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Research Article

Research on *Passiflora foetida* L. as a Pharmaceutical Treatment for Neurasthenia in Humans

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Abstract

Background and Objective: This plant is known in many parts of the world and is used as medicine in some countries. In Vietnam, people know how to use *Passiflora foetida* L., as an herb. There are many research works in the world, most of them focus on medicinal properties. The objective of this study was to determine the genetic diversity of samples of *Passiflora foetida* L., yellow fruit based on agronomic traits and the gene region of "rbcL". **Materials and Methods:** Seed samples were arranged in a completely randomized design with three replications. The trial was a sample of *Passiflora foetida* L., species. The distance between sample plant is 3×2 m, so the total number of trees is 1,330 trees ha⁻¹. The genetic relationship between them was determined through the construction of a phylogenetic tree in the "rbcL" gene region sequence. Data analysis and processing methods done by using X software. **Results:** The PCR results that amplify the "rbcL" gene region around 670 bp, the PCR products were then sequenced. The sequence results were compared with the sequences on the NCBI gene bank, showing that the sequences of the nine varieties/species all coincided with the *Passiflora foetida* Sims species sequences with a high similarity coefficient from 98.77 to 99.9%. Genetically shows that all nine species samples belong to *Passiflora foetida* which can be classified into three genetic samples of An Giang (LA1), Ca Mau (LA2) and Can Tho (LA3) which are genetically close to each other, have the same index. **Conclusion:** The heritability in the broad sense is high in the two traits of stem height and leaf length, so it can be considered that these two traits are controlled by genes. The cultivation of algae seeds to improve the productivity of these medicinal plant varieties is important in providing raw materials for the oriental medicine industry.

Key words: Agronomy, genes, neurasthenia, phylogenetic, *Passiflora foetida* L.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Passiflora foetida L., of the family *Passiflora foetida* L., Passifloraceae, order Violales, genus *Passiflora* currently has more than 400 species, including about 60 species that produce edible fruit. *Passiflora foetida* L., is a vine, small, cylindrical with longitudinal grooves, sparsely hairy. The tree grows up to tens of meters long, the leaves are alternate and the leaves are attached at each node. *Passiflora foetida* L., has two species of yellow and purple fruit. This plant is known in many parts of the world and used as medicine in some countries of South America, China and Southern Europe. In Vietnam, people know how to use *Passiflora foetida* L., and some other plant as herbs¹. Therefore, it has been grown in many different localities throughout the country as, including the Southern Vietnam, Northern and Central Vietnam. However, *Passiflora foetida* L., has been grown and used the most in Southern Vietnam, especially in the Mekong Delta. Many doctors have used products from *Passiflora foetida* L., as an effective therapy to support the treatment of digestive and respiratory diseases. The fruit of passion fruit contains small amounts of alkaloids, including derivatives harmol, harmin, harman, harmaline and harmalol. Barcoding DNA technique relies on the use of one or more DNA fragments ranging in size from 400 to 800 bp as a standard for rapid and accurate species identification. The barcoded DNA fragments can be those located in the nuclear genome (18S, 5.8S, 26S, ITS...)²⁻⁴, mitochondrial genome (Cytb, CO1...)⁵⁻⁷, chloroplast genome (*matK*, *rbcl*, *trnH-psbA*, *rpo*, *trnL-trnF*, *ycf*...)^{7,8}. Depending on the research object, the barcoded DNA fragments will be used appropriately^{3,7,9}.

Although there are many research works, most of them focus on medicinal properties, while the systematic study related to plant genetics has hardly been focused, therefore, studying the genetic characteristics of nine specimens/species of *Passiflora foetida* L., mainly yellow fruit collected in the Mekong Delta, were carried out to determine the genetic relationship between the samples/species *Passiflora foetida* L., based on the characteristics of *Passiflora foetida* L., agronomic scores and "*rbcl*" gene region sequences^{10,11}. The whole plant, leaves, vine and fruit are used as a medicine, except for the roots or tubers of *Passiflora foetida* L., which cannot be used. *Passiflora foetida* L., has long been used in oriental medicine to treat insomnia, neurasthenia, heart palpitations and dreams, in addition to helping to treat early menstruation, abdominal pain due to apple heat, helps cool the liver, cool down the body. Thus, researching the medicinal values of *Passiflora foetida* L., is

important in helping people balance circulatory, respiratory, digestive and reproductive physiological functions. At the same time, it supports the treatment of diseases related to important life functions of the body. To help more people use the valuable medicinal properties of *Passiflora foetida* L., surveying the agronomic characteristics of plant morphology and genetics to help farmers expand planting areas in different locations. Therefore, this is a study that has theoretical and practical significance in treating human diseases.

