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Research Article
Single Nucleotide Polymorphisms Marks of *Coccinia grandis* L., in the Mekong Delta

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Abstract

Background and Objective: Despite its ecological and agricultural importance, *Coccinia grandis* remains understudied in the Mekong Delta, particularly regarding morphological diversity and genetic variation. This study aims to analyze the species' morphological characteristics and genetic diversity using SNP markers. Specifically, it seeks to assess variations in morphological traits among different populations and evaluate genetic diversity and relationships through SNP analysis to better understand its population structure. **Materials and Methods:** The eight samples of *Coccinia grandis* L., the method of observing and describing the external morphology and microsurgery of *Coccinia grandis* L., was carried out based on the botanical research method with improvements to suit the experimental conditions. The quality of the DNA was checked by electrophoresis on a 1% agarose gel using safe-view dye. **Results:** The stem height, leaf length and root length show that there are ecological and nutritional influences leading to the above morphological differences. The results of comparing the sequences of 8 *Coccinia grandis* L., varieties on the NCBI GenBank show that the samples are similar to the sequence of the species *Coccinia grandis* L. **Conclusion:** With molecular biology techniques, it has been determined that all 8 *Coccinia grandis* L., varieties belong to the species *Coccinia grandis* L. From there, it can be applied to the pharmaceutical production industry to be more diversified.

Key words: Agronomy, morphology, SNP marker, gene *rbcL*, *Coccinia grandis*

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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

The *Coccinia grandis* L., is a plant that commonly appears in the South of Vietnam. In the past, they grew wild on hillsides throughout Asian countries, but today they are widely grown around the world.

Coccinia grandis L., has many health benefits, the leaves and stems help prevent spasms and are expectorants and the fruit has the effect of reducing symptoms of ulcers on the tongue. In India, the leaf and root juice are used to treat diabetes, wounds and snake bites. The leaves of the plant can inhibit the activity of the enzyme glucose-6-phosphatase. In folk medicine, the plant is also used to treat jaundice, bronchitis, psoriasis, herpes zoster and sexually transmitted diseases such as syphilis, gonorrhea[1,2].

With the advancement of science and technology, DNA barcodes have been developed and applied in practice based on using one or more DNA segments of different sizes as a standard for the rapid and accurate identification of species. The DNA barcode segments can be those located in the nuclear genome (18S, 5.8S, 26S, ITS, ...)[3-5]; mitochondrial genome (Cytb, CO1, ...)[6,7]. chloroplast genome (matK, rbcL, trnH-psbA, rpo, trnL-trnF, ycf, ...) Hollingsworth). Depending on the research object, barcode DNA segments will be used appropriately[7,8]. The *rbcL* sequence encodes the Rubisco enzyme involved in the carbon fixation process in plants, with the advantage of this sequence being easy to amplify in most plant species and is known as a standard locus in phylogenetic studies.

Therefore, a study on the genetic characteristics of the species of *Coccinia grandis* L., collected in the Mekong Delta was conducted to determine the genetic relationship between samples of this *Coccinia grandis* L., based on agronomic characteristics and the sequence of the "*rbcL*" gene region.

MATERIALS AND METHODS

Study area and materials: The study was conducted in the provinces of the Mekong Delta, Vietnam, from 2022 to 2024. Eight samples of *Coccinia grandis* L., were collected in the provinces of Ca Mau, Can Tho, Dong Thap, An Giang, Kien Giang, Tra Vinh, Hau Giang and Vinh Long. The samples were arranged in a completely random experiment with three replications. The experimental treatments were samples of *Coccinia grandis* L., collected in the provinces of the Mekong Delta.

Methods of morphological and agronomic observation: The method of observing and describing the external morphology and microsurgery of *Coccinia grandis* L., was carried out based on the botanical research method of Sanger *et al.*[9], with improvements to suit the experimental conditions. The parts described include the stem, leaves, roots and fruits; all of these agronomic indicators were measured and averaged over 10 sample plants. The measurement method is as follows:

- **Stem height (cm):** Entire stem above ground, from the ground to the end of the shoot tip was measured (Fig. 1a)
- **Leaf length (cm):** Length from the leaf petiole to the leaf tip and the width of the largest leaf blade was measured (Fig. 1b)
- **Root length (cm):** Underground part (mature region) to the root tip was measured (Fig. 1c)
- **Fruit length (cm):** Fruit bottom to the fruit stem was measured (Fig. 1d)

The microscopic method is as follows:

- Use a razor blade to cut the sample very thinly
- **For leaf samples:** Take 1/3 of the midrib from the junction with the petiole and a part of the leaf blade on both sides
- **Stem samples:** Cut at the internode
- **Root samples:** Cut at the middle part. Then use the carmine-iodine green double staining method (alum-iodine green double staining) to stain plant cells by: Soaking the cut slices in Javel solution for 15-30 min and washing them with distilled water many times

Soak the cut slices in 1-3% acetic acid solution for 2 min to remove the remaining Javel and wash with distilled water. Continue to soak in alum solution for about 15-30% min. Wash with distilled water until the washing solution is colorless. Mount the slide with 30% glycerin solution and observe under a microscope (OM157 40X-1000X, 1222 McDowell Avenue NE, Roanoke, Virginia 24012, USA) shown in Fig. 2a-c.

Molecular methods: Leaf samples were collected and frozen at -20°C. The samples were extracted and purified according to the correct procedure, the product was electrophoresed on a 1% agarose gel. The DNA extraction was performed at the Laboratory of Molecular Biology, Institute of Biotechnology and Food, Can Tho University. The total DNA of the samples was extracted from fresh leaf samples using the modified CTAB extraction procedure[10]. The quality of the DNA was checked by electrophoresis on a 1% agarose gel using safe view dye. Next, the DNA was amplified by PCR with primers specific for the "*rbcL*" gene region (F:5'-ATGTCACCACAAAC AGAGACTAAAGC-3'; R:5'-GTAAAATCAAGTCCACCRCG-3') and finally the amplified product was sequenced.

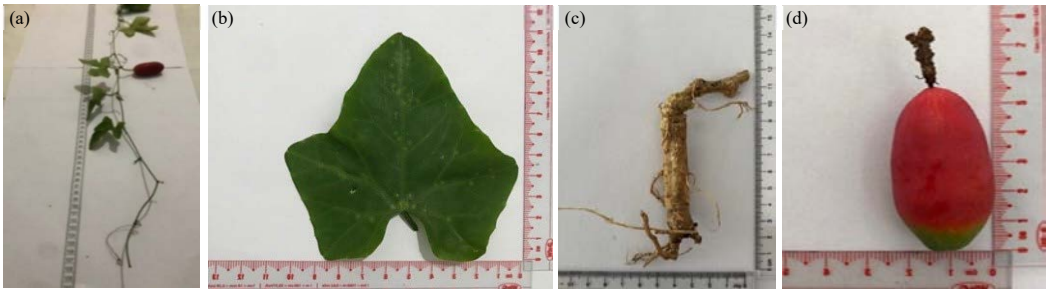

Fig. 1(a-d): Morphology of the *Coccinia grandis* L., (a) Stem, (b) Leaves, (c) Roots and (d) Fruit

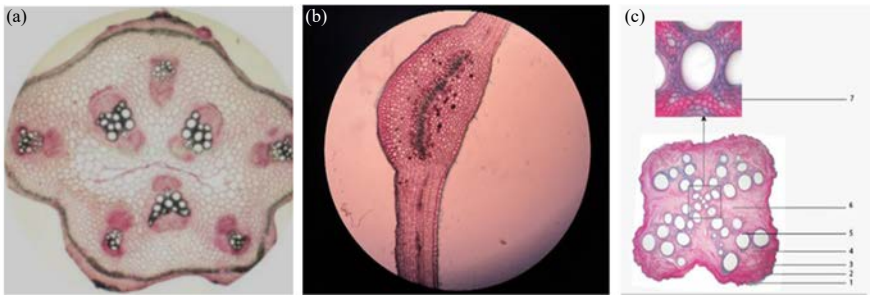

Fig. 2(a-c): Microscopic image of the *Coccinia grandis* L., (a) Stem, (b) Leaf and (c) Root

The thermal cycling protocol used in the PCR reaction consisted of 35 cycles with the following stages: Denaturation stage at 94°C for 4 min, 94°C for 30 sec, Annealing stage at 55°C for 30 sec, Extension stage at 72°C for 60 sec, finally, the stabilization stage of the chain product at 72°C for 10 min and finally the product was stored at 10°C. Molecular data related to the "*rbcL*" gene region sequences were analyzed and grouped using Mega 11-Software[11].

