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RESEARCH ARTICLE

Genomic variation in self-pollinated clove (*Syzygium aromaticum* (L.) Merr. & Perry) accessions from the Western Ghats

Sreekala G S[1], Avinash M[2], Reshma P[1*], Reddappa JB[1], Anargha T[1], Nainu Joseph[1] & Deepa S Nair[1]

[1]Department of Plantation, Spices, Medicinal and Aromatic Crops, College of Agriculture, Vellayani, Kerala, India

[2]College of Horticulture, University of Horticultural Sciences, Bagalkot, Karnataka, India

*Email: reshmap9497@gmail.com

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Abstract

Clove, the dried, unopened flower buds of *Syzygium aromaticum* (L.) Merr. & Perry, is the mainstay of income for farmers in India, Indonesia, Tanzania and Sri Lanka and is a self-pollinated crop. A study conducted in the Southern Western Ghats of India identified phenotypic variations in the clustering of clove flower buds. Four clove accessions (Acc.1, Acc.3, Acc.5 and Acc.7) that showed superior yield and variation in floral characteristics were selected and DNA was isolated from the floral tissue using the CTAB method. Random Amplified Polymorphic DNA (RAPD) analysis using Operon primers (OPB 01 to OPB 10) detected polymorphism among the accessions, with OPB-01 showing the highest Polymorphic Information Content (PIC), followed by OPB-04 and OPB-06. Similarity coefficients were calculated based on the presence or absence of polymorphic bands, with coefficients ranging from 0.47 to 0.72. The resulting phylogenetic tree classified the accessions into two main groups based on their bud clustering habit (branching): branching (Acc. 1 and Acc. 5) and non branching (Acc. 3 and Acc. 7). Acc. 3 showed distinct separation from the other genotypes at a coefficient of 0.47, while Acc. 7 separated from Acc. 1 and Acc. 5 at a coefficient of 0.60. Acc. 5 appeared genetically distinct, as it showed separation from the other accessions based on RAPD analysis. The distinctiveness of Acc.5 was further confirmed through 18S rRNA sequencing, suggesting potential intra-species genomic variations among the four clove phenotypes and hinting at the possibility of cross pollination.

Keywords

amplification; bud clustering; clove; RAPD

Introduction

Clove, scientifically known as the flowering buds of *Syzygium aromaticum* (L.) Merr. & L.M. Perry, or *Eugenia caryophyllata* Thunb, stands as a notable emblem in the realm of spices and medicinal flora (1). *Syzygium*, the largest genus in the Myrtaceae family, includes around 1200-1800 flowering plants. Nestled within the Myrtaceae family, *Syzygium* claims a significant place as the largest genus, encompassing a vast spectrum of approximately 1200-1800 flowering plant species (2). Originating from the Maluku Islands in Eastern Indonesia, colloquially referred to as the "Spice Islands," clove has played a significant role in the spice trade's history. Clove has been valued for its captivating scent and medicinal properties, leaving an indelible mark on various cultures and culinary practices worldwide (3). Eugenol, the primary

bioactive compound found in cloves, exists in concentrations ranging from approximately 9381.70 to 14,650.00 mg per 100 grams of the plant material (1).

Across history, cloves' inherent antiseptic and anesthetic qualities have given them significant value, intertwining them intimately with culinary customs and age -old medicinal applications. In the modern era, the cultivation of cloves spans continents, with contributions from Indonesia, India, Malaysia and Sri Lanka. The West Indies, Madagascar and Tanzania, especially the renowned island of Zanzibar, are also major regions known for their high clove production (3). Despite its significant role in agricultural economies, clove faces notable challenges, such as limited genetic diversity. This limitation is likely due to centuries of deliberate selective breeding to enhance desirable traits like increased eugenol levels and strong disease resistance. The plant's tendency towards self-pollination also contributes to reduced genetic variation and limits evolutionary adaptation.

In India, clove cultivation thrives predominantly in the mountainous areas of the Western Ghats, particularly at elevated altitudes within Tamil Nadu and Karnataka, alongside Kerala's rich red soils. The lineage of clove populations in India can be traced back to a select few trees initially introduced from other regions (4). This restricted genetic base impacts the species' adaptability and resilience, making it more susceptible to diseases, pests, and environmental changes. Preserving and understanding the genetic diversity of clove populations is essential to ensuring the sustainability and productivity of cultivation in the region. While previous studies (4-10) have primarily focused on phenotypic characteristics and geographic distribution of clove varieties, there is an escalating demand for in-depth genomic analysis to unravel underlying genetic variations. Efficient extraction of DNA from any plant is of paramount importance across many scientific disciplines and applications, spanning taxonomy, biodiversity assessment, conservation efforts, ecological research, evolutionary studies, biotechnology, and molecular investigations (11). In a comprehensive survey, the taxonomic classification and phylogenetic relationships of two prominent clove varieties from North Maluku, namely 'Afo' from Ternate Island and 'Sibela' from Bacan Island, were meticulously analyzed using Internal Transcribed Spacer (ITS) sequence data. The investigation unveiled critical genetic linkages and evolutionary lineages, positioning the 'Afo' clove as the ancestral progenitor of all clove varieties indigenous to North Maluku. These findings significantly advance our understanding of cloves' genetic heritage and diversification processes within this region (12). However, there currently needs to be more information regarding the taxonomic position and phylogenetic relationships among the local clove varieties in India.