MATERIALS AND METHODS

Study area: The experiment was carried out from January, 2019 to January, 2022, at the Medicinal Garden of Tay Do University.

Plant sample: Nine samples of seeds/species *Passiflora foetida* L., grown in provinces such as An Giang, Ca Mau, Can Tho, Dong Thap, Hau Giang, Kien Giang, Soc Trang, Tien Giang and Vinh Long were collected and planted. Survey planting at Can Tho Experimental Farm for a period of 20 months (from March, 2019 to November, 2021). Seed samples were arranged in a completely randomized design with three replications. The trial was a sample of the variety/species *Passiflora foetida* L., collected in the Mekong Delta provinces. The planting distance is 3×2 m, so the total number of trees is 1,330 trees ha⁻¹. The soil is plowed to a depth of about 30-35 cm and then weeds and other plant residues are removed.

The organic fertilizers are suitable for *Passiflora foetida* L., especially composted manure. The amount of fertilizer for plants according to the growth stages is: Manure is used 15-20 tons ha⁻¹, powdered lime is applied with the amount of 1 ton. In addition, the amount of chemical fertilizer is used 170 kg N-170 kg P₂O₅-145 kg K₂O.

During the cultivation process, it is necessary to spray more foliar fertilizers containing trace elements such as Ca, Mg, S, B, Mo and Fe, with a dosage of about 40-50 mg in the period of 30 days after planting and before flowering by spraying on leaves, in order to promote plant growth and stimulate flowering and fruiting after harvests.

Research methods: Young and fresh leaf samples were collected and refrigerated at -20°C. The DNA extraction was carried out at the Laboratory of Molecular Biotechnology, Biotechnology Research and Development Institute, Can Tho University. The whole DNA of samples/species *cf2* was

extracted from fresh leaf samples according to the extraction procedure by modified CTAB method⁵. Check the quality of DNA by electrophoresis on 1% agarose gel^{12,13}. The sequence of primer pairs is as follows:

F: 5'ATGTCACCAACAGAGACTAAAGC-3'
R: 5'-GTAAATCAAGTCCACRCG-3'

The PCR reaction consists of 35 heating cycles, a denaturation period of 5 min at 95°C, 60 sec at 95°C, a pairing time is 50 sec at 54°C, an elongation time is 90 sec at 72°C and chain elongation in 5 min at 72°C and the product was stored at 10°C for 20 min. The PCR products were electrophoresed and purified using the Wizard SV gel kit and PCR Clean-up System (Promega), based on the Sanger method¹⁴.

This technique is based on the Sanger method¹⁴. The sample is sent to the Molecular Biology Laboratory. The *rbcl* gene region sequence was solved by the Sequencing Company of Korea⁶.

Data analysis and processing methods: To determine the genetic relationship between nine varieties/species of *Passiflora foetida* L., a pedigree tree was constructed using Mega X software¹⁵ with the maximum likelihood method, 2-parameter Kimura model and bootstrap index is 1000 times. The sequences of the "*rbcl*" gene region of the nine varieties/species *Passiflora foetida* L., used to build the pedigree, were aligned with BioEdit 7.2 software and removed some confounded sequences in the two groups, beginning of each sequence.

Genotypic and phenotypic variation coefficients are calculated according to Burton's and Devane¹⁶ formula and are divided into three levels: Low (<10%), medium (10-15%) and high (>15%) in which:

$$GCV = \frac{\sqrt{V_g}}{\bar{X}} \times 100$$

$$PCV = \frac{\sqrt{V_p}}{\bar{X}} \times 100$$

Where:

V_g = Genotype variance
 V_p = Phenotypic variance
 $\bar{X}$ = Overall average

Heritability in the broad sense (h^2_b) was calculated as the ratio of the genotype and phenotypic variance as a percentage:

$$h^2_b = \frac{V_g}{V_p} \times 100$$

Where:

V_g = Genotype variance
 V_p = Phenotypic variance

Heritability was assessed as low (<30%), moderate (30-70%) and high (>70%). The high heritability (h^2_b) of a trait indicates that the gene-regulated trait is less affected by environmental conditions.

