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GENETIC ANLYSIS OF SEVEN GARLIC VARIETIES FROM PROMINENT GROWING AREAS IN SOUTHERN VIETNAM

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Abstract

This study aimed to assess the genetic similarity and diversity of seven garlic accessions from Vietnam using Simple Sequence Repeat (SSR) markers. Whole garlic bulbs were used for DNA extraction, which was performed using the GeneJET Plant Genomic DNA Purification Kit. DNA concentration and purity were determined using a NanoVue Plus spectrometer. PCR procedures for each primer set were optimized, and the best amplification temperature regime was selected. Of the ten SSR primer sets used, Asa04, Asa06, Asa07, Asa08, Asa16, Asa18, and Asa20 showed good amplifications with bright, clear bands. In contrast, Asa10 and Asa14 did not amplify the DNA of any samples, and Asa17 did not amplify the DNA from the NhonHai, VanHai, and NinhHoa samples but did for the other samples. The genetic distance matrix revealed high similarity among the garlic samples, ranging from 0.90 to 1.00. The phylogenetic analysis grouped the samples into two clusters: Group I (VinhHai1, VinhHai2, VinhHai3, and LySon) and Group II (NhonHai, VanHai, and NinhHoa), suggesting that the samples within each group likely originated from the same source. These findings highlight the necessity of using more molecular markers and conducting in-depth biochemical analyses to further understand the genetic and biochemical differences between garlic varieties in Vietnam. This study provides a foundation for future research on garlic breeding and cultivar improvement.

Key words: Genetic similarity, Garlic varieties, Genetic Analysis, Phylogenetic Tree.

1. Introduction

Garlic (Allium sativum L.) belongs to the family Alliaceae and is native to Central Asia, where many wild varieties thrive in regions with abundant sunlight, low humidity, and significant diurnal temperature variations. Garlic is renowned for its health benefits, including cancer prevention, anti-hypertensive properties, and lipid-lowering effects (Do T.L., 2003). Garlic is cultivated globally, with significant production in Asia, particularly in Vietnam, China, Indonesia, Pakistan, South Korea, Thailand, and India. In Vietnam, garlic cultivation has a long history and is widespread, especially in the provinces of Hai Duong, Vinh Phuc, Bac Ninh, Quang Ngai, Ninh Thuan, and Nha Trang, covering a total area of 6,000 hectares. However, the genetic similarity and diversity of the garlic population in Vietnam are still unknown. While many garlic varieties in Vietnam are of foreign origin, a number of valuable local varieties have emerged through natural selection. The Phan Rang garlic variety has gained significant reputation in Vietnam, but its market reputation is likely affected by the impurity of the variety. Assessing the genetic diversity and purity of garlic varieties is crucial for establishing an effective cultivar improvement program. Molecular marker-based analysis provides a robust basis for variety identification and supports the legal development of garlic varieties, especially for the Phan Rang garlic variety brand. Among the current molecular methods, Simple Sequence Repeat (SSR) markers are notable for their high diversity and widespread distribution within the genome (Frascaroli et al., 2013). Due to these characteristics, SSR markers are widely used in breeding, developing gene maps, studying genetic linkage, and population genetics (Goldstein et al., 1995; Gonzalo et al., 2005; Wight, 1959; Wu et al., 1993). The objective of this study is to assess the genetic similarity and diversity of seven garlic accessions using SSR markers.

Materials and methods

The study utilized seven garlic accessions (see Table 1). Ten SSR genetic markers were employed to assess the genetic variation among the collected samples.

Table 1. Seven garlic accessions used in the study.

Table 2. SSR primer pairs used in the study.

DNA extraction

Whole garlic bulbs were used for DNA extraction. DNA was extracted using the GeneJET Plant Genomic DNA Purification Kit (Thermo Fisher, Protocol: Document Connect [thermofisher.com]). After extraction, DNA concentration and purity were determined using a NanoVue Plus spectrometer. Sample con-

Table 1. Seven garlic accessions used in the study

Number	Accession name	Collected place
1	VinhHai1	Vinh Hai 1 – Ninh Hai
2	VinhHai2	Vinh Hai 2 – Ninh Hai
3	VinhHai3	Vinh Hai 3 – Ninh Hai
4	NhonHai	Nhon Hai – Ninh Hai
5	VanHai	Van Hai - PRTC
6	NinhHoa	Ninh Hoa – Khanh Hoa
7	LySon	Ly Sơn – Quang Ngai

centrations ranging from 150 to 550 ng/ìL were deemed satisfactory for further PCR amplification. The samples were then diluted to 10 ng/ìL prior to PCR amplification. PCR amplification of the garlic samples and data analysis The PCR thermal cycle followed the recommendations by Camila et al. (2012) with minor modifications. The electrophoresis procedure was based on protocols from Vipin et al. (2009) and Mukesh et al. (2019). The phylogenetic tree was constructed using the genetic similarity coefficient (Dice coefficient) and the UPGMA clustering method with NTSYSpc 2.10m software.

