010	0.010	0.010	0.010	0.010	0.011	0.010	0.011	0.010	0.011	0.011	0.010	0.010	0.010	0.003	0.002	0.011		
M20	0.020	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.011	0.011	0.000	0.011	

Phylogenetic Trees Construction

The phylogenetic tree of 20 Hue *Ochna* samples and reference species was generated by using the Mega 11.0 software and the Maximum likelihood method (Figure 3). The phylogenetic relationship analysis results showed that two main different clusters were formed by 20 Hue Ochna samples and reference species: The first cluster contained the sequences of 2 reference species and the second cluster contained 20 Hue *Ochna* samples. This cluster split into two sub-clusters: one with 17 Hue *Ochna* samples and another with 3 samples M16, M17, and M19. This result was similar to the result of nucleotide variations. Álvarez and Wendel et al. (2003) reported that the *ITS* sequence data has been used in about 66% of studies to analyze the phylogeny and 34% of all published phylogenetic hypotheses have relied solely on *ITS* sequences. This study identified 20 Hue *Ochna* samples at the species level by analyzing region sequences, using *ITS1/ITS4* primers and comparing them with NCBI GenBank databases. However, the lack of species-level variation may limit the precision of species identification. Therefore, to achieve accurate identification at the species level, the use of *ITS* along with *rbc*, *matK*, and *trnH-psbA* is suggested. Moreover, for accurate identification of various species, the use of specific and distinctive DNA regions from both the plastid genome and the nuclear genome (*ITS*) is recommended.

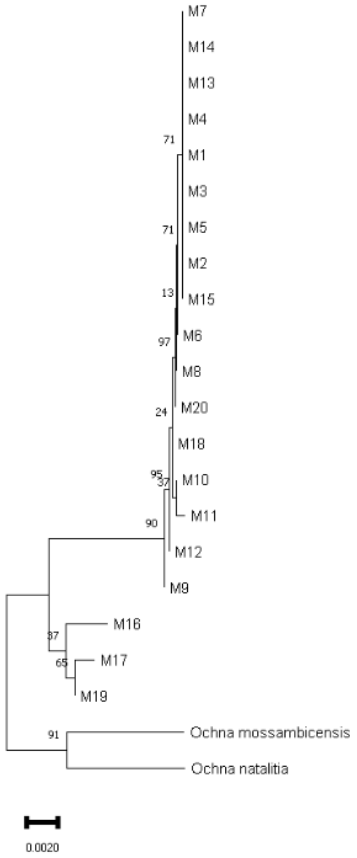

Figure 3. Phylogenetic tree species of 20 Hue *Ochna* samples with the reference species using the Neighbour-joining method. Numbers near nodes represent bootstrap proportions.

Genetic structure of Hue Ochna population

The results of the analysis of the ability to group the Hue *Ochna* samples with genetic similarity using the Structure software are shown in Figure 4

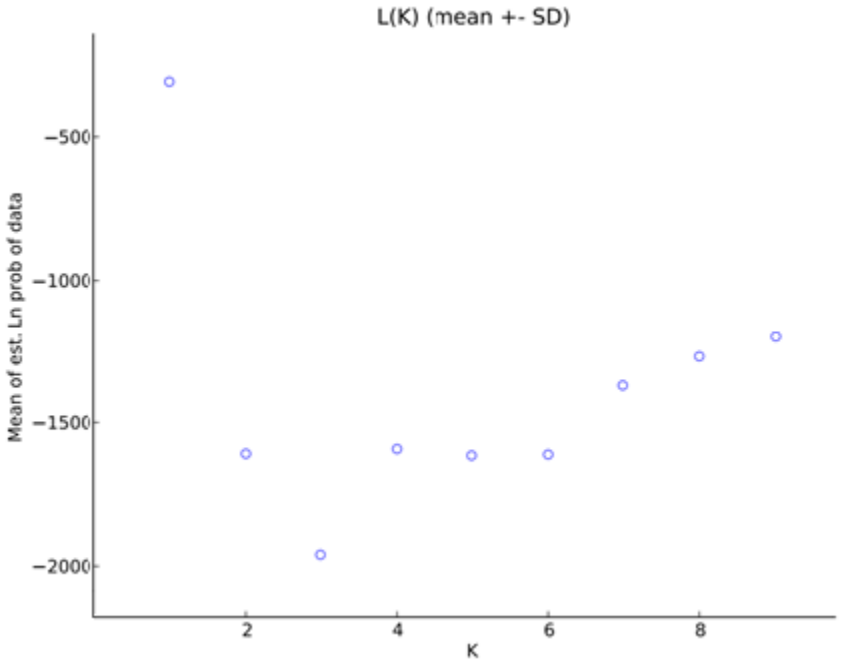

Figure 4. Genetic clustering of the Hue yellow apricot population.

