Available online www.jsaer.com

***Journal of Scientific and Engineering Research*, 2023, 10(8):7-12**

Research Article

ISSN: 2394-2630
CODEN(USA): JSERBR

Identification of the medicinal plant "Phjac chac" in Cao Bang province, Vietnam using morphological and chloroplast DNA barcodes

Nguyen Ha Anh[1], Nguyen Thanh Nhung[2], Nguyen Van Nguyen[3*], Tran Dang Khanh[2*]

[1]Ha Noi-Amsterdam High School for the Gifted, Cau Giay, Ha Noi, Vietnam
[2]Agricultural Genetics Institute, Vietnam Academy of Agricultural Sciences, Ha Noi, Vietnam
[3]Vietnam Institute of Agricultural Engineering and Post-Harvest Technology, Cau Giay, Ha Noi, Vietnam

Abstract Phjac chac is considered as a native plant discovered in Cao Bang province. This plant holds valuable flavoring agents and potential essential oils and is widely used in relieving common cold symptoms in traditional local communities. Despite its significant uses, there has been a scarcity of information to identify this plant species. Therefore, the study aimed to identify this plant using morphological characteristics and *trn*L–*trn*F markers. The young stems of this plant display a green and smooth appearance, while the older branches exhibit a dark green to dark coloration. Additionally, the older branches frequently possess numerous nodules on their surface, which have round or oval cross sections and are found either individually or in series. Distinctively, the plant itself emits a noticeable and distinct odor. By chloroplast DNA analysis, this plant was closely related to Neocinnamomum mekongense species (94.81% similarity) which belongs to the Lauraceae family. The finding may provide useful information to further exploit and conserve this plant for developing the local economic activity of this province.

Keywords Phjac chac plant, identification, medicinal plant, Neocinnamomum mekongense, Cao Bang province.

1. Introduction

Vietnam is considered as one of the world's most prosperous in terms of rich and diverse plant genetic resources due to diverse climate conditions in both template and tropical plant species [1]. Over 4,000 species are being used as medical plants [2]. In Vietnam, there are 54 ethnic minority groups, and many of them such as Dao, Tay and Nung tribes, etc., often live in the mountainous areas with thousands of years experiencing using traditional plants as medicinal treatments due to a lack of access to modern medicine.

Cao Bang province is a typical geographical location with special climatic conditions, where is rich and very diverse abundant flora, typically medicinal species. A recent survey report documented approximately 940 medicinal species, of which 577 plant species have been traditionally treated for various ailments such as asthma, diabetes, hemorrhage, hepatitis, scrofulous, sterile, syphilis, toothache, tranquilizer, urolithiasis, and vaginitis, etc [3].

The plant species named "Phjac chac" was derived from the ethnic minority of "Dao" people, who are often sheltering in these areas. The term "Phjac" means vegetable, "Chac" implies aromatic during crushing. Therefore, Phjac chac is a woody plant that well grows on high rocky mountains over 1000m above sea level. It considers to be native to Bao Lac district, Cao Bang province. According to the report of Cao Bang Department of Science and Technology, this plant is challenging to domesticate. Out of 15 attempts to domesticate this plant, only one was successful. The leaves of Phjac chac contain about 1% essential oil with a mild fragrance similar to the scent of lemongrass. It is used to develop fragrances, deodorize, purify the air, repel mosquitoes, and relieve cold symptoms in Oriental medicine. Local residents have also used Phjac chac leaves for bathing

and food flavoring due to its aromatic nature property. Notably, this plant is especially used for treating coughs and common colds.

Traditionally, the identification of plant species has relied on the expertise of botanists to make classifications based on morphology or histological microscopy. However, identification based on morphological characters alone is difficult due to morphological similarity among species [4-5]. Moreover, it is virtually impossible to discriminate the species once the commodity loses its physical form; for example, when supplied as a powder.