Genetic progress was calculated according to the formula of Johnson *et al.*¹⁷.

$$GA = i.h^2_b.\sigma_p$$

Where:

i = Selective differential (2.06) at 5% selective intensity
 h^2_b = Heritability in the broad sense
 σ_p = Standard deviation of the phenotype

RESULTS AND DISCUSSION

Sequence of the "*rbcl*" gene region: The PCR results that amplify the "*rbcl*" gene region around 670 bp were shown in Fig. 1. The PCR products were then sequenced. The sequence results were compared with the sequences on the NCBI gene bank, showing that the sequences of the nine varieties/species all coincided with the *Passiflora foetida* Sims species sequences with a high similarity coefficient from 98.77 to 99.9%.

Sequencing the *rbcl* gene region of nine samples of *Passiflora foetida* L., showed that the nucleotides (including A, T, C and G) were clearly expressed with high peaks (pick) and were not blurred from the position. The 50th onwards, for example, the sequence of samples LA01 (Fig. 2), LA04 (Fig. 3), LA07 (Fig. 4).

Based on the sequence of these nine genera/species, the genetic relationship between them was determined through the construction of a phylogenetic tree in the "*rbcl*" gene region sequence, the results were presented in Fig. 5. Thereby, it shows that nine varieties/species can be classified into three genetic samples of An Giang (LA1), Ca Mau (LA2) and Can Tho (LA3) which are genetically close to each other, have the same index. Group II includes 2 varieties of Vinh Long (LA8) and Dong Thap (LA6). Group III includes 4 samples of varieties/species in Kien Giang (LA7), Hau Giang (LA4), Soc Trang (LA5) and Tien Giang (LA9). Although there are ecological differences between provinces, in which provinces

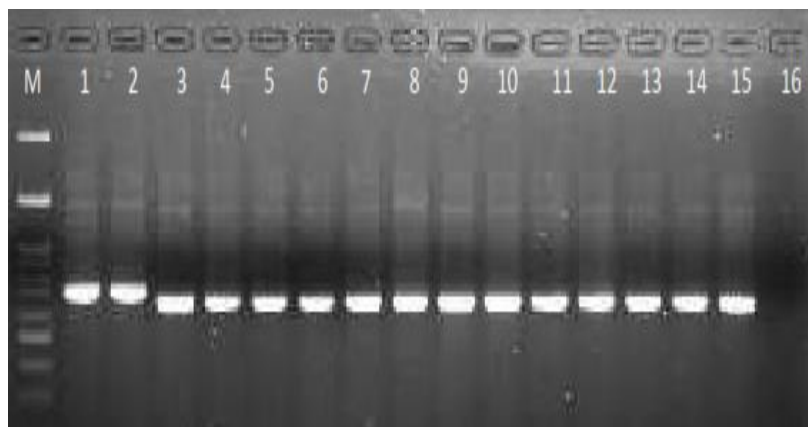


Fig. 1: PCR of the *rbcL* gene region of nine specimens of *Passiflora foetida* L.

M: standard scale 100 bp, Well 1, 2: Control sample, Well 3-12: Sample La 1-10, Well 13-15: La samples run repeatedly and Well 16: Negative control sample