Further research is imperative to fill this knowledge gap and to elucidate the genetic underpinnings and evolutionary trajectories of Indian clove varieties. Thus, this study aims to analyze genomic variation in self-pollinated clove accessions from the Southern Western Ghats, recognized as a biodiversity hotspot. The research employs advanced genomic tools to delineate genetic relationships and evaluate the extent of genetic diversity within this pivotal crop. PCR amplification of genomic DNA was conducted to ensure the quality of the extracted DNA following the optimized protocol. Various techniques were utilized for this purpose, including RAPD (Random Amplified Polymorphic DNA) and ITS. The findings of this study are anticipated to provide valuable insights supporting the development of improved clove varieties, thereby bolstering the resilience and sustainability of clove cultivation in the Southern Western Ghats.

Materials and Methods

Four clove accessions from the Western Ghats labelled as Acc. 1, Acc. 3, Acc. 5 and Acc. 7, were selected for this study based on their superior yield characteristics and distinct morphological traits (Table 1, Fig. 1). Genomic DNA was extracted from the floral regions of the selected clove accessions. Immediately after being plucked, the fresh flowers were placed in sealable polythene bags and promptly transported to the laboratory for DNA extraction. While fresh samples are recommended, samples stored in a freezer for 1 to 2 days are also suitable for DNA extraction. The samples were washed with distilled water and dried using sterile filter paper.

DNA Extraction and PCR Amplification

Genomic DNA was extracted from the floral tissues of the clove accessions (Acc. 1, 3, 5 and 7) using the cetyltrimethylammonium bromide (CTAB) method. The 18S ribosomal RNA (rRNA) region was amplified utilizing ITS4 and ITS5 primers. The intergenic spacer between the 18S and 28S rRNA regions is pivotal for identifying genomic variation at the rRNA level in eukaryotes (Fig. 2). The polymerase chain reaction (PCR) conditions comprised an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds and extension at 72°C for 1 minute, culminating in with a final extension at 72°C for 10 minutes.

Table 1. Details of the clove accessions selected for the study

Clove accessions	Superior or distinct characteristics	Location	Geographic coordinates of location		
			Latitude	Longitude	Altitude (m)
Acc. 1	Local type produces bold and large flower buds, a combination of 1,2,3,4,5 flower bud per cluster and is a high yielder.	Braemore estate, Braemore, Trivandrum	8°45´51"	77°05´00"	453
Acc. 3	Local type produces 1,2,3 buds per cluster, high yielder	Braemore estate, Braemore, Trivandrum	8°45´51"	77°05´00"	441
Acc. 5	Robusta type produces a combination of 1,2,3,4,5 flower bud per cluster, which is a high yielder.	Merchiston estate, Ponmudi, Trivandrum	8°44´35"	77°07´38"	653
Acc. 7	Local type produces 1,2,3 bud per cluster, high yielder	Merchiston estate, Ponmudi, Trivandrum	8°44´34"	77°07´38"	651

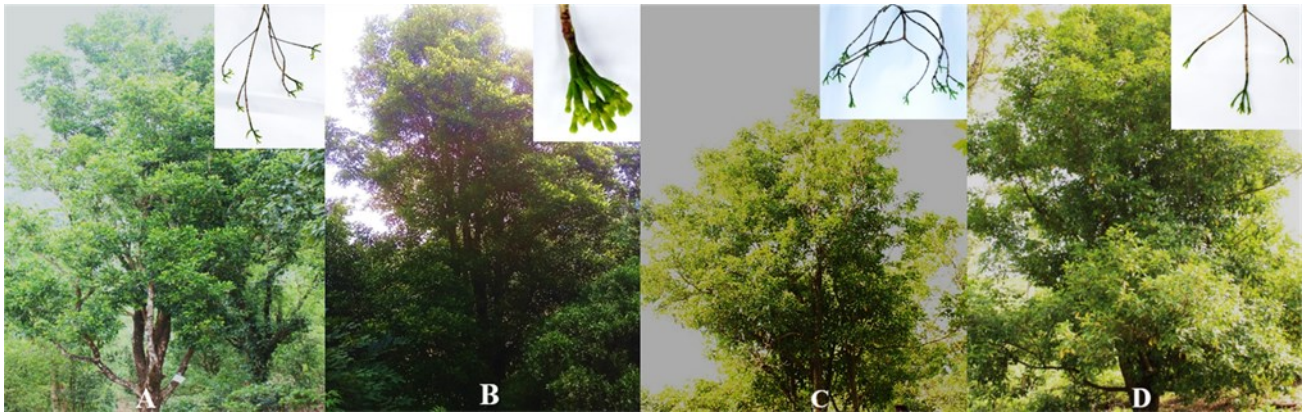

Fig. 1. Clove accessions **A.** Acc 1 **B.** Acc 3 **C.** Acc 5 **D.** Acc 7

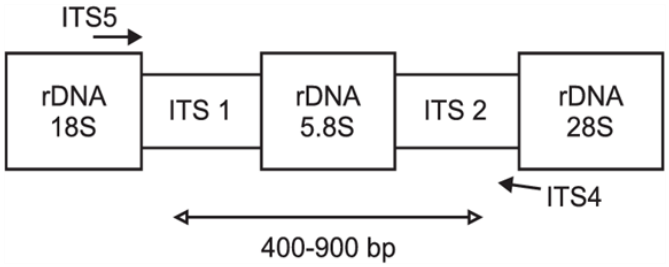

Fig. 2. Schematic representation of the 18SrRNA region in angiosperms

Sequencing and phylogenetic analysis

The PCR products were sequenced using Sanger's method. The resulting sequences were aligned and subjected to analysis to assess genetic variation. Phylogenetic trees were constructed using Maximum Parsimony and Neighbour-Joining algorithms to elucidate the genetic relationships among the accessions.