2. Results and discussion

Gene applification level of the SSR primers in the seven collected garlic samples

Fig 3.2 Electrophoresis graph of the PCR products from seven garlic samples with primer Asa06. M2: ZipRuler Express DNA Ladder 2; arrows indicate the target bands.

Fig 3.3 Electrophoresis graph of the PCR products from seven garlic samples with primer Asa07. M2: ZipRuler Express DNA Ladder 2; arrows indicate the target bands.

PCR procedures for each primer set amplification were optimized, and the

Table 2. SSR primer pairs used in the study

Primer name	Primer sequence (5' - 3')	T_a (^{0}C)	Allele size (bp)
Asa04	F: AGACTTTTGGAGGCTAGGGC	54	264
	R: CCCTGGTCTCTTTCAACCAA		
Asa06	F: GGGGTGTTACATTCTCCCCT	57	192
	R: ACCGCCTGATTTTGCATTAG		
Asa07	F: CTCGGAACCAACCAGCATA	58	229 - 235
	R: CCCAAACAAGGTAGGTCAGC		
Asa08	F: TGATTGAAACGAATCCCACA	56	209-257
	R: GGGGGTTACCTGAACCTGTTA		
Asa10	F: TTGTTGTTCTGCCATTTT	48	225 - 239
	R: GATCTAAGCCGAGAGAAA		
Asa14	F: TCTATCTCGCTTCTCAGGGG	48	220 - 234
	R: GCTGACAGAAGTAGTCTTTCC		
Asa16	F: CACGACTTTTCCTCCCATTT	48	148-154
	R: GCTAATGTTCATGTCCCCAGT		
Asa17	F: TCCACGACACACACACACAC	56	126 - 196
	R: ATGCAGAGAATTTGGCATCC		
Asa18	F: TCAAGCTCCTCCAAGTGTCC	45	254-264
	R: TCGGGATATGACAGCATTTG		
Asa20	F: GAAGCAGCAAAGATCCAAGC	48	260
	R: CGTGCAGAACTTAACCTT		

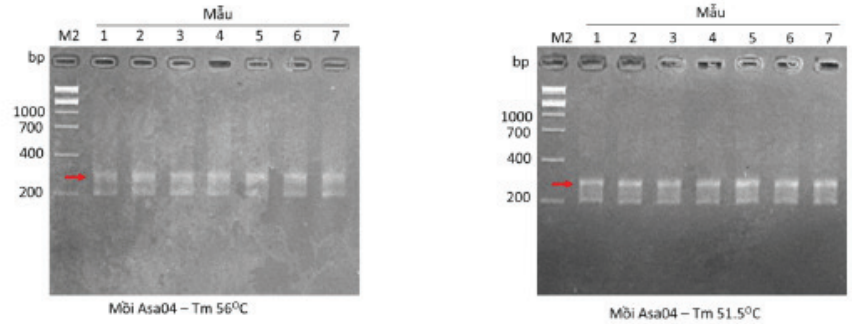

Fig 3.1 Electrophoresis graph of the PCR products from seven garlic samples with primer Asa04. M2: ZipRuler Express DNA Ladder 2; arrows indicate the target bands.

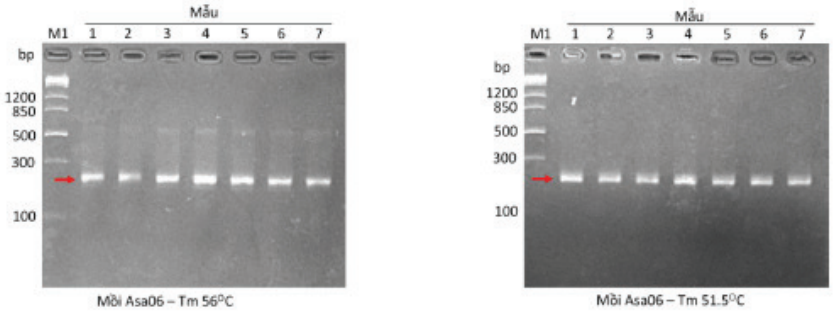

Fig 3.2 Electrophoresis graph of the PCR products from seven garlic samples with primer Asa06. M2: ZipRuler Express DNA Ladder 2; arrows indicate the target bands.