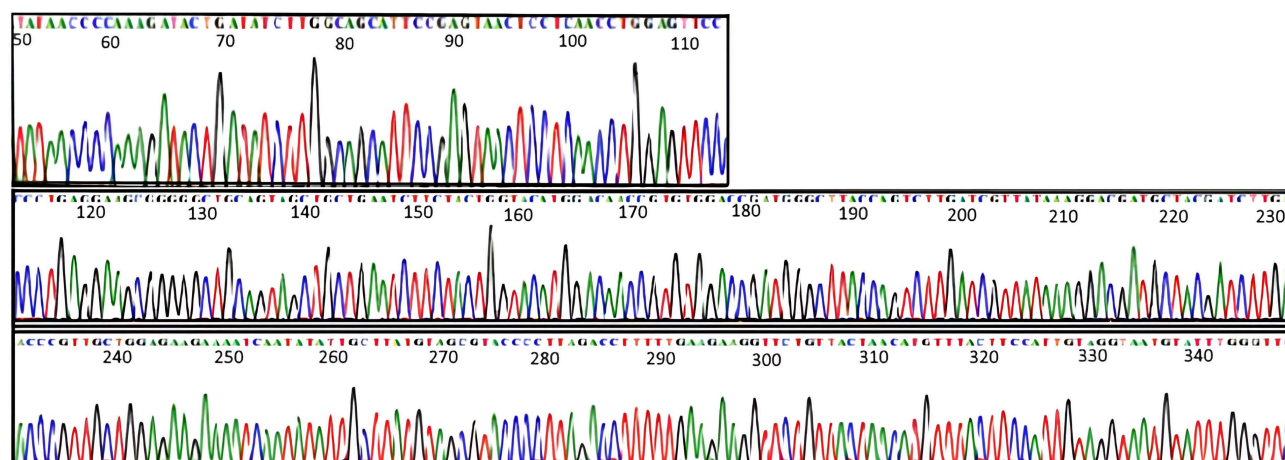


Fig. 2: Sequence of sample *rbcL* gene region LA01

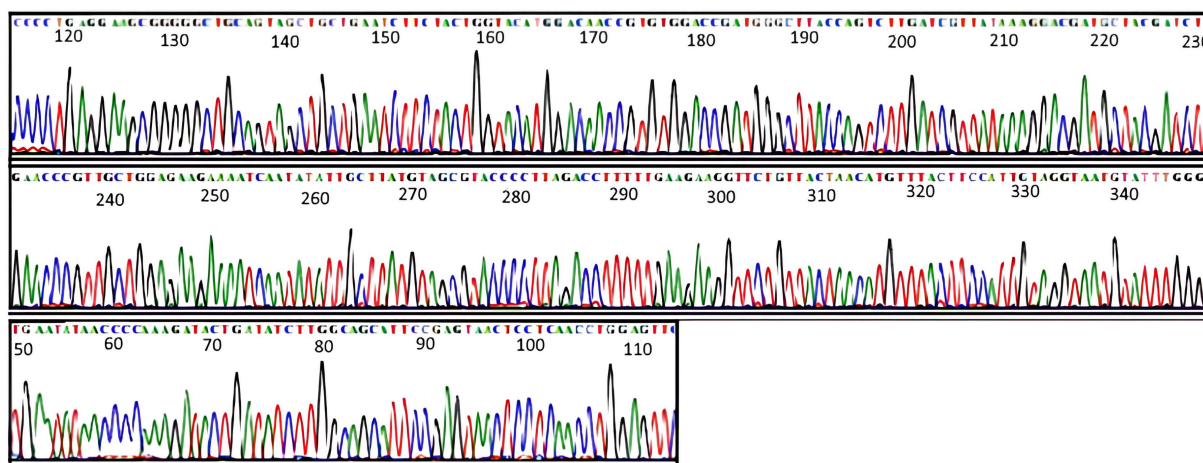


Fig. 3: Sequence of the LA04 sample *rbcL* gene region

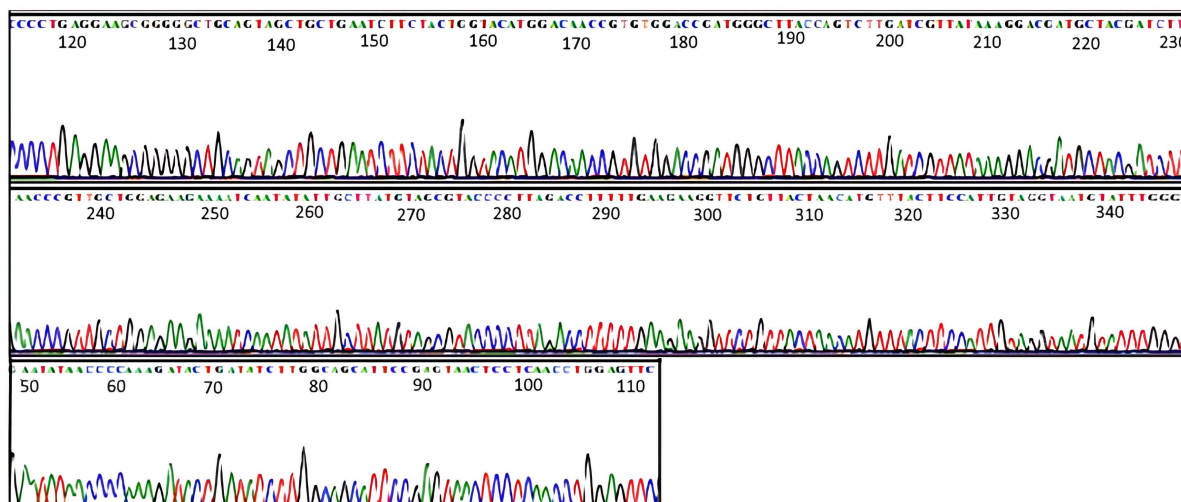


Fig. 4: Sequence of *rbcL* gene region sample LA07

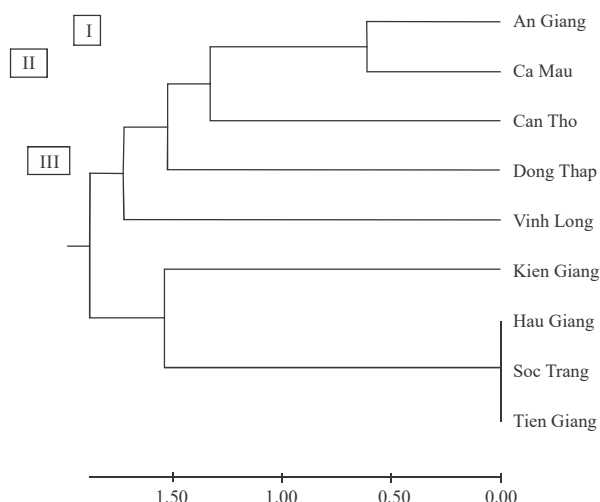


Fig. 5: Grouping of nine varieties/species *Passiflora foetida* L., based on the "*rbcL*" gene region sequence

belong to freshwater ecological zones such as An Giang, Can Tho, Dong Thap, Vinh Long, Tien Giang and Hau Giang, remaining provinces, such as Kien Giang, Ca Mau and Soc Trang, are saltwater and brackish ecoregions, but the cultivar/species samples may be similar as they are moved from one region to another in a different manner.

Thus, the research results show, even though in different ecological regions, the variety/ species patterns may be similar because they are moved from one region to another in a different way. Compare these newly discovered results with some morphological studies by other authors such as Duvall *et al.*¹⁸ and Kellogg and Juliano¹⁹ or genetics by Chase *et al.*¹⁰, Hasebe *et al.*²⁰, Savolainen *et al.*²¹ and Newmaster *et al.*¹¹. These results are beneficial in being able

to propagate medicinal plants to different ecological regions. However, more extensive research is needed, with a larger sample size, which will be more meaningful in the future.

CONCLUSION

Results showed that the nine varieties/species all coincided with the *Passiflora foetida* L. species sequences with a high similarity coefficient from 98.77 to 99.9%. Hence, this study is significant in confirming that it is possible to increase the productivity of medicinal plants by using therapy that affects their genes. The cultivation of algae seeds to improve the productivity of these medicinal plant varieties is important in providing raw materials for the oriental medicine industry. Thus, in the future, the oriental pharmaceutical industry can proactively source raw materials for production in large quantities.

SIGNIFICANCE STATEMENT

This research is essential for identifying medicinal plants genetically adapted to living conditions in the Mekong Delta, with the aim of building a raw material area of plant origin to supply the pharmaceutical industry products in Vietnam. Building raw material areas for the pharmaceutical industry towards large production requires stability in input materials, research to increase medicinal plant productivity is to meet this need. This study will help the researchers to uncover the critical areas of *Passiflora foetida* L., as a medicinal herb in the Mekong Delta. Vietnam where many researchers were not able to explore in this area.

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