RAPD analysis

RAPD analysis was conducted using operon primers OPB1 to OPB10. The presence or absence of polymorphic bands was recorded and similarity coefficients were computed. A phylogenetic tree was generated using the RAPD data to classify the accessions according to their inflorescence patterns.

Results

Analysis of 18SrRNA Region and Phylogenetic Analysis

This study undertook the sequencing of the 18SrRNA region to discern genetic variations among distinct clove accessions. Despite the availability of clove rRNA sequences in public databases, a systematic classification based on the 18S rRNA region and specific genotypes still needs development. To facilitate PCR amplification, ITS4 and ITS5 primers targeting the 18S rRNA region were employed. Sequencing revealed marked genetic diversity among the four analyzed clove accessions (Fig. 3). PCR amplification generated products ranging from 700 to 800 base pairs. Verification of these amplified products was conducted via agarose gel electrophoresis, using a 100 base pair size marker as a reference (Fig. 4). This methodological approach validated the size and integrity of the PCR products, thereby ensuring the robustness of the sequencing data. These findings underscore the considerable genetic variation within the 18S rRNA region among clove accessions, suggesting promising directions for further genetic and phylogenetic investigations in clove. This research establishes a framework for classifying clove genotypes based on the 18S rRNA region and enriches the understanding of the genetic diversity within this

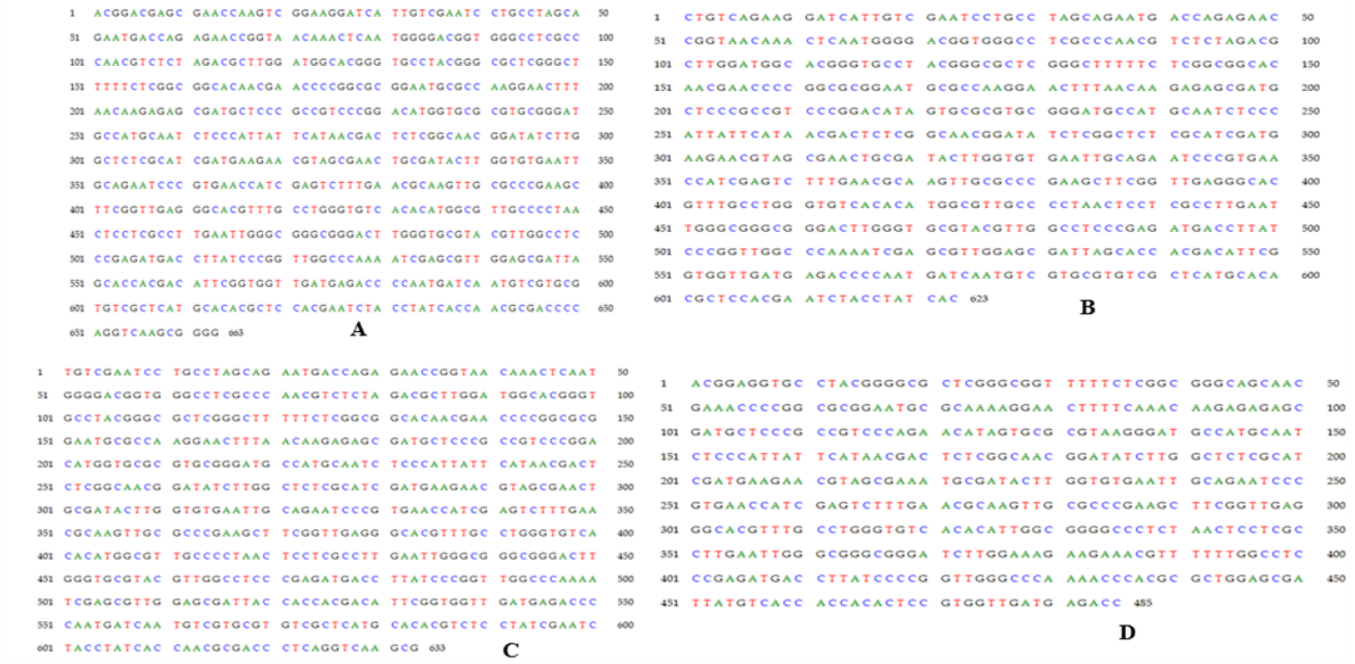

Fig. 3. The forward and reverse assembled sequence of ITS region from **A.** Acc. 1 **B.** Acc. 3 **C.** Acc. 5 **D.** Acc. 7

