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Research Article

Genetic Study of Medicinal *Sauropus androgynus* (L.) Merr. used in Mekong River, Southern Vietnam

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Abstract

Background and Objective: *Sauropus androgynus* (L.) Merr., also known as coriander, is a shrub that grows wild in tropical Asia but is also cultivated as a vegetable in some countries. Determining the agronomic characteristics of 8 varieties of Bodhi tree, thereby seeing the genetic relationship between them, proposing a breeding strategy and effectively exploiting this genetic resource. **Materials and Methods:** Samples of grouper varieties were planted in eight provinces in Mekong Delta. Each seed sample was collected and measured the agronomic and morphological indicators were on 6 sample plants. Tree morphology observation and description based on improved plant research methods. **Results:** The genetic relationships of 8 samples/cultivars based on the ITS sequence showed that samples of Vinh Long and Thuan Hung, Soc Trang were in the same group, samples of Hau Giang and Dong Thap in the second group, the remaining Kien Giang, Ca Mau, Can Tho and Chau Thanh and Soc Trang belong to each group. **Conclusion:** Samples/cultivars collected from eight provinces in the Mekong Delta showed variation in agronomic characteristics, but only stem height and length changes were statistically significantly different.

Key words: ITS marker, phylogenetic tree, *Sauropus androgynus*, agronomic

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

INTRODUCTION

Sauropus androgynus (L.) Merr.), also known as coriander, is a shrub that grows wild in tropical Asia but is also cultivated as a vegetable in some countries, especially in Vietnam. India, Malaysia, Indonesia, China^{1,2}. In Vietnam, greens are grown a lot, especially in the Northern provinces and the Mekong Delta provinces. This vegetable is often grown as medicine, fence or used as a vegetable in daily meals.

Since ancient times, along with Western medicine, traditional Eastern medicinal values have had great significance in curing diseases, so the research and breeding of *Sauropus androgynus* (L.) as raw materials for traditional medicine is invaluable equally important.

The Mekong Delta is the largest in Vietnam, where there is a rich ecosystem, especially flora. Therefore, it is necessary to study the genetic relationship between them. There have been several studies on this issue such as "Optimization of DNA isolation and PCR protocol for analysis and evaluation of genetic diversity of the medicinal plant, *Anemopsis californica* using RAPD"³ or "Optimizing the pure genomic DNA isolation procedure for *Plectranthus amboinicus*"⁴. Through isolating plant DNA from fresh tissue, it quickly spreads rare and precious plant varieties that need to be preserved for replication to different localities. Study on "Vietnamese traditional medicine from a pharmacist's perspective"⁵. This is a new study at the molecular level of the relationship between plants in the Mekong Delta. The results of Woerdenbag *et al.*⁵ research show that the role of each plant species in the prevention and treatment of common diseases and diseases specific to the tropics is very effective, especially with the varieties of *Sauropus androgynus* (L.) in the Mekong Delta.

However, up to now, research on these varieties has not been focused and systematic⁶. To meet that practical need, research on morphological characteristics and genetic relationships of the lupus seed samples was conducted in eight provinces in the Mekong Delta. This is a new study at the molecular level of the relationship between plants in the Mekong Delta. The objective was, initially to identify morphological characteristics and the agronomy of the eight varieties of *Sauropus androgynus* (L.) variety through which the genetic relationship between them will be seen, from that, will propose a suitable strategy to propagate this genetic resource effectively. This study also contributes to the conservation and development of rare and precious varieties of *Sauropus androgynus* (L.) in Vietnam.

MATERIALS AND METHODS

Materials: Samples of grouper varieties planted in eight provinces including Vinh Long, Kien Giang, Soc Trang, Ben Tre, Ca Mau, Hau Giang, Can Tho and Dong Thap was collected, planted, surveyed and evaluated. Each seed sample was collected and measured the agronomic and morphological indicators were on 6 sample plants. The time was made from February, 2018 to June, 2020.

Morphological method: Tree morphology observation and description based on improved plant research methods⁶, descriptive and metering components including roots, stems, leaves, flowers, fruits and seeds.

Layout: The experiment was arranged in a completely randomized format with a sampling factor. Each seed sample was planted in 3 pots, with 2 plants in each pot. The pot has a height of 30.5 cm, a diameter of 35 cm with a cylindrical shape with a volume of 29329 cm³. The basin is made of nylon and is black which many drainage holes. The amount of soil is more than 2/3 of the height of the pot, each pot contains about 10 kg of clean soil, for a total of 24 pots. The pots are arranged at a distance of 70×50 cm.

Young and fresh leaf samples were collected and frozen at -20°C. The DNA extraction was performed at the Laboratory of Molecular Biotechnology, Biotechnology Research and Development Institute, Can Tho University. Total DNA of the lupus cultivars was extracted from fresh leaf samples following an improved CTAB extraction procedure (Lingling Zhang)⁷.