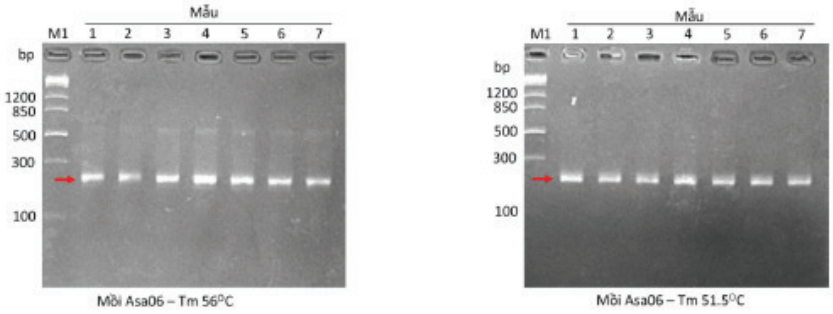

Fig 3.2 Electrophoresis graph of the PCR products from seven garlic samples with primer Asa06. M2: ZipRuler Express DNA Ladder 2; arrows indicate the target bands.

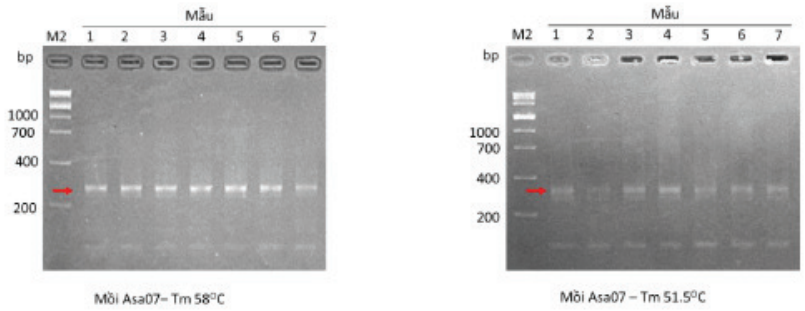

Fig 3.3 Electrophoresis graph of the PCR products from seven garlic samples with primer Asa07. M2: ZipRuler Express DNA Ladder 2; arrows indicate the target bands.

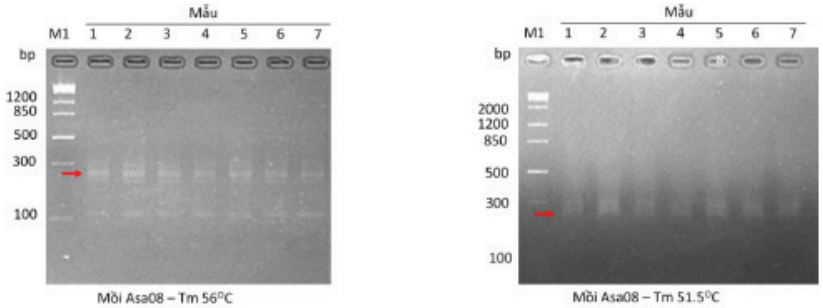

Fig 3.4 Electrophoresis graph of the PCR products from seven garlic samples with primer Asa08. M2: ZipRuler Express DNA Ladder 2; arrows indicate the target bands.

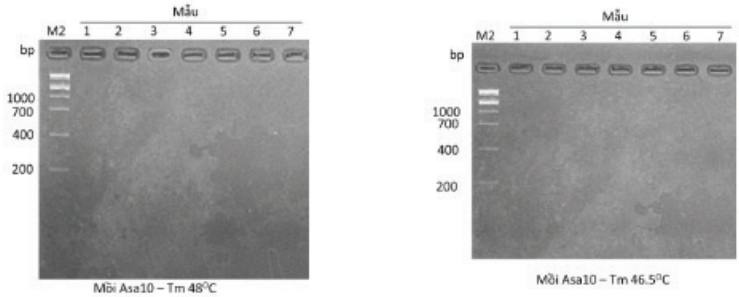

Fig 3.5 Electrophoresis graph of the PCR products from seven garlic samples with primer Asa10. M2: ZipRuler Express DNA Ladder 2; arrows indicate the target bands.