Figure 4 shows that the Hue *Ochna* samples could be assigned to different genetic groups with probability (LnPr) depending on the number of groups (K). The probability was lowest when K = 3 and highest when K = 1. This indicated that the Hue *Ochna* samples were genetically homogeneous. A field survey in seven localities of Thua Thien Hue province revealed that the main seed source was Hue *Ochna*, propagated by seeds. Some households applied eye grafting to accelerate flowering. The breeding and propagation process generated some point mutations that differentiated the samples within the population. Moreover, cross-pollination occurred between different individuals in the population in nature, due to the pollination of bees or wind. Therefore, some genetic variations appeared in the process of reproduction to develop varieties and species.

Comparison between genetic distance and geography

The Mantel correlation test was performed by using GenAlEx 6.2 to analyze the relationship between the similarity matrix of molecular data for 20 Hue *Ochna* samples. The correlation value was 0.1842, indicating low uniformity between the relationships assessed based on geographic distance and the genetic relationships revealed by molecular characterizations among the Hue *Ochna* population. This finding is consistent with the research of the medicinal plant *Glehnia littoralis* in China. The study showed that populations within the same province, despite their close geographic proximity, did not show clustering. Instead, certain populations from distinct provinces, even over substantial geographic distances, showed clustering in the same group (Wang et al., 2016).

Conclusion

In conclusion, within the scope of this study, the *ITS* marker was more sensitive and resolute than the *trnL*-F marker in detecting genetic diversity in Hue *Ochna* samples. The Hue *Ochna* samples had low genetic diversity and were genetically homogeneous, possibly due to the breeding and natural selection process. This study provided useful information for the research on the phylogeny and conservation of the yellow *Ochna* varieties in Thua Thien Hue.

References

Álvarez, I. J. F. W., & Wendel, J. F. (2003). Ribosomal ITS sequences and plant phylogenetic inference. *Molecular phylogenetics and evolution*, *29*(3), 417-434.

Estrada-Bárcenas, D. A., Vite-Garín, T., Navarro-Barranco, H., de la Torre-Arciniega, R., Pérez-Mejía, A., Rodríguez-Arellanes, G., ... & Toriello, C. (2014). Genetic diversity of Histoplasma and Sporothrix complexes based on sequences of their *ITS1-5.8 S-ITS2* regions from the BOLD System. *Revista Iberoamericana De Micologia*, *31*(1), 90-94.

Filiz, E., Uras, M. E., Ozyigit, I. I., Sen, U., & Gungor, H. (2018). Genetic diversity and phylogenetic analyses of Turkish rice varieties revealed by ISSR markers and chloroplast *trnL-F* region. *Fresenius Environmental Bulletin*, *27*(12), 8351-8358.

Freeman, S., Minz, D., Maymon, M., & Zveibil, A. (2001). Genetic diversity within *Colletotrichum acutatum* sensu Simmonds. *Phytopathology*, *91*(6), 586-592.

Ha, T. T. T., Khanh, T. D., & Trung, K. H. (2020). Evaluation of genetic diversity and identification of *Huperzia* species collected in some different areas in Vietnam by molecular markers. *International Letters of Natural Sciences*, *80*.

Hall, T. A. (1999, January). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In *Nucleic acids symposium series* (Vol. 41, No. 41, pp. 95-98).

Hoang, N., Nhung, N. T., Minh, N. T. N., Trang, V. T. H., Trung, K. H., Khanh, T. D., & Van Hung, H. (2023). Morphological Traits and Nuclear Genetic Diversity of *Coptis* sp. in Hoang Lien National Park, Lao Cai Province, Vietnam. *Journal of Advanced Zoology*, *44*(3), 1061-1068.

Hoang, N., Nhung, N. T., Trang, V. T. H., Khanh, T. D., & Van Hung, H. (2023). Molecular Analysis of Genetic Diversity and Genetic Relationship of *Polygonatum kingianum* Samples Collected in Northern Mountainous Regions in Vietnam. *European Chemical Bulletin*, 12(8):9296-9304

Huong, B.T.T., Anh, D.X., Cuong, N.H., An, N.T., Gioi, D.H., Tuong, H.M., Ha, C.H., Ha, T.T.T., Trung, K.H., Khanh, T.D. (2022). Morphological characteristics and DNA barcoding in bach hop (*'Lilium poilanei'Gagnep*) in Vietnam. *Australian Journal of Crop Science*, 16(4), 471-478.