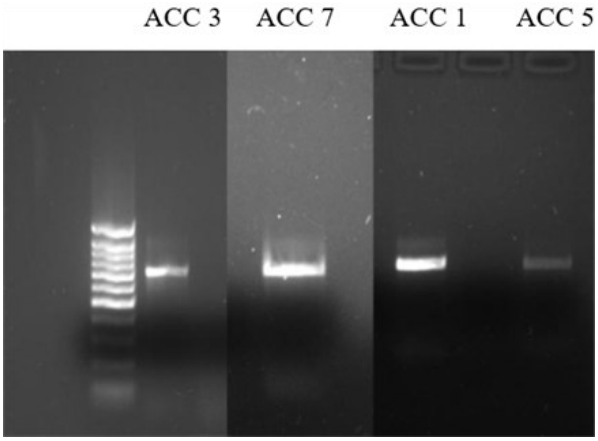

Fig. 4. Agarose gel electrophoresis of PCR amplified region between ITS5 and ITS4 from the four clove accessions

economically significant species. (Table 2) delineates a comparative analysis of the ITS regions among the four clove accessions, presenting patristic distances, percentage identity and the count of nucleotide differences and identities. The percentage identity matrix elucidates a pronounced genetic concordance among Acc. 1, Acc. 3 and Acc. 7 while highlighting Acc. 5 as the most genetically divergent. This matrix underscores the intricate genetic relationships and variations, providing a comprehensive view of the genetic landscape across these clove accessions.

The genetic relationship analysis of clove accessions, leveraging ITS sequences and employing Maximum Parsimony and Neighbour-Joining algorithms, yielded phylogenetic trees elucidating the relatedness of each accession (Acc. 1, Acc. 3, Acc. 7 and Acc. 5) in comparison with sequences from three additional Syzygium aromaticum specimens and other related genera retrieved from the NCBI database (Fig. 5.).(Fig. 6) presents an exhaustive phylogenetic tree encompassing these four clove accessions. The phylogenetic analysis revealed that Acc. 5 is markedly distinct from the remaining accessions, aligning with its unique phenotypic characteristics. In contrast, Acc. 1, Acc. 3 and Acc. 7 exhibited closer genetic affinities, albeit with discernible genetic distinctions. Notably, Acc. 5, identified as the robusta type, contrasts with the other three accessions, which are local varieties endemic to Kerala. Acc. 3 occupies the earliest divergent position within the phylogenetic framework, underscoring its foundational place in the evolutionary hierarchy of the studied clove accessions.

Table 2. Comparison of ITS region of four clove accessions

Patristic distances				
	ACC5	ACC3	ACC7	ACC1
ACC5		0.073	0.081	0.073
ACC3	0.073		0.017	0.008
ACC7	0.081	0.017		0.008
ACC1	0.073	0.008	0.008	
Percentage identity				
	ACC5	ACC3	ACC7	ACC1
ACC5		82.409	82.409	82.409
ACC3	82.409		98.361	99.197
ACC7	82.409	98.361		98.578
ACC1	82.409	99.197	98.578	
Number of differences				
	ACC5	ACC3	ACC7	ACC1
ACC5		92	92	92
ACC3	92		10	5
ACC7	92	10		9
ACC1	92	5	9	
Number of identities				
	ACC5	ACC3	ACC7	ACC1
ACC5		431	431	431
ACC3	431		600	618
ACC7	431	600		624
ACC1	431	618	624	

The present study was inconclusive regarding the branching and non-branching of the inflorescence based solely on the ITS region. Consequently, to further investigate the genetic variance among the clove clones, it was decided to employ Random Amplified Polymorphic DNA (RAPD) analysis.

RAPD analysis and phylogenetic relationships based on RAPD data

To further explore genetic variance among the clove accessions, RAPD analysis was employed, which categorized the accessions into two distinct groups based on their inflorescence characteristics: branching (Acc. 1 and Acc. 5) and non-branching (Acc. 3 and Acc. 7). Agarose gel electrophoresis of RAPD fragments using Operon primers (OPB 01 to OPB 10) revealed significant polymorphism among the accessions, with OPB-01 demonstrating the highest Polymorphic Information Content (PIC) (Table 3), followed by OPB-04 and OPB-06 (Fig. 7-10). Acc. 1 exhibited 13 polymorphic bands, indicating substantial genetic variability, whereas Acc. 5 displayed only two bands, suggesting a comparatively lower level of genetic diversity within this accession. Acc. 3 and Acc. 7 exhibited distinct polymorphic patterns, notably under primers OPB3 and OPB6. Despite sharing some genetic similarities, discernible differences were observed among Acc. 3, Acc. 7 and Acc. 1. Polymorphic bands detected in Acc. 1 using OPB1 were also present in Acc. 3 and Acc. 7, indicating shared genetic markers among these accessions. Additionally, Acc. 3 revealed unique polymorphic bands absent in Acc. 7, Acc. 1 and Acc. 5, underscoring its genetic distinctiveness.