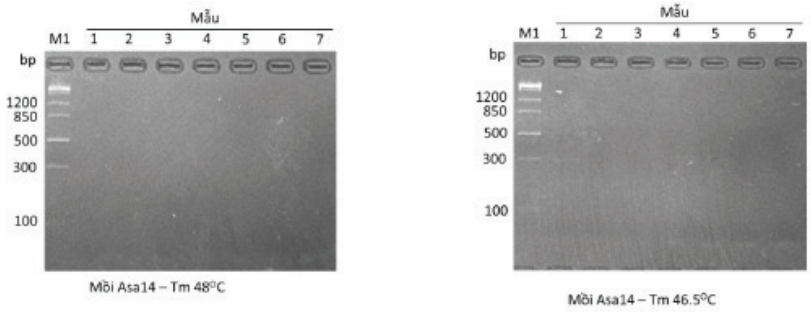

Fig 3.6 Electrophoresis graph of the PCR products from seven garlic samples with primer Asa14. M2: ZipRuler Express DNA Ladder 2; arrows indicate the target bands.

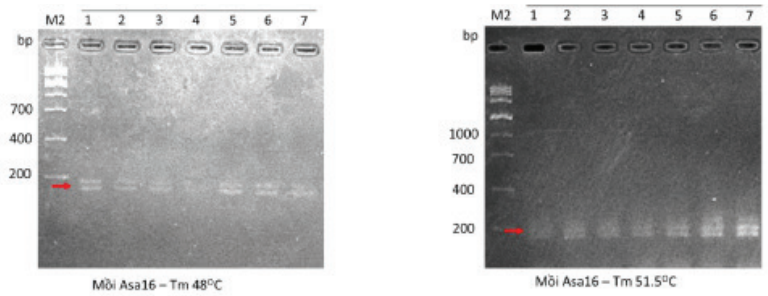

Fig 3.7 Electrophoresis graph of the PCR products from seven garlic samples with primer Asa16. M2: ZipRuler Express DNA Ladder 2; arrows indicate the target bands.

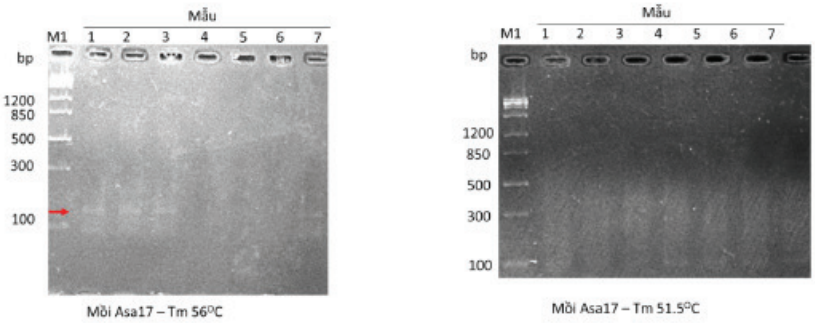

Fig 3.8 Electrophoresis graph of the PCR products from seven garlic samples with primer Asa17. M2: ZipRuler Express DNA Ladder 2; arrows indicate the target bands.

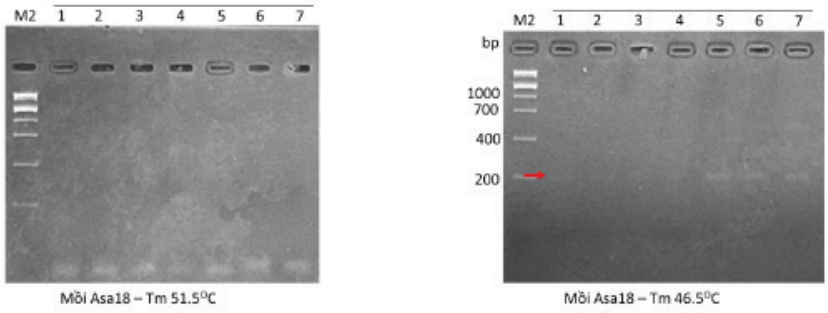

Fig 3.9 Electrophoresis graph of the PCR products from seven garlic samples with primer Asa18. M2: ZipRuler Express DNA Ladder 2; arrows indicate the target bands.