DNA barcoding is widely used and is an effective technology that enables rapid and accurate identification of plant species [6-7]. Specifically, the non-coding sequence *trn*L–*trn*F in chloroplast genomes has been proven to be useful and valuable for analyzing phylogenetic relationships at the intergenetic level [8]. Numerous studies have utilized intergenic regions, especially between two *trn*L–*trn*F genes, for species identification and phylogenetic analysis [7, 9]. The plant *trn*L–*trn*F intergenic spacer (*trn*L–*trn*F IGS) is less than 500 bp in length size. From the conservative region, universal primers were designed to amplify this region in various species. With the high polymorphism in the *trn*L–*trn*F intergenic region, these primers have become a good marker for analysis studies in many plant species [9].

Currently, there is a lack of scientific evidence to identify the origin of the Phjac chac plant. Therefore, this study aims to precisely determine the scientific name of the Phjac chac by using the main morphological characteristics and chloroplast *trn*L–*trn*F intergenic spacer DNA sequence. The results of this study prove useful in supplementing information that may contribute to the advancement of further developing and conserving this medicinal plant in Vietnam.

2. Materials and Equipment Used

Samples collection

Five young leaf samples of Phjac chac used in this study were collected from different locations at Bao Lac town, Cao Bang province, Vietnam. All samples were immediately transferred to the Laboratory and were kept in 100% ethanol and stored at - 20°C before DNA extraction.

Morphological features evaluation

The morphology of Phjac chac was described and observed based on the previously described method of Thin [10] with some modifications. The described parts including the trunk, leaves, and stem were presented.

DNA extraction, amplification, analysis of DNA sequence

A total of DNA extraction was carried out following the CTAB method according to Doyle and Doyle [11] with some minor modifications. The yielded DNA products were checked on the agarose gel (1%). Samples were then diluted to 100 ng/µL working stocks.

The PCR reaction was performed on the Veriti 96 wells Thermal cycler with the nucleotide sequences of *trn*L/*trn*F primers: GGT TCA AGT CCC TCT ATC CC / ATT TGA ACT GGT GAC ACG AG [12]. PCR amplification was performed to a final volume of 20 µL in a 96 wells Thermal cycler (Applied Biosystems, Waltham, MA, USA). The reaction mixture contained 100 ng genomic DNA, 1X PCR buffer, 0.4 mM dNTPs mix, 0.6 µM forward and reverse primers, and 1 U Kapa Taq DNA Polymerase (Kapa Biosystems). PCR cycling was carried out with an initial denaturation step at 94 °C for 3 min, followed by 35 cycles at 94 °C for 30s, 55 °C for 30s, and 72 °C for 1 min, and a final extension step at 72 °C for 5 min. The DNA of all samples was sent to Apical Scientific (Malaysia) for sequencing by Sanger dideoxy sequencing technology.

Sequence alignment and data analysis

The original chromatograms were visually checked in the BioEdit software to avoid reading errors. Low-quality chromatograms were excluded from further analysis [13]. The DNA sequences were searched on NCBI using Nucleotide BLAST to confirm their availability in the GeneBank or to make a new identification submission. The best BLAST hit for the query sequence was checked. The DNA sequences were further analyzed using MEGA 11 software to generate a phylogenetic tree with neighbor-joining methods.

3. Results

Morphological characteristics and growth traits of Phjac chac

The Phjac chac plant can be distinguished by its morphological characteristics. It is a shrub that typically reaches a height of 1.5 – 2.5m in regions with warm and humid climates, on altitudes from medium to high, in mountainous cinnamon forests. Young stems of this plant unveil a green and smooth appearance, while the older branches are characterized by dark green to dark coloration and frequently possess numerous nodules on the surface with round or oval cross sections, and are located individually or in series. Distinctively, the plant itself emits a noticeable and distinct odor.

The leaves of Phjac chac are arranged in a distichous and glabrous. The leaf blade is ovate in shape with pointed ends, approximately 6-15cm in length, 5-7 cm in width. The leaves are and crisp in texture with the upper side revealing a shiny and smooth appearance. While the upper side has a darker shade of green compared to the underside. The leaf venation structure is illustrated by three prominent veins on both sides of the leaf, including the midrib and two large and distinct secondary veins. The secondary veins are slightly curved and extend onward the length of the leaf, whereas tertiary veins are relatively sparse. The petiole or leaf stalk has a cross-section that is slightly indented on the upper half, while the other half is round; it is grayish-green and reaches approximately 0.5 – 1cm in length.