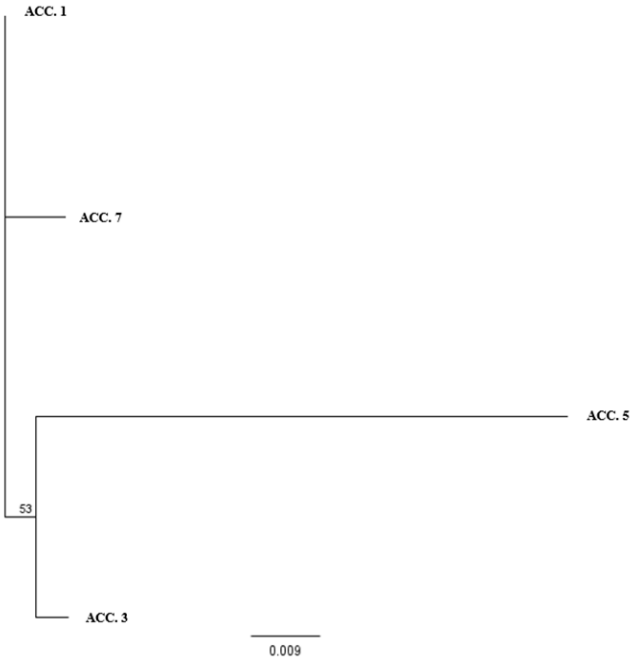

Fig. 5. Phylogenetic tree showing relatedness of clove accessions with sequences from the NCBI database

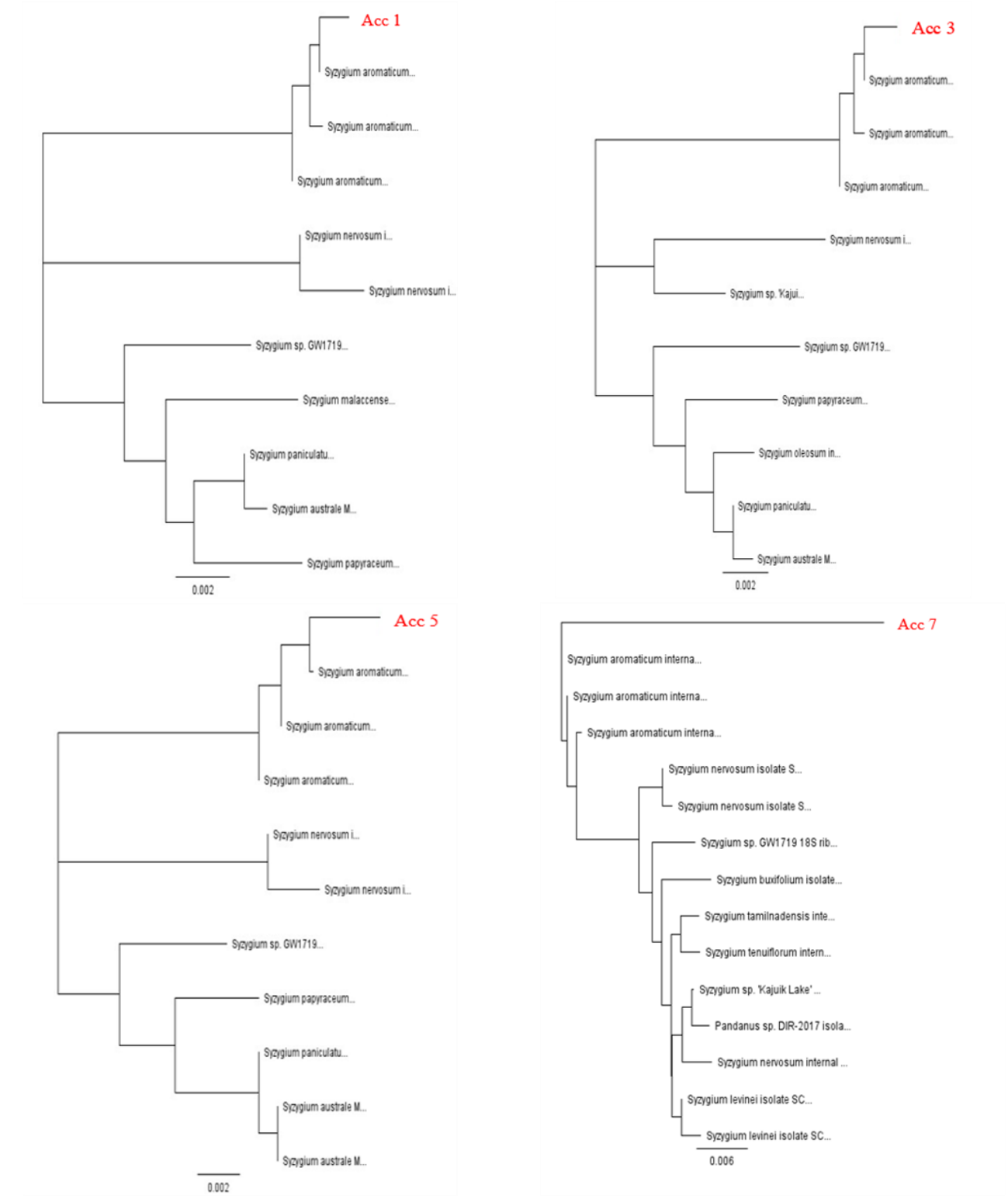

Fig. 6. Phylogenetic tree showing relatedness of four clove accessions

Table 3. Gel polymorphism details of OPB1 to OPB 10

Sl. No.	Primer	Total number of bands	Number of polymorphic bands	Percentage of polymorphic loci	PIC value
1	OPB 1	9	9	100	0.5
2	OPB 2	5	4	80	0.48
3	OPB 3	2	2	100	0.46
4	OPB 4	11	8	72.72	0.49
5	OPB 5	6	4	66.66	0.46
6	OPB 6	7	7	100	0.49
7	OPB 7	6	5	83.33	0.48
8	OPB 8	7	7	100	0.48
9	OPB 9	1	1	100	0.37
10	OPB 10	3	1	33.33	0.15