Data processing method: Agronomic data recorded such as stem height, leaf length and width, root length, flower length and fruit length were averaged using Microsoft Excel 2016 Software. To determine the genetic relationship between the eight samples of the Vietnamese cucurbita plant, the experimental results were constructed into a phylogenetic tree using Mega X-CC 3S Software with the Maximum likelihood method, 2-parameter Kimura model and a bootstrap index of 1000 times. The "*rbcL*" gene region sequences of the eight Vietnamese cucurbita plant samples used to construct the phylogenetic tree were aligned using BioEdit 7.2 Software and some noisy sequences at both ends of each sequence were removed.

Statistical analysis: The statistical method used for the analysis of the results is IBM SPSS Statistics 20 Software.

RESULTS AND DISCUSSION

Results of morphological and agronomic observations: The results of the agronomic characteristics of the eight samples are presented in Table 1. Specifically, stem height varied from 115.73 cm (Ca Mau) to 193.60 cm (Tra Vinh), which was the variable with the most variation. Statistical analysis showed a significant difference at the 1% significance level through the Duncan test. Phenotypic and genotypic variances were similar, indicating that stem height was contributed by genes. Leaf size among the samples of *Coccinia grandis* L., showed that leaf length varied more than width, from 9.0 cm (Can Tho) to 14.02 cm (Vinh Long). The results of the analysis of the coefficient of variation between phenotype and genotype were also similar and the heritability coefficient in the broad sense was also relatively high. Roots are important organs because they have the function of providing water and nutrients to the plant. Therefore, a well-developed root system will help the plant grow tall and strong. Root length between the samples varied from 8.85 cm (Dong Thap) to 21.13 cm (An Giang), according to Duncan's test, the difference was at a significance level of 5%. In terms of fruit morphology, the results of 10 replications at each sampling location showed a variation from 4.47 cm (Hau Giang) to 6.0 cm (Tra Vinh).

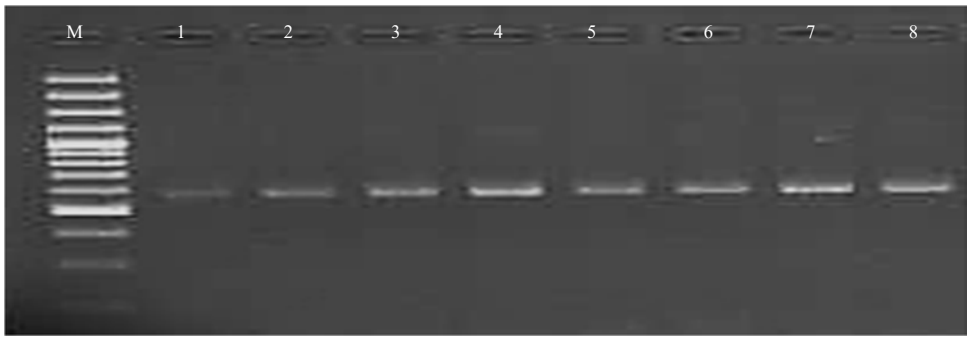

Fig. 3: Electrophoresis results of PCR products of 8 varieties of *Coccinia grandis* L.
M: Standard scale; 1: An Giang, 2: Ca Mau, 3: Can Tho, 4: Dong Thap, 5: Kien Giang, 6: Vinh Long, 7: Tra Vinh and 8: Hau Giang

Table 1: Agronomic characteristics of 8 samples of *Coccinia grandis* L.

Sampling location	Symbol	Stem height (cm)	Leaf length (cm)	Leaf width (cm)	Root length (cm)	Fruit length (cm)
An Giang	AG	136.87	11.37	4.05	21.13	4.71
Ca Mau	CM	115.73	12.01	10.16	14.73	5.36
Can Tho	CT	120.27	9.00	6.73	14.71	4.87
Đong Thap	DT	121.30	12.15	4.29	8.85	5.77
Kien Giang	KG	134.87	11.93	4.54	21.13	4.71
Vinh Long	VL	125.23	14.02	4.47	14.78	5.50
Tra Vinh	TV	193.60	11.19	10.25	20.69	6.00
Hau Giang	HG	187.35	10.15	4.39	9.75	4.47
Average		141.90	11.48	6.11	15.72	5.17
Audit		**	*	ns	*	*
Var. G		952.61	2.20	7.09	24.14	0.31
Var. P		833.54	1.92	6.20	21.12	0.27
GCV		6.71	0.19	1.16	1.54	0.06
PCV		5.87	0.17	1.01	1.34	0.05
H2 (%)		1.14	1.14	1.14	1.14	1.14
GA (%)		72.66	3.49	6.27	11.57	1.32