Huong, T.V., Quang, L.T., Huy, V.N., An, P.Y., Khanh, T.D. (2024). Analyses of genetic diversity and relationships of some *Polyscias* samples collected in Vietnam using morphological and molecular markers. *African Journal of Biological Sciences*, 6(Si3), 1964-16 pages.

Huynh, V. T. (2004) Techniques of planting and grafting apricot. *Young Publishers.*

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of molecular evolution*, *16*, 111-120.

Makhafola, T. J. (2012). Five *Ochna* species have high antibacterial activity and more than ten antibacterial compounds. *South African Journal of Science*, *108*(1), 1-6.

Miller, M. P. (1997). Tools for population genetic analysis (TFPGA) 1.3: a Windows program for the analysis of allozyme and molecular population genetic data. *Computer software distributed by the author*.

Nhat, N, N., Hoang, D. N., Minh, T. V., & Do, H. D. K. (2023). New Insights Into The Evolution of Chloroplast Genomes in *Ochna* Species (Ochnaceae, Malpighiales). *Evolutionary Bioinformatics*, *19*, 11769343231210756.

Nilsson RH, Kristiansson E, Ryberg M, Hallenberg N, Larsson KH. Intraspecific *ITS* variability in the kingdom fungi as expressed in the international sequence databases and its implications for molecular species identification. *Evol Bioinformatics*. 2008;4:193–201.

Pham, D. L., Nguyen, T. B., Vu, C. C., & Jean, C. M. (2008). Using microsatellite technique to assess the genetic diversity and genetic structure of cattle population in Ha Giang province. *VNU Journal of Science: Natural Sciences and Technology*, *24*(4).

Schneider, J. V., Jungcurt, T., Cardoso, D., Amorim, A. M., Paule, J., & Zizka, G. (2022). Predominantly eastward long-distance dispersal in pantropical Ochnaceae inferred from ancestral range estimation and phylogenomics. *Frontiers in Ecology and Evolution*, *10*, 813336.

Schneider, J. V., Paule, J., Jungcurt, T., Cardoso, D., Amorim, A. M., Berberich, T., & Zizka, G. (2021). Resolving recalcitrant clades in the pantropical Ochnaceae: insights from comparative phylogenomics of plastome and nuclear genomic data derived from targeted sequencing. *Frontiers in Plant Science*, *12*, 638650.

Sevindik, E., Veysel, U. Z. U. N., & Coşkun, F. (2016). Sequence Analysis of the Internal Transcribed Spacer (*ITS*) Region of the Nuclear Ribosomal DNA (*nrDNA*) and Chloroplast *trnL-F* Region (cpDNA) of Some *Lactuca* L.(Asteraceae) Species in Turkey. *Notulae Scientia Biologicae*, *8*(4), 444-450.

Shah, T., Mashimba, F. H., Suleiman, H. O., Mbailwa, Y. S., Schneider, J. V., Zizka, G., ... & Darbyshire, I. (2022). Phylogenetics of *Ochna* (Ochnaceae) and a new infrageneric classification. *Botanical Journal of the Linnean Society*, *198*(4), 361-381.

Tamura, K., & Nei, M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular biology and evolution*, *10*(3), 512-526.

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: molecular evolutionary genetics analysis version 11. *Molecular biology and evolution*, *38*(7), 3022-3027.

Trang, D. H., Van Dong, D., Chung, B. H., Gioi, D. H., & Khanh, T. D. (2018). Identification of Vietnamese *Ochna integerrima* (Lour.) Merr species based on ribosomal DNA internal transcribed spacer sequence. *International Letters of Natural Sciences*, *68*.

Vaez-Sarvari, H., Emamjomeh, A., & Fazeli-Nasab, B. (2022). Evaluation of genetic diversity of Cantaloupe landraces based on the internal transcriptional spacer regions (ITS1, 4). *International Journal of Vegetable Science*, *28*(6), 541-552.

Vijayan, K. and Tsou, C.H. (2010) DNA Barcoding in Plants: Taxonomy in a New Perspective. Current Science (Bangalore), 99, 1530-1541

Wang, A., Zhang, P., Liu, X., Liang, J., & Li, W. (2016). Genetic structure and diversity of *Glehnia littoralis*, an endangered medicinal plant in China. *Biochemical Systematics and Ecology*, 66, 265-271.