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Research Article
Genetic Relationships of Some Samples of Ginger (*Zingiber officinale* (Wild) Roscoe) as Medicinal Herbs in the Cuu Long River Delta, Vietnam

[1]Thieu Van Duong, [1]Do Van Mai, [2]Truong Trong Ngon and [3]Mai Van Hung

[1]Faculty of Pharmacy, Tay Do University, Vietnam
[2]Institute for Research and Development of Biotechnology, Can Tho University, Vietnam
[3]Department of Natural Sciences, VNU University of Education, Hanoi, Vietnam

Abstract

Background and Objective: Ginger (*Zingiber officinale* (Wild). Roscoe) is a plant that has long been used as a medicinal herb for humans, so it has been grown and popularized in the Mekong Delta Provinces, but the research systematic genetics has not been given much attention. The current study evaluated the genetic characteristics of some ginger samples collected in eight provinces in the Mekong Delta based on morphological characteristics and ITS gene sequence regions. **Materials and Methods:** Samples of Ginger varieties were collected in eight provinces, fresh leaf samples were collected and stored frozen at -20°C. Ginger morphology observation and description based on improved plant research methods. Total DNA extraction of ginger leaf samples was extracted from fresh leaf samples according to the extraction procedure by the modified CTAB method. **Results:** The phenotypes of the samples like length, leaf width, flower length and fruit diameter were significantly different between growing regions due to different environmental and farming conditions. Genetic relationship analysis showed that there are two distinct groups. Group I has 5 ginger samples from G8-Vinh Long, G4-Ben Tre, G2-Ca Mau, G5-Dong Thap and G6-Hau Giang provinces and group II includes ginger varieties belonging to G7-Ho Chi Minh City, G1-An Giang Province and G3-City. Can Tho. **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. Genotyping most of the samples belonged to the species *Zingiber officinale* (Wild) Roscoe.

Key words: Ginger, medicinal herbs, genetics, morphology

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Corresponding Author: Mai Van Hung, Department of Natural Sciences, VNU University of Education, Hanoi, Vietnam

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

Ginger (*Zingiber officinale* (Wild) Roscoe) is a plant that has long been used as a medicine in Vietnam, different types of ginger have been grown a lot, especially in the Northern Provinces and the Mekong Delta Provinces[1-3]. These plants are often grown to make medicine. In addition to the morphology studied by many authors[4,5]. DNA markers are now considered useful tools for species identification in plants as well as in animals[6]. Sequencing the ITS region of nuclear rDNA has been widely used for the identification of traditional Chinese medicinal plants[7,8]. In addition, the ITS region of rDNA has been defined as the genetic unit, which can be amplified by PCR (Polymerase Chain reaction) with specific primers[9,10]. On that basis, many authors have provided some data on the distribution area, life form and ecology as well as the use-value of each species. However, up to now, the research about this ginger variety has not been paid much attention and the research is not systematic. To meet that practical need, an experiment to study the morphological characteristics and genetic relationships between ginger varieties collected in eight provinces in the Mekong Delta, Vietnam was carried out, in the initial step. To determine the morphological characteristics and genetic relationships of 8 samples of ginger, this study investigated appropriate methods to propagate, conserve and exploit genetic resources effectively.

MATERIALS AND METHODS

The study was carried out from February to November, 2021 in South Vietnam, Ginger varieties were collected from eight provinces in the Mekong Delta, including Ca Mau, Dong Thap, Hau Giang, Ben Tre, Vinh Long, Ho Chi Minh (HCM) city, An Giang and Can Tho were surveyed and sampled. Sampling locations across 8 provinces and cities were presented in Table 1.

At each collection site, each ginger root has an equivalent weight of about 200-250 g, then brought back and planted in pots. The experiment was arranged in a completely randomized design in a greenhouse. After being brought back, ginger root samples were planted in pots with 4 replicates for each seed sample. The size of the pot is 35 cm in diameter, 30.5 cm in height. Soil is clean soil rich in nutrients TRiBAT produced by Saigon Green Biotechnology Company. The soil is mixed with a ratio of 2/3 of TRiBAT clean soil with 1/3 of rice husk ash and mixed well before being placed in pots for planting. The period was from January, 2020 to June, 2021.

Morphological method: Gingers morphology observation and description based on improved plant research methods[4,11], descriptive and metering components including roots, stems, leaves, flowers, fruits and seeds.