Var. G (variance of genotype): Genotypic variance, Var. P (variance of phenotype): Phenotypic variance, GCV (genotypic coefficient of variation): Genotypic coefficient of variation, PCV (phenotypic coefficient of variation): Phenotypic coefficient of variation, H2 (Heritability in a broad sense): Heritability in a broad sense, GA (genetic advance): Genetic advance and *Significance at 5% while **Significance at 1%

Extraction and purification results: After the samples were extracted and purified according to the correct procedure, the product was electrophoresed on a 1% agarose gel for thick, medium-bright bands, with no secondary bands, indicating that the DNA obtained was almost unbroken. The DNA concentration and purity were checked using a NanoDrop One spectrophotometer (Thermo Scientific, UK)[11] for DNA concentration results from 28.73-42.23 ng/µL and purity (OD: A260/A280) in the range of 1.81-2.1, which was sufficient to ensure the implementation of the following molecular techniques.

The PCR product of the *rbcL* sequence region of the *Coccinia grandis* Levine samples was about 600 bp in size (Fig. 3), the product was clear and clean with only 1 band, which was sequenced and used Blast N software to compare the DNA sequences obtained from 8 *Coccinia grandis* Levine samples in the NCBI data bank to confirm the samples that had been previously surveyed for morphology.

Sequencing of the "*rbcL*" gene region: This technique is based on the Sanger method[9]. Sent to the DNA Sequencing company, the DNA sequence results of the "*rbcL*" gene are shown in Fig. 4.

The results of comparing the sequences of 8 *Coccinia grandis* L., varieties on the NCBI GenBank show that the samples are similar to the sequence of the species *Coccinia grandis* L., with a coefficient of variation from 99.01 to 100%. This shows that the reliability of the "*rbcL*" sequence is quite high. The scientific names of the samples are presented in Table 2.

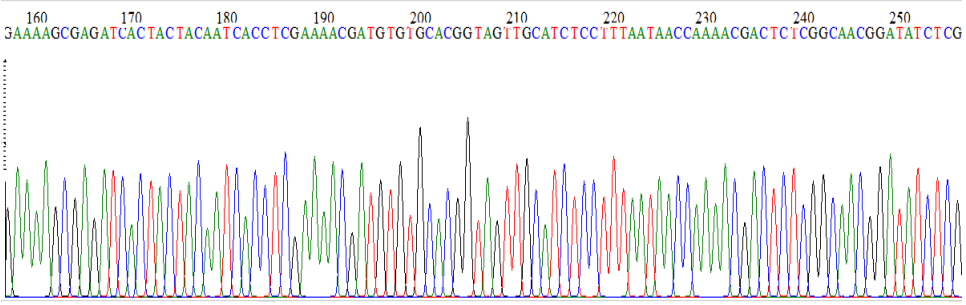

Fig. 4: Sequence signal of *rbcL* gene region of the *Coccinia grandis* L.

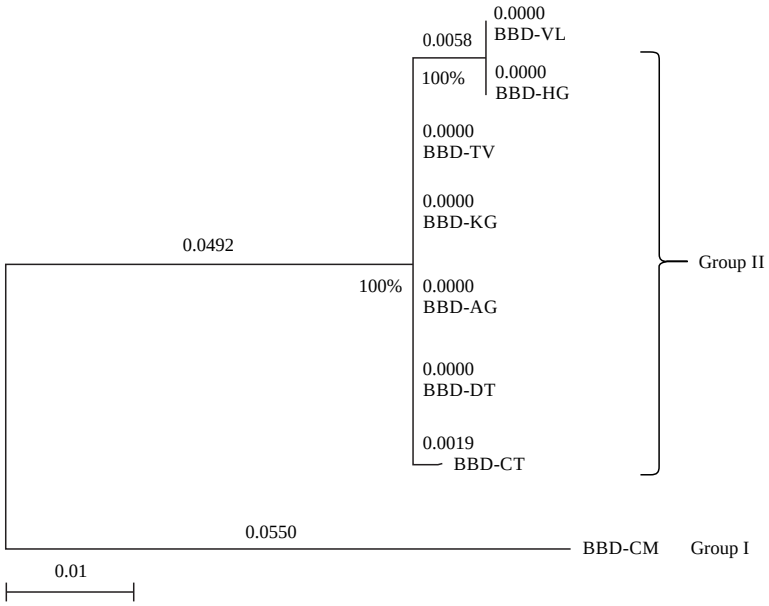

Fig. 5: Phylogenetic tree diagram of 8 samples of *Coccinia grandis* L., based on the sequence of the "*rbcL*" gene region