Phjac chac's flowers emerge from the nodes or buds of the stem and are minutely sericeous. They are hermaphroditic and grow in clusters. Flower buds are darker shades of green. Peduncles are 0.3 – 0.4 cm in length and green in color (data not shown). Its pollination process of Phjac chac is facilitated by insects. As the flowers are fertilized and afterward developed into fruits. The fruits are oval-shaped or round and are eaten by birds which subsequently distribute in the dispersal of their seeds.

Figure 1: Phjac chac's morphological characteristics

(a) Natural habitat; (b) Trunk; (c) Leaf placement; (d) Trunk height; (e) Front side of leaf; (f) Backside of leaf; (g) Leaf breadth; (h) Leaf length; (i) Flower buds on stem; (j) Stem; (k) Branch.

DNA sequencing and data analysis

In this study, we collected a total of 5 leaf samples of Phjac chac from 5 different regions in Bao Lac, Cao Bang for analysis. Firstly, the primer pairs of *trn*L–*trn*F IGS were successfully applied to amplify the DNA samples. The amplified products yielded a single band of approximately 400 base pair (bp) in length size, which showed good enough quality and quantity for further sequencing. Based on data of the nucleotide sequence, it showed that the length of the *trn*L–*trn*F IGS region varied from 397-400 bp for all samples. We used 380 bp to compare with the published sequence since they were clear. Interestingly, all samples had the same sequences. The G-C ratio was 36%, and the A-T ratio was 64%, respectively. To compare the attained sequence with the published sequences in the gene bank, we applied the NCBI/Blast tool. The results revealed that the gene sequence obtained had a similarity level of 94.81% with reference samples NC_036003.1 *Neocinnamomum mekongense* and NC_036003.1 *Neocinnamomum delavayi*.

The *trn*L gene sequence of Phjac chac was subjected to phylogenetic tree analysis along with the reference sequences obtained from Genebank and aligned by the BioEdit program and MEGA-11 software [13]. The evolutionary tree was constructed using the Neighbour-Joining method. According to the analysis, Phjac chac was found to be closely related to *N. mekongense* and *N. delavayi* with a confidence value of 71% (Figure 2). In phylogenetic analysis, the bootstrap technique is employed to assess the accuracy of statistical estimates According to the report of Hillis and Bull [14], this proportion of bootstrap (>70%) is considered as very good confidence value.

Figure 2: Phjac chac's phylogenetic tree. A phylogenetic tree showing the relationships between Phjac chac samples and reference species. The phylogenetic tree is derived from the neighbor-joining method. The numerals at the respective branching points indicate bootstrap values (%) based on 1,000 replications. The scale bar indicates 0.001 substitutions per nucleotide position.

4. Discussion

Plant species grow naturally and are distributed across various regions, often referred to by their common names. However, there can be confusion when multiple common names are used for the same plant or when a single common name is attributed to different plants. To avoid such confusion, the use of scientific names is crucial when communicating about plants. Scientific names provide a standardized and internationally recognized nomenclature, eliminating ambiguity and ensuring clear and accurate communication in plant-related discussions.

Some certain species exhibit morphological similarities, which can make it challenging, and in some cases, impossible to differentiate between them based solely on external characteristics [15]. Moreover, variations in