DNA extraction method and PCR reaction: Fresh leaf samples were collected and stored frozen at -20°C. The DNA extraction was carried out at the Laboratory of Molecular Biotechnology, Biotechnology Research and Development Institute, Can Tho University. The total DNA of ginger-like leaf samples was extracted from fresh leaf samples according to the extraction procedure by the modified CTAB method[12,13]. After purification, check the quality of DNA by electrophoresis on 1% agarose gel. After electrophoresis, gels were stained with Redsafe dye (Biobasic, UK). The PCR reaction was performed with primer pairs and ITS region gene sequencing[14]. The sequence of the two primer pairs is as follows:

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

The PCR reaction consisted of 35 heating cycles, a denaturation phase with 5 min at 95°C, 60 sec at 95°C, 50 sec pairing at 54°C, 90 sec time at 72°C, 5 min chain elongation 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). Including Taq polymerase of Promega (Singapore). PCR reaction was based on Sanger method[15,16]. Each dideoxynucleotide is labelled with

Table 1: Ginger sampling locations in eight provinces and cities in the Mekong Delta

Provinces/city	Longitude	Latitude
G1-An Giang	105°21′89″ E	10°29′94″ N
G2-Ca Mau	105°07′29″ E	9°09′24″ N
G3-Can Tho	105°42′20″ E	10°05′54″ N
G4-Ben Tre	106°35′15″ E	10°05′37″ N
G5-Dong Thap	105°38′45″ E	10°28′82″ N
G6-Hau Giang	105°43′38″ E	10°04′25″ N
G7-HCM city	120°41′05″ E	10°46′03″ N
G8-Vinh Long	105°49′19″ E	10°04′03″ N

a different coloured fluorescent agent. Thus, oligonucleotides ending at a Dideoxynucleotide will have the same colour. The DNA sequencing was performed by DNA Sequencing Services General Order Information[9,17], in Korea based on an automated sequencer.

Data processing methods: The morphological and agronomic data were calculated as the mean of 4 samples for each variety, then the standard deviation (SD). All data were calculated using Microsoft Excel 2016. The molecular weight of DNA was calculated using Gel Analyzer software. ITS fragment sequencing results were saved as FASTA and then analyzed using BioEdit ver software 7.0.5. Then use BLAST software on the NCBI gene bank system (National Center for Biotechnology Information) to evaluate the similarity. The genetic relationships of eight ginger cultivars were plotted using Mega 7.0 software.3.

RESULTS

Morphological and agronomic characteristics: The agronomic characteristics of eight ginger samples were presented in Table 2. Stem height ranges from 41.60 cm (Ben Tre) to 64.50cm (Can Tho). This result was consistent with previously published results[3,4,6]. Meanwhile, the average leaf length varies from 15.20 cm (G3-Can Tho) to 19.50 cm (G1-An Giang) and leaf width varies from 2.25 cm (G6-Hau Giang) to 2.85 cm (G1-An Giang). The development of stems and leaves is more or less influenced by the root system, especially in ginger, which is why root length is important. The variation in root length from 3.90 cm (Can Tho) to 4.69 cm (Ca Mau), this result shows that it is also consistent with previously published results[5,17,18].

For plants in general and ginger in particular, the growth of the plant goes through two stages. The vegetative stage of the plant develops roots, stems and leaves, then the plant moves to the stage of sexual development, flowering and fruiting. The average flower length of ginger flowers varies from 3.38 cm (G5-Dong Thap) to 5.10 cm (G4-Ben Tre).

ITS gene region sequencing results and genetic relationships: The PCR products of the eight ginger samples all gave amplification bands in the 700 bp range. The results were shown in Fig. 1. It's sequencing ITS region in eight samples of ginger, approximately 650-690 nucleotides. These results are consistent with the previous studies[5,6,9].

Table 2: Agronomic characteristics of eight ginger varieties (unit: cm)

Provinces/city	Stem height	Leaf length	Leaf width	Root length	Flower diameter	Flower length
G1-An Giang	56.20±17.94	19.50±1.41	2.85±0.28	4.42±0.55	4.50±0.62	6.10±0.65
G2-Ca Mau	50.50±12.25	19.41±1.78	2.36±0.41	4.69±0.51	4.15±0.58	6.20±0.75
G3-Can Tho	64.50±12.61	15.20±2.28	2.50±0.61	3.90±0.27	3.50±0.48	7.20±0.64
G4-Ben Tre	41.60±3.01	18.60±1.14	2.60±0.49	3.82±0.26	5.10±0.43	6.40±0.71
G5-Dong Thap	50.10±11.44	19.41±1.65	2.36±0.40	4.10±0.36	3.38±0.57	7.35±0.47
G6-Hau Giang	52.09±15.97	18.19±1.64	2.25±0.31	4.50±0.35	4.10±0.32	7.10±0.85
G7-HCM city	43.40±15.50	18.60±1.33	2.35±0.34	4.37±0.39	4.47±0.37	6.30±0.78
G8-Vinh Long	43.70±15.32	18.50±0.86	2.40±0.33	4.40±0.40	3.80±0.59	6.65±0.79