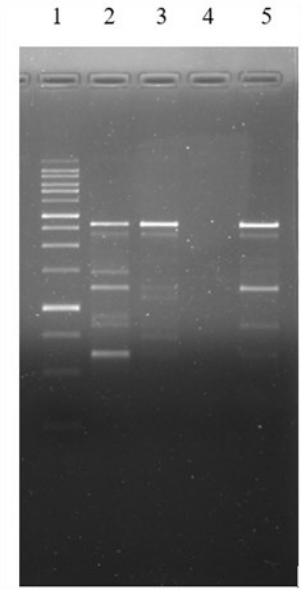

Fig. 7. Agarose gel electrophoresis of RAPD fragments in 1.2% agarose gel Primer OPB1 (1- High range ladder; 2- Acc.3; 3- Acc.1; 4- Acc.5; 5- Acc.7)

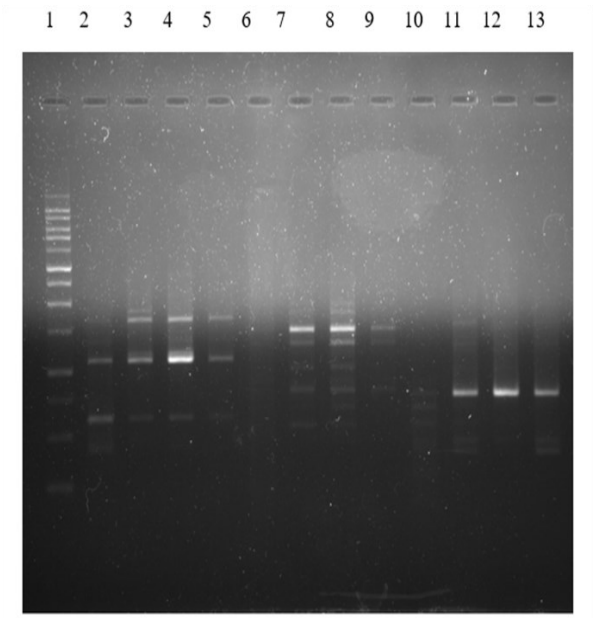

Fig. 9. Agarose gel electrophoresis of RAPD fragments in 1.2% agarose gel Primer OPB5 (lane 2-5), OPB6 (Lane 6-9) and OPB7 (Lane 10-13) 1- High range ladder; 2- Acc.3; 3- Acc.1; 4- Acc.5; 5- Acc.7; 6- Acc. 3; 7- Acc.1; 8- Acc.5; 9- Acc.7; 10-Acc.3; 11-Acc.1; 12-Acc.5; 13-Acc.7

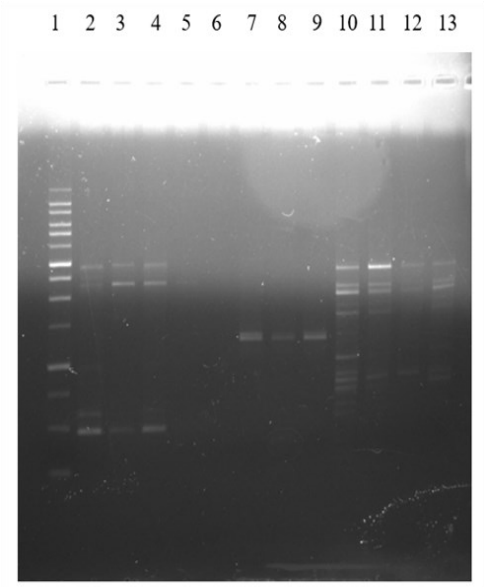

Fig. 8. Agarose gel electrophoresis of RAPD fragments in 1.2% agarose gel Primer OPB2 (lane 2-5), OPB3 (Lane 6-9) and OPB4 (Lane 10-13) 1- High range ladder; 2- Acc.3; 3- Acc.1; 4- Acc.5; 5- Acc.7; 6- Acc. 3; 7- Acc.1; 8- Acc.5; 9- Acc.7; 10-Acc.3; 11-Acc.1; 12-Acc.5; 13-Acc.7

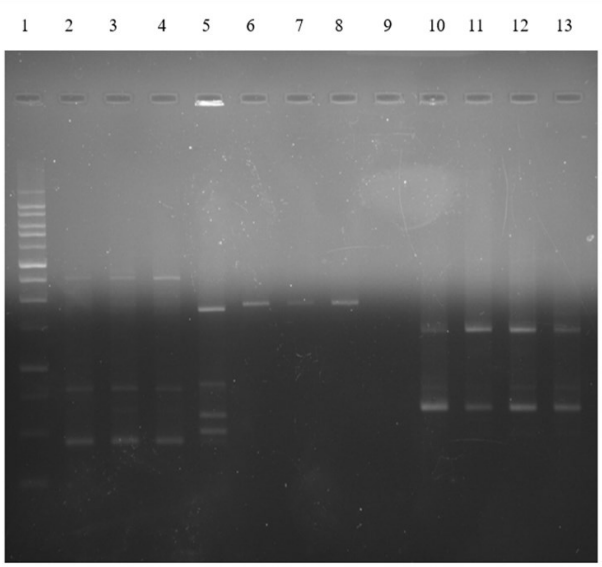

Fig. 10. Agarose gel electrophoresis of RAPD fragments in 1.2% agarose gel Primer OPB 8 (lane 2-5), OPB9 (Lane 6-9) and OPB10 (Lane 10-13) 1- High range ladder**;** 2- Acc.3; 3- Acc.1**;** 4- Acc.5**;** 5- Acc.7**;** 6- Acc. 3**;** 7- Acc.1**;** 8- Acc.5**;** 9- Acc.7**;** 10-Acc.3**;** 11-Acc.1**;** 12-Acc.5**;** 13-Acc.7