environmental conditions can lead to changes in the morphology of the same species as they adapt to their surrounding environments [16-17]. In this study, in order to determine the accurate scientific name of the "Phjac chac" a medicinal plant, our efforts have been made to utilize not only plant morphology but also DNA barcoding for this plant species identification. This approach allows for a more comprehensive and reliable means of identification, overcoming the limitations posed by external morphological features alone. Molecular phylogenetics based on DNA sequencing has become a regular approach in plant systematics. The application of DNA barcodes has successfully identified many plant species. Recently, the applicability of some molecular markers including the *trn*L–*trn*F IGS region for plant species identification has been reported [18-20]. In which, Tsai et al. [18] used the sequences of the *trn*L–*trn*F IGS and established a DNA sequence database to identify plant species in Taiwan for forensic purposes. Moreover, Kwon et al. [21] (2022) identified 19 poisonous plants native to Jeju Island using seven DNA barcodes. Among them, the *trn*L–*trn*F barcode was the most convenient marker for PCR amplification and sequence retrieval, and the combination of *trn*L–*trn*F and ITS1-ITS4 barcodes enabled single species identification in 18 out of 19 plants.

In this study, the Phjac chac plant was identified as closely related to *N. mekongense* and *N. delavayi* based on the *trn*L–*trn*F IGS sequence. The Neocinnamomum genus is one of the most mysterious genera in the Lauraceae family [22]. This result was unexpected since *N. delavayi* and *N. mekongense* are very closely related and similar morphologically [23-24]. Indeed, it is difficult to distinguish between these two species due to overlapping characters. According to Kostermans (1974), *N. delavayi* has alternate leaves that are sub-coriaceous, ovate-elliptic, 1 x 2 - 2 x 5.5 cm, rarely broadly ovate, 4.5 x 7 cm. This characteristic is important when compared to *N. mekongense* which has larger leaves measuring 1.5 x 4 to 5 x 9.5 cm, caudate-acuminate, with a slender acumen up to 15 mm in length. Based on the description by Kostermans, Phjac is likely to have the characteristics of an ovate leaf with a length of 6-15cm and a width of 5-7cm, which may belong to the species *N. mekongense*.

5. Conclusion

Our study, for the first time reported on the identification of Phjac chac in Cao Bang province, Vietnam as a member and belonging to the species *N. mekongense*. This study provides valuable information for distinguishing and utilizing this economically promising medicinal plant in this province. However, further research needs to be done on analyzing the bioactive components of this plant for human health improvement.

References

[1]. Anh, D.T & Toan, V.D. (2019). Results of biodiversity policy in Vietnam: Conservation and exploitation of plant genetic resources. In FFTC Agricultural Policy Platform (FFTC-AP), food and fertilizer technology center for the Asian and Pacific region. Article No. 1640. https://ap.fftc.org.tw/article/1649.

[2]. Chen, J., Tauer, C., & Huang, Y. (2002). Paternal chloroplast inheritance patterns in pine hybrids detected with trnL–trnF intergenic region polymorphisms. Theoretical and Applied Genetics, 104, 1307-1311.

[3]. Nguyen, H.T.T., Le, H.N., Bui, H.T., & Tran, B.T. (2023). Medicinal flowering plants in Cao Bang province, Vietnam: The diversity and medicinal uses. GSC Biological and Pharmaceutical Sciences, 23(01), 240–248.

[4]. Doh, E. J., Kim, J. H., Oh, S. E., & Lee, G. (2017). Identification and monitoring of Korean medicines derived from Cinnamomum spp. by using ITS and DNA marker. Genes & Genomics, 39, 101-109.

[5]. Trung, K.H., Ha, D.T.T., My, N.D.H., Hoang, D.T., Hanh, D.H., Diep, N.T., Dung, K.T., Quan, N.T., Nhung, N.T., Minh, L.H.N. Minh, Khanh, T.D., & Ha, T.T.T. (2023). Chloroplast analysis of genetic diversity of Dolichandrone spathacea collected in the Central Coast region. Advanced Studies in Biology, 15(1): 37-45.

[6]. Li, H., Xiao, W., Tong, T., Li, Y., Zhang, M., Lin, X et al., & Guo, X. (2021). The specific DNA barcodes based on chloroplast genes for species identification of Orchidaceae plants. Scientific Reports, 11(1), 1424.