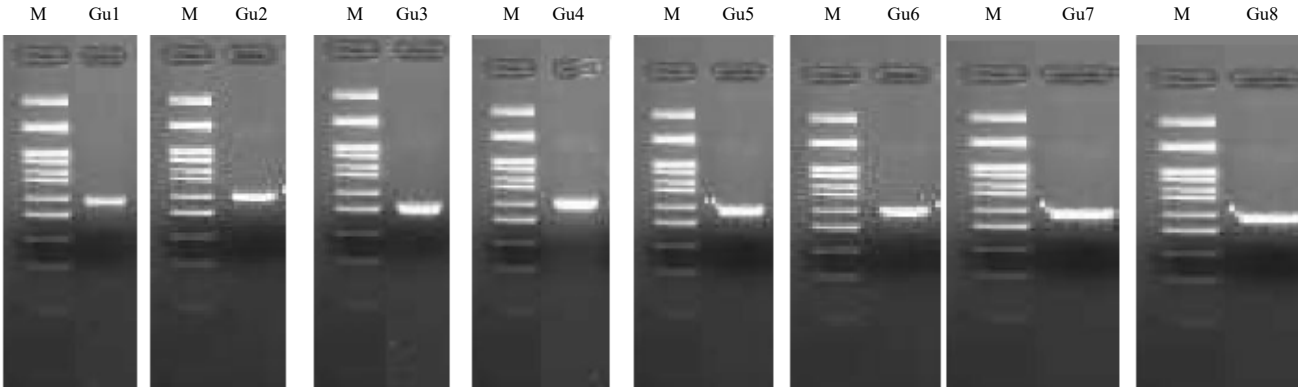

Fig. 1: PCR products of eight ginger samples collected in the Mekong Delta
M: Ladder 1000 bp, G1: An Giang, G2: Ca Mau, G3: Can Tho, G4: Ben Tre, G5: Dong Thap, G6: Hau Giang, G7: HCM and G8: Vinh Long

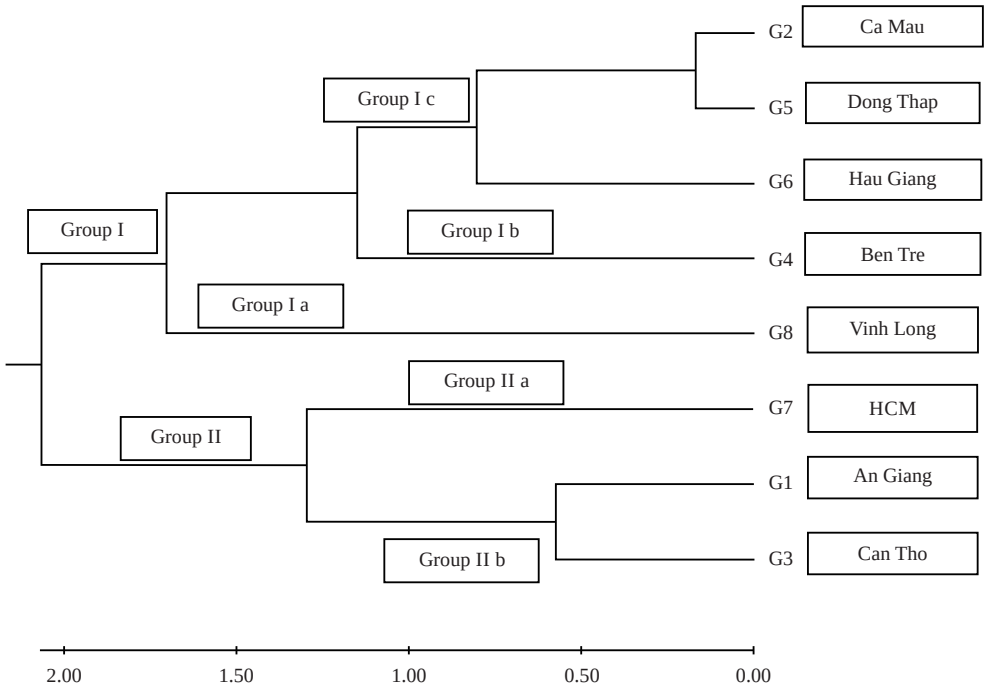

Fig. 2: Genetic relationships of 8 ginger samples from the Mekong Delta Provinces based on ITS sequences