After purification the DNA quality test by electrophoresis on 1% agarose gel, after electrophoresis, the gel was dyed with a Redsafe dye (Biobasic, UK). The PCR amplification was performed with primers and ITS region sequencing⁸. The sequence for the two primers is as follows:

- ITS 1: 5'-TCCGTAGGTGAACCGCGG-3'
- ITS 4: 5'-TCCTCCGCTTATTGATATGC-3'

The PCR reaction includes 35 heating cycles, a denaturation phase with 5 min at 95°C, 60 sec at 95°C, hits 50 sec at 54°C, lasts 90 sec at 72°C, extends the chain for 5 min at 72°C and the product is stored at 10°C for 20 min. Electrophoresis of PCR products and then purified with Wizard SV gel and PCR Clean-up System (Promega) kit. Based on the Obrador-Sánchez José Abraham method. The DNA sequencing by DNA Sequencing Services General Order Information Company, Korea on automatic sequence reader.

Data processing method: The morphological and agronomic data have calculated the mean of the 6 seed samples and the standard deviation (Standard Deviation (SD) using Microsoft Excel 10.0 software. The DNA molecular weight was calculated using the Gel Analyzer software. The results of the ITS free explanation were saved in FASTA format and then analyzed using Bioedit ver. 7.0.5. Then using BLAST on the NCBI gene bank system (National Center for Biotechnology Information) was used to evaluate the similarity. The genealogical tree was drawn using Mega 7.0 software according to the UPGMA (Unweighted Pair-Group Method, Arithmetic Average) method to determine the genetic relationship between eight mudflows collected in the river delta. Kowloon.

RESULTS AND DISCUSSION

Morphological and agronomic properties: The morphological and agronomic characteristics of the *Sauropus androgynus* (L.) varieties in eight provinces of the Mekong Delta are shown in Table 1. Body height ranges from 23.11 (Soc Trang) to 81.0 cm (Kien Giang). This result is consistent with the results previously published^{5,9,10}.

The average leaf length varied from 4.32 (Ca Mau) to 5.75 cm (Soc Trang). Leaf width varies from 3.15 (Soc Trang) to 3.19 cm (Vinh Long). Meanwhile, the shortest average root length is 11.62 cm (Hau Giang) and the longest 21.8 cm (Dong Thap).

The average root length varied from 12.12 (Hau Giang) to 22.50 cm (Dong Thap). This result is consistent with the results previously published^{1,5,6}.

The average flower diameter varies from 3.31 (Can Tho) to 4.14 cm (Ca Mau). This result is relatively consistent with the results published previously. The average flower length varied from 0.85 (Ca Mau) to 1.14 cm (Hau Giang). The average fruit length varied from 0.63 (Kien Giang) to 1.13 cm (Hau Giang). This result is consistent with the results previously published^{1,5,6}.

Through the statistical analysis, the results showed that only the stem height and root length of the eight cultivars were statistically significant at the 0.05 level.

Comparison of the similarity of the ITS sequence: The ITS is one of the most common DNA markers used for genetic analysis and the identification of plant varieties/species. The results of the comparison of ITS sequence on NCBI bank are presented in Table 2. Most of the sequences of eight varieties have the lowest similarity value of 91.25% (B4-Ben Tre province) and the highest 99.44% (B5-Hau Giang province). Thereby, it shows that these varieties belong to the species *Sauropus androgynus* (L.) Merr.

These results are also found in the study of before^{1,5,6,8} that the other the Medical plants, also as for *Sauropus androgynus* (L.) Merr⁹⁻¹¹.

Table 1: Morphological and agronomic characteristics of the eight varieties of lupus (cm)

Location	Stem height	Leaf length	Leaf width	Root length	Flower diameter	Flower length	Fruit length
Vinh Long	75.60 ^a	5.16 ^a	3.19 ^a	13.60 ^b	3.60 ^a	0.92 ^a	0.88 ^a
Soc Trang	23.11 ^d	5.75 ^a	3.15 ^a	15.42 ^b	3.47 ^a	0.86 ^a	1.13 ^a
Can Tho	78.00 ^a	5.10 ^a	3.18 ^a	13.61 ^b	3.31 ^a	0.94 ^a	0.84 ^a
Ben tre	43.22 ^c	4.85 ^a	3.16 ^a	15.22 ^b	3.44 ^a	0.84 ^a	1.06 ^a
Hau Giang	71.20 ^{ab}	5.31 ^a	3.11 ^a	12.12 ^b	3.34 ^a	1.14 ^a	1.13 ^a
Ca Mau	64.21 ^b	4.32 ^a	3.12 ^a	12.09 ^b	4.14 ^a	0.85 ^a	1.12 ^a
Dong Thap	36.40 ^c	5.16 ^a	2.70 ^a	22.50 ^a	3.32 ^a	0.56 ^a	1.20 ^a
Kien Giang	81.00 ^a	5.41 ^a	2.67 ^a	13.82 ^b	3.86 ^a	0.88 ^a	0.63 ^a
F	34.1023	0.468 ^{ns}	1.368 ^{ns}	6.708	1.008 ^{ns}	0.521 ^{ns}	1.126 ^{ns}
CV (%)	14.21	16.68	11.81	18.30	12.76	2.12	19.45

ns: Non-significant and different alphabets showed a different significant level

Table 2: Similarity values of 8 lupus seed samples when comparing sequence on NCBI bank