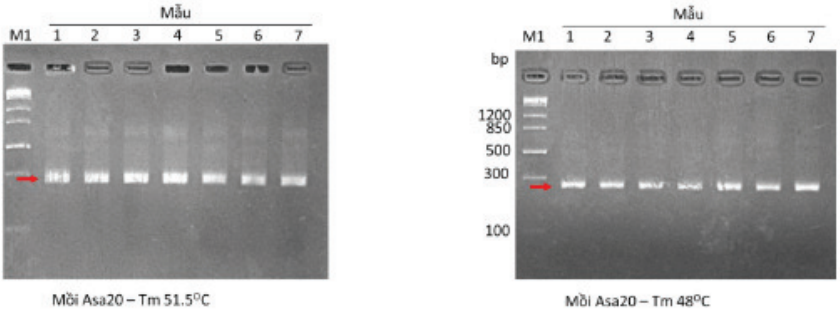

Fig 3.10 Electrophoresis graph of the PCR products from seven garlic samples with primer Asa20. M2: ZipRuler Express DNA Ladder 2; arrows indicate the target bands.

best amplification temperature regime was chosen for final amplification. The results of the PCR amplification of the seven garlic samples showed that the primer sets Asa04, Asa06, Asa07, Asa08, Asa16, Asa18, and Asa20 had good amplifications with bright, clear bands. In contrast, primer sets Asa10 and Asa14 did not amplify the DNA of any of the garlic samples. Primer set Asa17 did not amplify the DNA from the NhonHai, VanHai, and NinhHoa samples, but it gave good amplification for the other samples. Genetic distance matrix of seven garlic samples based on SSR analysis.

Table 3. Genetic distance matrix based on the PCR products of 10 SSR primer pairs for the seven collected garlic samples

	VinhHai1	VinhHai2	VinhHai3	NhonHai	VanHai	NinhHoa	LySon
VinhHai1	1.000000						
VinhHai2	1.000000	1.000000					
VinhHai3	1.000000	1.000000	1.000000				
NhonHai	0.900000	0.900000	0.900000	1.000000			
VanHai	0.900000	0.900000	0.900000	1.000000	1.000000		
VanNinh	0.900000	0.900000	0.900000	1.000000	1.000000	1.000000	
LySon	1.000000	1.000000	1.000000	0.900000	0.900000	0.900000	1.000000

The results presented in Table 3 show that the seven collected garlic samples had high similarity, ranging from 0.90 to 1.00. Among these, the VinhHai1, VinhHai2, VinhHai3, and LySon samples had 100% similarity, indicating that they originated from the same source or are of the same species. Similarly, the NhonHai, VanHai, and NinhHoa samples also had 100% similarity, suggesting that these samples are potentially from the same origin.

Phylogenetic tree of seven collected garlic samples

Fig. 3.11: The phylogenetic tree of seven collected garlic samples constructed based on the PCR products of 10 SSR primer pairs.

Based on the average genetic similarity coefficient of 0.96 and the results from the family tree in Fig. 3.11, the garlic samples can be divided into two groups: Group I includes VinhHai1, VinhHai2, VinhHai3, and LySon, while Group II includes NhonHai, VanHai, and NinhHoa. This indicates that VinhHai1, VinhHai2, VinhHai3, and LySon have a close origin, as do NhonHai, VanHai, and NinhHoa. Therefore, the samples in each group likely originated from the same source and diversified in different geographical areas over time. The difference between these two groups is due to the Asa17 primer set used in the PCR reaction, which produced no products in the NhonHai, VanHai, and NinhHoa samples, while producing bright, clear bands in the other samples. This primer set was crucial in revealing the difference between the two garlic

groups and serves as a foundation for future in-depth studies when combined with other markers.

Conclusions and suggestions

Based on the genetic distance matrix results, the samples VinhHai1, VinhHai2, VinhHai3, and LySon had 100% similarity. Similarly, the samples NhonHai, VanHai, and VanNinh also had 100% similarity. The phylogenetic tree split the samples into two groups: Group I included VinhHai1, VinhHai2, VinhHai3, and LySon, while Group II included NhonHai, VanHai, and NinhHoa.

Based on the analysis results, it is suggested that more molecular markers should be used for the analysis and identification of garlic varieties in Vietnam. Additionally, it is necessary to conduct in-depth biochemical analyses to identify the similarities in biochemical compositions and genetic differences between the garlics of the two groups identified in the phylogenetic tree developed in this study.

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