Table 2: Identification results of *Coccinia grandis* L., samples

Province	Identification result	Similarity (%)
An Giang	*Cucumis hystrix*	99.68
Ca Mau	*Coccinia grandis*	100
Can Tho	*Cucumis melo*	99.36
Đong Thap	*Cucumis myriocarpus*	100
Kien Giang	*Coccinia grandis*	100
Vinh Long	*Cotus Coccinia grandis*	100
Tra Vinh	*Cucumis myriocarpus*	100
Hau Giang	*Cucumis myriocarpus*	99.01

The genetic relationship of 8 samples of *Coccinia grandis* L., was determined through the construction of a phylogenetic tree based on the sequence of the "*rbcL*" gene region (Fig. 5). With the analysis results from the determined phylogenetic tree, the samples of *Coccinia grandis* with similar genetic indices were classified into two main groups with a genetic distance of 0.0992 including group I is the sample in Ca Mau (BBD-CM) and group II remaining samples. Within Group II, there are 3 subgroups including subgroup 1 is the samples in Vinh Long (BBD-VL), Hau Giang (BBD-HG); subgroup 2 is the sample in Can Tho (BBD-CT); the remaining group is the samples in Tra Vinh (BBD-TV), Kien Giang (BBD-KG), An Giang (BBD-AG) and Dong Thap (BBD-DT). Molecularly, the samples of *Coccinia grandis* Levine in Ca Mau (BBD-CM) have the longest genetic distance among the surveyed samples. Although the samples are from different localities, they are genetically similar, possibly because they were transferred from one region to another.

The study showed that stem height, leaf length and root length, indicate ecological and nutritional influences leading to morphological differences, this result is much different from the studies of some other authors[5,6,9]. Sequencing of 8 *Coccinia grandis* L., varieties on the NCBI GenBank showed that the samples had sequences similar to the sequence of *Coccinia grandis* L. These results are equivalent to previous studies[2,3]. The analysis of single nucleotide polymorphism markers of *Coccinia grandis* L., is considered a new finding compared to many published papers. Therefore, this result supports previous works, they do not contradict existing studies[10,11]. However, this study has some limitations such as the number of trees is not representative of all provinces in the Mekong Delta. Therefore, the research team proposed to expand the study to the remaining localities in the country. The research results are of great significance in providing a scientific basis for the conservation and propagation of valuable medicinal plants in the Mekong Delta and the Southern Provinces of Vietnam. The collected data are intended to recommend localities to expand the cultivation area and increase the productivity of these plants. However, there are currently many difficulties in propagating and determining their adaptability to the soil, climate and ecological conditions of each province.

The research results are also important to supplement the bank for the conservation of valuable medicinal plants in Vietnam. Based on the data from this bank, it is recommended that localities expand the cultivation area of these plants. However, there are still many difficulties in determining the compatibility between soil and climate factors in each ecological region and the biological characteristics of the plants.

CONCLUSION

The results of the morphological survey as well as the agronomic characteristics of the 8 *Coccinia grandis* L., varieties show differences. With the initial sequencing of the *rbcL* gene region on the 8 *Coccinia grandis* L., varieties, it was shown that the *Coccinia grandis* L., samples coincide with the species *Coccinia grandis* L., with a high similarity coefficient of over 99%. Based on the pedigree, the 8 *Coccinia grandis* L., varieties are classified into two groups, showing differences in ecological regions.

Based on the above conclusions, it is recommended to expand research on other medicinal plants in the provinces as in this study to develop a rich source of medicinal plants for the Mekong Delta.

SIGNIFICANCE STATEMENT

This study mainly focuses on the Single Nucleotide Polymorphism Marker of *Coccinia grandis* L., in the Mekong Delta. The results of the study have revealed genetic diversity among *Coccinia grandis* L., varieties, which may be beneficial for crop improvement and conservation strategies. This study will help researchers explore important areas of genetic variation and ecological adaptation. As a result, a new theory of evolutionary relationships and taxonomy of *Coccinia grandis* L., can be put forward. This study will help researchers explore important areas of *Coccinia grandis* L., such as herbalism and biodiversity. Therefore, the study is very necessary, especially for the pharmaceutical raw material areas in South Vietnam, where there is no data on the Asian pharmaceutical raw material market.

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