By surveying the genetic relationships of eight ginger samples grown in eight provinces in the Mekong Delta based on the ITS sequence. The ginger samples were divided into 2 main groups: Group I has 5 samples of ginger varieties belonging to different regions. Vinh Long province (belonging to subgroup Ia). Ben Tre Province to subgroup Ib, Ca Mau, Dong Thap and Hau Giang to subgroup Ic, in which ginger samples from Ca Mau and Dong Thap Provinces are genetically close to each other. Groups Ia and Ib showed average values across 6 agronomic characteristics. Meanwhile, Group II includes ginger samples from HCM City (subgroup IIa). The remaining An Giang and Can Tho cultivars that are genetically close to each other belong to subgroup IIb. In group Iib, ginger samples collected from Can Tho and An Giang had relatively high stem height. The rest of group IIa only had ginger samples from HCM city showed average values compared to other groups across 6 agronomic characteristics. This can be explained by the fact that these ginger samples are grown in geographical areas with different ecological conditions. So there is a slight change in the nucleotide sequence in the ITS region so that they can live and grow well. However, it is necessary to pay attention to ginger samples such as Vinh Long. Ben Tre and Hau Giang due to very different ecological conditions. so although they are classified in subgroups Ia. Ib and Ic are separate in each group. In group II, there were samples of ginger from HCM City that is very different (subgroup IIa) compared to two ginger samples from Can Tho and An Giang Provinces (both in subgroup IIb) in Fig. 2.

DISCUSSION

The result of the study are presented in Table 2 showed was relatively consistent with previously published results[5,18,19]. The process of pollination and fertilization occurs after flowering for the plant to produce fruit and seeds. The diameter of the fruit varies from 6.10 cm (G1-An Giang) to 7.20 cm (G3-Can Tho). The variation in fruit diameter did not change much across the collected ginger varieties. This shows that fruit diameter was relatively less affected by environmental factors[20].

Compare similar sequences: The results of comparing ITS sequences on NCBI bank were showed that most of the sequences of eight Ginger samples showed high similarity values. the lowest 92.44% (G4-Ben Tre) and the highest, 95.60% (G1-An Giang). Theoretically. when comparing the similarity value with the original sequence sample on NCBI. the percentage is high or low depending on the length of the base pairs-bp sequence. The results of the study of the ITS fragment has a relatively short length (670-700 bp). So the percentage above 90% was considered a relatively high value according to many authors published[7,19,20].

Sample G1 in An Giang Province compared with *Zingiber officinale* voucher BBLZ5G13 18S. Internal transcribed spacer 1 has a similar value of 95.60%, sample G2 in Ca Mau province compared with *Zingiber officinale* voucher BBLZ5G13 18S. internal transcribed spacer 1 has a similar value of 92.50% and Sample G7 in HCM city compared with *Zingiber officinale* voucher BBLZ5G13. internal transcribed spacer 1 has a similar value of 93.56% and Sample G8 in Vinh Long Province compared with *Zingiber officinale* voucher BBLZ5G13. Internal transcribed spacer 1 has a similar value of 94.60% (Das, A., R.K. Sahoo and E. Subudhi, 2015. ITS region of Odisha ginger).

Sample G3 in Can Tho Province compare with *Zingiber officinale* voucher IMGHPKN051 18S. Internal transcribed spacer 1 has a similar value of 94.89%.

Sample G4 in Ben Tre province compare with *Zingiber officinale* isolate 35.1 clone 18S. internal transcribed spacer 1 has a similar Value of 92.44%. Sample G5 in Dong Thap province compare with *Zingiber officinale* isolate 35.2 clone 18S. internal transcribed spacer 1 has a similarity value of 94.43% and Sample G6 in Hau Giang province compared with *Zingiber officinale* isolate 35.1 clone 18S. Internal transcribed spacer 1 has a similarity value of 95.43%. Past evolutionary history dampens barcode success in Indian Zingiberaceae. So these results showed that the ginger varieties belong to the same group species *Zingiber officinale.*

CONCLUSION

Ginger seed samples collected in eight provinces in the Mekong Delta showed variation in agronomic properties. this difference is because the ginger cultivar was grown individually in each pot. So it was more or less affected micro-environmental factors such as temperature humidity as well as light intensity in each pot. In addition. when collecting samples of ginger root in each place with different cultivation conditions by growers. It also affects the quality of ginger seeds more or less. In general, the ginger varieties in group Ic had higher values than those in group II in all 6 agronomic characteristics. Particularly. groups Ia and Ib gave average values through 6 agronomic characteristics. In group IIb, ginger cultivars collected from Can Tho and An Giang had relatively high stem height. Remaining for group IIa. only the ginger variety HCM City showed average values compared to other groups across 6 agronomic characteristics.

SIGNIFICANCE STATEMENT

This study will help the researchers to uncover the critical areas of ginger (*Zingiber officinale* (Wild) Roscoe) as medicinal herbs in the Mekong Delta, Vietnam where many researchers were not able to explore this area.

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