Sample	Location	NCBI Bank	Similarity value (%)	References
B1	Vinh Long	<i>Sauropus androgynus</i> 18S, ITS 1	98.75	Pruesapan <i>et al.</i> ¹¹
B2	Soc Trang	<i>Sauropus androgynus</i> Genes for ITS 1	96.51	Pruesapan <i>et al.</i> ¹¹
B3	Can Tho	<i>Sauropus androgynus</i> Genes for ITS 1	97.52	Pruesapan <i>et al.</i> ¹¹
B4	Ben tre	<i>Sauropus androgynus</i> Genes for ITS 1	91.25	Pruesapan <i>et al.</i> ¹¹
B5	Hau Giang	<i>Sauropus androgynus</i> 18S, ITS 1	99.44	Pruesapan <i>et al.</i> ¹¹
B6	Ca Mau	<i>Sauropus androgynus</i> 18S, ITS 1	98.89	Pruesapan <i>et al.</i> ¹¹
B7	Dong Thap	<i>Sauropus androgynus</i> 18S, ITS 1	98.96	Pruesapan <i>et al.</i> ¹¹
B8	Kien Giang	<i>Sauropus androgynus</i> 18S, ITS 1	98.62	Pruesapan <i>et al.</i> ¹¹

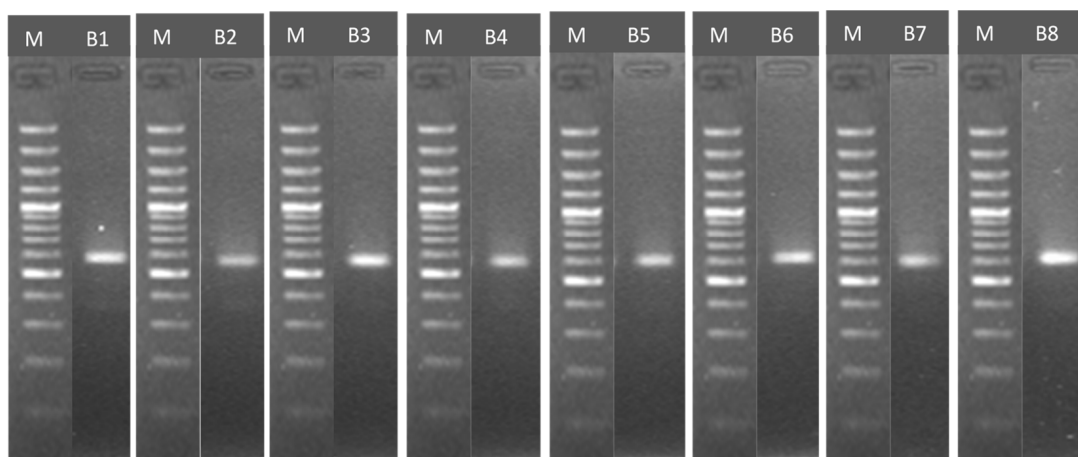


Fig. 1: Amplification band in the range 600-670 bp

M: Ladder 1000 bp and B1-B8: Samples collected in eight provinces of the Mekong Delta

Results of the ITS segment sequence and the genetic relationship: The products of the eight varieties of lupus all gave an amplification band in the range 600-670 bp, this result is shown in Fig. 1. Its sequencing ITS region in eight samples of lupus, approximately 640-670 nucleotides. These results are consistent with the previous studies¹².

Investigate the genetic relationship of the lupus variety based on the given ITS sequence eight seed samples were found, divided into 5 groups.

Group I has 2 subgroups. Sub-group 1 consists of two samples of Bo Gau variety grown in Vinh Long and Soc Trang, Sub-group 2 has seed samples grown in Hau Giang and Dong Thap. The remaining varieties grown in Kien Giang, Ca Mau, Can Tho and Ben Tre are grouped separately. This can be explained that these varieties are grown in geographical areas with different ecological conditions, so there is a little change in the nucleotide sequence in the ITS, typically Kien Giang, Ca Mau and Soc Trang is located near the sea, so the land is salty in the dry months, while Can Tho is a region with better climatic conditions because it is located in the centre of the Mekong Delta, the land is almost not salty and copper the weather has a more temperate climate

CONCLUSION

Samples/cultivars collected from eight provinces in the Mekong Delta showed variation in agronomic characteristics, but only stem height and length changes were statistically significantly different. millet rate is 0.05, which is due to different ecological and environmental conditions including different soil, temperature and humidity. In addition, the farming conditions by growers in each place is also different.

Genetically, the Can Tho, Ca Mau, Kien Giang and Ben Tre groups are quite different from the groups in Vinh Long, Hau Giang, Soc Trang and Dong Thap provinces. Analysis results using Mega 7.0 software according to the UPGMA method showed that eight samples/cultivars based on ITS sequence could be divided into 5 groups.

SIGNIFICANCE STATEMENT

This study will help the researchers to uncover the critical areas of medicinal characteristics that many researchers were not able to explore in the Mekong Delta. Thus a new theory on environmental conditions may be arrived at.

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