Conversely, OPB6 identified polymorphic bands specific to Acc. 5, distinguishing it from the other accessions. These findings from the RAPD analysis provide deeper insights into the genetic diversity and relationships among the studied clove accessions, highlighting both shared and unique genetic markers that contribute to understanding their evolutionary history and agricultural characteristics. (Table 4) presents a correlation matrix assessing the relationships among different clove accessions based on RAPD analysis. Similarity coefficients, calculated from the presence or absence of polymorphic bands, ranged from 0.47 to 0.72. The phylogenetic tree (Fig. 11) resulting from the study categorized the clove accessions into two main groups based on their inflorescence characteristics: branching types (Acc. 1 and Acc. 5) and non-branching types (Acc. 3 and Acc. 7). This classification highlights distinct genetic traits associated with how buds cluster among these accessions. Acc. 3 exhibited a distinct separation from the other genotypes with a similarity coefficient of 0.47, indicating significant genetic divergence compared to Acc. 1, Acc. 5 and Acc. 7, suggesting unique evolutionary paths or selective pressures shaping its genetic makeup. Similarly, Acc. 7 also showed distinct genetic separation from Acc. 1 and Acc. 5, with a coefficient of 0.60. This underscores the genetic distinctiveness of Acc. 7 within the studied clove varieties. RAPD analysis provided further insights into the genetic relationships among the accessions, revealing a more significant number of shared bands

Table 4. Assessment of relationship among different accessions of clove-Correlation matrix

	Acc.3	Acc.7	Acc.1	Acc.5
Acc.3	1			
Acc.7	0.473	1		
Acc.1	0.473	0.649	1	
Acc.5	0.473	0.543	0.719	1

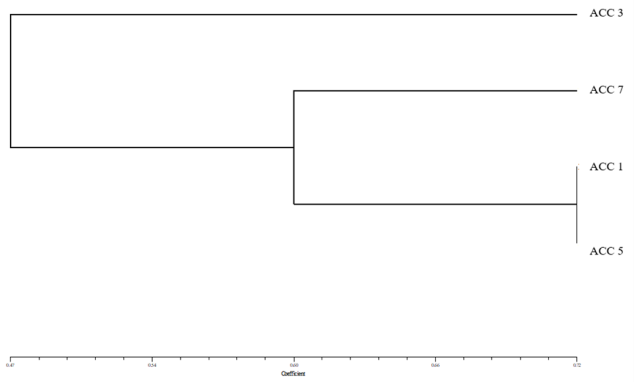

Fig. 11. Dendrogram showing phylogenetic relationships among the four accessions of clove based on RAPD- PCR data by OPB 1 to 10

between Acc. 1 and Acc. 5. This observation suggests a closer genetic affinity between these branching types compared to the non-branching types (Acc. 3 and Acc. 7), which exhibited fewer shared genetic markers. The genetic distinctiveness of Acc. 3 was further substantiated by its clear separation in the phylogenetic tree based on RAPD data, indicating a unique genetic profile among the studied accessions. This uniqueness was further explored through additional analyses, such as 18S rRNA sequencing, highlighting potential genomic variations within the species among the four clove phenotypes. These variations imply that past cross-pollination events or other evolutionary factors have influenced genetic diversity.

Discussion

The present study on genomic variation among four clove accessions from the Western Ghats provides valuable insights into the genetic relationships and variability. The findings suggest that Acc. 1, Acc. 3 and Acc. 7 exhibit substantial genetic similarity, whereas Acc. 5 is genetically distinct from the other accessions. The genetic distinctiveness of Acc. 5 is evident from the lower percentage identity values and higher counts of nucleotide differences compared to Acc. 1, Acc. 3 and Acc. 7 (13). This suggests that Acc. 5 has undergone unique evolutionary pathways or selective pressures that have shaped its ITS regions differently from its counterparts. According to (14), the development of varieties in distinct agro-climatic zones shows significant levels of variation due to the differing selection pressures in these zones. This is important because clove populations in India have limited genetic diversity, likely due to self-pollination and the fact that only a few trees were originally introduced (4). The genetic distinctiveness of Acc. 5 is a significant finding, as it highlights the potential for unique genetic variation within the clove population from the Western Ghats, often characterized by limited genetic diversity (13, 15).

