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RESEARCH ARTICLE

Integrated molecular and phytochemical characterization of lemongrass (*Cymbopogon* spp.) germplasm from Vietnam and Laos

Hai Thi Hong Truong · Nhi Thi Hoang Ho · Han Ngoc Ho · Thuy Thanh Duong

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Abstract This study evaluated the genetic and phytochemical diversity of 35 *Cymbopogon* accessions from Vietnam and Laos using an integrated approach combining *ITS* sequencing, random amplified polymorphic DNA (RAPD) markers, and GC–MS profiling. *ITS* analysis identified 33 accessions as *Cymbopogon citratus* and two as *Cymbopogon winterianus*, revealing only two major haplotypes and indicating low intraspecific variation, likely due to clonal propagation. RAPD analysis using eight highly polymorphic primers generated 86 bands with 61.28% polymorphism, indicating moderate genetic diversity and forming two distinct clusters corresponding to two species boundaries. The lack of clear geographic structuring suggests extensive exchange of planting materials, resulting in the genetic homogenization of cultivated germplasm. The GC–MS analysis of n-hexane extracts identified 27 volatile compounds, with citral isomers being the most prevalent. The E-citral (geranial) content ranged from 22.480 to 67.577%, while Z-citral (neral) ranged from 10.027 to 25.107%. This observation indicates significant chemotypic variation among the accessions. Notably, there was also variation in geraniol, β-myrcene, and geranyl acetate. Accessions SK, BK, V1, V3, TN1, and PY1 exhibited high levels of citral and geraniol making them strong candidates for breeding, production, and chemotype stabilization. In contrast, accessions such as V2, QN2, HCM1, QT3, and BD3 significantly higher levels of geraniol, indicating distinct geraniol-rich chemotypes within the germplasm. This study highlighted accession SK as a superior high-citral genotype, with a citral content of 86.530%. Additionally, accessions with elevated geraniol levels were identified, including PY1 at 8.567%, underscoring the valuable chemotypic diversity present in the germplasm. The integration of molecular and chemical data helped clarify the relationships between different genotypes and chemotypes. This provides a solid foundation for selecting elite germplasm with consistent essential oil quality, thereby supporting the sustainable breeding and conservation of lemongrass genetic resources in Southeast Asia.

Keywords *Cymbopogon* · Genetic diversity · Phytochemical profiling · RAPD and ITS markers · Vietnam and Laos germplasm

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Introduction

Lemongrass (*Cymbopogon* sp.) is an economically and medicinally significant aromatic grass that is widely cultivated in tropical and subtropical regions. The lemongrass essential oil is extensively utilized in the food, cosmetic, and pharmaceutical industries due to its distinctive lemon scent and a variety of biological activities (Valková et al. 2022; Saide et al. 2025; Soares et al. 2013; Jeong et al. 2009; Gurusamy et al. 2021). The oil primarily contains citral, a mixture of the geometric isomers geranial (E-citral) and neral (Z-citral). Additionally, the oil contains varying amounts of myrcene, geranyl acetate, linalool, and other mono- and sesquiterpenes. These components are known to affect the quality, aroma profile, and biological activity of the oil (Avoseh et al. 2015).

The phytochemical characterization of *Cymbopogon* spp. has been extensively studied using gas chromatography-mass spectrometry (GC–MS) (Gurusamy et al. 2021; Dangol et al. 2023; Mohamed et al. 2022). This technique allows for the reliable identification and comparison of volatile metabolites among different accessions from various geographical regions. Previous research has consistently shown significant variation in the essential oil composition of *C. citratus*. This variation is attributed to factors such as genetic background, environmental conditions, cultivation practices, and the stage of plant development and harvesting (Madi et al. 2021; Saraswathi et al. 2017; Ali et al. 2017; Van et al. 2018; Fekri et al. 2021; Zolotilov et al. 2022; Lal et al. 2025; Pingle et al. 2015; Furlan et al. 2010). Such chemical variability highlights the importance of phytochemical markers for distinguishing between chemotypes and assessing the quality of germplasm.