[7]. Trang, L.T.T, Ha, D.T.T., N.T. Diep., Nghia, L.T., Duc, D.X., Khoa, N.T., Anh, L.H., Dung, K.T., Trung, K.H., Gioi, D.H., Huong, B.T.T., Khanh, T.D., & Hue, H.T. (2021). Genetic diversity and genetic relationships of Vietnamese Citrus varieties using internal transcribed spacer regions (ITS). Advanced Studies in Biology, 13(1): 1-9.

[8]. Kojoma, M., Kurihara, K., Yamada, K., Sekita, S., Satake, M., & Iida, O. (2002). Genetic identification of cinnamon (Cinnamomum spp.) based on the trnL–trnF chloroplast DNA. Planta Medica, 68(01), 94-96.

[9]. Chen, J., Tauer, C., & Huang, Y. (2002). Paternal chloroplast inheritance patterns in pine hybrids detected with trnL–trnF intergenic region polymorphisms. Theoretical and Applied Genetics, 104, 1307-1311.

[10]. Thin, N.N. (2006). Methods of plant research. Vietnam National University Press, 165p.

[11]. Doyle, J. J. & Doyle, J. L. (1987). A Rapid DNA Isolation Procedure for Small Quantities of Fresh Leaf Tissue. Phytochemical Bulletin, 19: 11 –15.

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

[13]. Lee, T. H., Guo, H., Wang, X., Kim, C., & Paterson, A. H. (2014). SNPhylo: a pipeline to construct a phylogenetic tree from huge SNP data. BMC Genomics, 15, 1-6.

[14]. Hillis, D. M., & Bull, J. J. (1993). An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. Systematic Biology, 42(2), 182-192.

[15]. Sukontason, K., Bunchu, N., Chaiwong, T., Moopayak, K. & Sukontason, K. L. (2010). Forensically important flesh fly species in Thailand: Morphology and developmental rate. Parasitology Research, 106, 1055–1064.

[16]. Lemic, D., Benítez, H. A., Püschel, T. A., Gašparić, H. V., Šatvar, M., & Bažok, R. (2016). Ecological morphology of the sugar beet weevil Croatian populations: Evaluating the role of environmental conditions on body shape. Zoologischer Anzeiger-A Journal of Comparative Zoology, 260, 25-32.

[17]. 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, 80:13-23.

[18]. Tsai, L. C., Yu, Y. C., Hsieh, H. M., Wang, J. C., Linacre, A., & Lee, J. C. I. (2006). Species identification using sequences of the trnL intron and the trnL–trnF IGS of chloroplast genome among popular plants in Taiwan. Forensic Science International, 164(2-3), 193-200.

[19]. 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 Gapnep) in Vietnam. Australian Journal of Crop Science, 16(4): 471-478.

[20]. Cong, D.V., Anh, D.T., Huong, T.T.H., Nhung, N.T., Ha, T.T.T, Khanh, T.D., & Toan, V.D. (2023). Genetic diversity of Job's tear (Coix lacryma-jobi L.) germplasms based on the morphological traits and SSR markers. European Chemical Bulletin, 12(6): 42-52.

[21]. Kwon, E., Kim, J. Y., Chang, M., Lee, M., Moon, S., & Lee, W. H. (2022). Identification of 19 Species of Poisonous Plants from Jeju Island and Construction of a Database Using DNA Barcoding. Korean Journal of Plant Resources, 35(2), 346-361.

[22]. Wang, Z. H., Li, J., Conran, J. G., & Li, H. W. (2010). Phylogeny of the Southeast Asian endemic genus Neocinnamomum H. Liu (Lauraceae). Plant Systematics and Evolution, 290, 173-184.

[23]. Kostermans, A.J.G.H. (1974). A monograph of the genus Neocinnamomum Liou Ho. Reinwardtia, 9, 85–96.

[24]. Li, H.W., Pai, P.Y., Lee, S.K., Wei, F.N., Wei, Y.T., Yang, Y.C., Huang, P.H., Tsui, H.P., Shia, Z.D., & Li, J.L. (1984). Lauraceae. Flora Reipublicae Popularis Sinicae. Vol. 31 Beijing: Science Press.