The genetic relationship analysis of clove accessions through ITS sequences and phylogenetic algorithms has provided valuable insights into the evolutionary dynamics and genetic diversity within *Syzygium aromaticum* (12). Our study, corroborated by sequences from NCBI databases and involving Maximum Parsimony and Neighbour-Joining methods, has delineated distinct genetic clusters among the studied accessions. This differentiation suggests potential adaptive strategies or selective pressures that have shaped their genetic makeup differently from the local Kerala varieties represented by Acc. 1, Acc. 3 and Acc. 7. The genetic divergence of Acc. 5 from the local varieties in Kerala suggests that it may have evolved under different environmental conditions or selection pressures, leading to the development of its distinct phenotypic and genotypic features (12). The phylogenetic tree illustrates varying branch lengths among the clove accessions in the present study. The longer branches observed for Acc. 5 suggest more genetic changes in the gene markers during their evolutionary processes (16). This implies that these accessions have undergone more significant genetic divergence, potentially due to adaptation to unique environmental conditions or selective pressures.

Consequently, the genotype on this more extended branch, such as Acc. 5, can be considered more advanced evolutionarily, reflecting more significant genetic differentiation and complexity. The findings of this study are corroborated by the research on the clove genome, which has provided insights into the evolutionary history and diversification of the Myrtaceae family, to which *S. aromaticum* belongs. The comparative genomic analysis between *S. aromaticum* and *Eucalyptus grandis,* another member of the Myrtaceae family, has revealed exemplary genome structure conservation and intrachromosomal rearrangements, suggesting the potential for further exploration of the genetic basis of economically important traits in clove (17).

Despite the inability of the ITS region to differentiate these accessions, RAPD analysis successfully identified several unique polymorphic bands, which serve as potential markers for genetic characterization. These results align with prior research by (12), indicating genetic diversity among local clove varieties in different geographical regions. The ancestral status of the Afo 1 clove, representing an early stage in speciation, contrasts with the more advanced characteristics observed in Sibela cloves from North Maluku. Similarly, in the Western Ghats accessions, a classification into two major groups based on inflorescence branching was observed, underscoring distinct genetic traits influencing bud clustering habits. The high level of polymorphism observed, particularly with primer OPB-01 exhibiting the highest PIC, indicates potential genetic diversity among the clove accessions. High diversity reflects environmental adaptation, benefiting propagation, resource conservation, wild species domestication and specific locus screening. Geographically isolated individuals accumulate genetic variations as they adapt to their environments (18). Polymorphism in RAPD assays can occur due to deletions, additions, or substitutions of bases within the priming site sequence (19). Acc. 1 demonstrated many polymorphic bands (13), reflecting its significant genetic variability. In contrast, Acc. 5 exhibited only two polymorphic bands, suggesting a lower genetic diversity within this accession. This finding is consistent with previous studies that have shown a correlation between the number of polymorphic bands and the level of genetic diversity within plant populations. Acc. 3 and Acc. 7 showed distinct polymorphic patterns under primers OPB-03 and

OPB-06, respectively, highlighting their unique genetic profiles despite some shared markers with other accessions. Unique bands in Acc. 3, absent in the other accessions, emphasize its genetic distinctiveness, which could be attributed to different evolutionary pressures or historical genetic events. RAPD analysis revealed significant levels of polymorphism among 6 genotypes of clove (20). Several reports confirmed that the RAPD technique has been applied to assess molecular polymorphism in several plants (21-25).

The utility of these polymorphic RAPD bands as potential sequence-characterized amplified region (SCAR) markers offers practical implications for nursery management and the cultivation of clove seedlings with desired inflorescence types. Early identification of branching traits through marker-assisted selection could enhance efficiency in clove breeding programs and ensure targeted agricultural practices. The phylogenetic tree derived from RAPD data categorized the accessions into two main groups based on their branching characteristics, corroborating the initial morphological classification. The similarity coefficients ranged from 0.47 to 0.72, indicating varying degrees of genetic relatedness. Notably, Acc. 3 exhibited the lowest similarity coefficient (0.47), signifying significant genetic divergence from the other accessions. This substantial divergence may suggest unique evolutionary trajectories or selective breeding pressures unique to Acc. 3. Acc. 7 also showed a distinct genetic separation from Acc. 1 and Acc. 5, with a similarity coefficient of 0.60, reinforcing its genetic uniqueness within the clove varieties studied. This finding aligns with the hypothesis that non-branching types (Acc. 3 and Acc. 7) possess distinct genetic traits compared to branching types (Acc. 1 and Acc. 5), which exhibited more shared genetic markers, suggesting a closer genetic affinity. In a study (20), five distinct clusters of clove accessions were identified from various geographical regions of Nilgiris, India. The phylogenetic analysis demonstrated a notable genetic affinity between samples S1 from Athipali and S6 from Muloor, Kotagiri, within the Nilgiris district, Tamil Nadu, India.

Conclusion

The study emphasizes the significance of exploring genomic variation in clove accessions to understand their genetic relationships and potential for improvement. The distinct genetic profile of Acc. 5 presents opportunities for further research into the evolutionary and selective pressures affecting its ITS regions, which can inform breeding programs to enhance genetic diversity and adaptability in clove populations. Identifying specific polymorphic bands lays the groundwork for developing SCAR markers, aiding in the early selection of desirable traits like branching inflorescence. Validation studies across a broader range of clove germplasm are necessary to ensure the robustness of these markers, ultimately refining breeding strategies to improve clove productivity and quality traits for different agricultural needs.

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Authors' contributions

SGS- planned and carried out the experiment

RP- wrote the manuscript with support from AM, RJB, AT, NJ, DSN

Compliance with ethical standards

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