Molecular marker techniques have been demonstrated to be effective in assessing genetic diversity among various *Cymbopogon* species (Bishoyi et al. 2016; Ganjewala 2008; Baruah et al. 2017; Kumar et al. 2009, 2007; Obaleye et al. 2023), and identifying significant intraspecific genetic variation in *C. citratus* that is linked to geographic origin and cultivation history (Shamsheer et al. 2022). The RAPD marker has been utilized to establish relationships (Khanuja et al. 2005; Shasany et al. 2000; Bishoyi et al. 2016) and assess genetic diversity among *Cymbopogon* species (Shasany et al. 2000; Baruah et al. 2017). Additionally, DNA barcoding regions, such as ITS 1 & ITS 2 spacers, matK, psbA-trnH and rbcL have been employed to discriminate and authenticate species at the species level (Bishoyi et al. 2016; Ma et al. 2023).

By integrating molecular data with phytochemical profiles, researchers can better understand the relationships between genotypes and chemotypes, thereby facilitating the identification of elite germplasm that exhibits stable, desirable traits. Therefore, the present study aims to assess the diversity of lemongrass (*Cymbopogon* spp.) germplasm collected from Vietnam and Laos using an integrated approach based on molecular and phytochemical markers. By combining molecular marker analysis with GC–MS-based phytochemical profiling, this research seeks to clarify genetic diversity and chemotypic variation, and their relationships within and across the two countries. This work will provide a scientific foundation for germplasm conservation, selection, and the sustainable utilization of lemongrass resources in Southeast Asia.

Materials and methods

Plant materials

A total of 35 lemongrass accessions were collected from different geographical locations across Vietnam (28 accessions) and Laos (7 accessions) presented in the Table 1.

Soil preparation and plant cultivation

The soil was collected from Hue city, Vietnam (16◦32′06.2″ N, 107◦31′39.7″ E) and characterized for electrolytic conductivity, pH, total C, available phosphorus, total N, water holding capacity, humidity and textural characteristics as previously reported (Truong et al. 2024). To fill a W23 cm × H40 cm pot, 18 kg of soil mixed with 20 g of organic compost (Que Lam, Vietnam) and 20 g of CaCO3. The mother plant of each accession was grown in a pot, in full sun positions with regular watering. After six months, new plants derived from the mother plants were used for phytochemical and molecular analysis.

Table 1 List of lemongrass (*Cymbopogon* spp.) accessions used in study

No	Accession name	Accession code	Genbank accession	Place of collection	Coordinates
1	HUIB_CC1	SK	PX699672	Savannakhet, Laos	16°32′38″ N 104°49′48″ E
2	HUIB_CC2	V1	PX699673	Vientiane, Laos	18°01′57″ N 102°36′00″ E
3	HUIB_CW3	V2	PX699674	Vientiane, Laos	18°01′57″ N 102°36′00″ E
4	HUIB_CC4	V3	PX699675	Vientiane, Laos	18°01′57″ N 102°36′00″ E
5	HUIB_CC5	L1	PX699676	Luang Prabang, Laos	19°45′05″ N 101°59′50″ E
6	HUIB_CC6	L2	PX699677	Luang Prabang, Laos	19°52′31″ N 102°08′06″ E
7	HUIB_CC7	BK	PX699678	Bolikhamsai, Lào	18°19′35″ N 103°51′40″ E
8	HUIB_CC8	DNo	PX699679	Lam Dong, Vietnam	12°13′59.0″ N 107°32′25.2″ E
9	HUIB_CC10	QT1	PX699680	Quang Tri, Vietnam	16°85′01.5″ N 107°09′30.2″ E
10	HUIB_CC11	HT	PX699681	Ha Tinh, Vietnam	18°27′25.7″ N 105°31′54.7″ E
11	HUIB_CC12	PY1	PX699682	Dak Lak, Vietnam	13°17′48.8″ N 109°02′29.4″ E
12	HUIB_CC13	PY2	PX699683	Dak Lak, Vietnam	13°21′22.5″ N 109°05′38.8″ E
13	HUIB_CC17	QT2	PX699684	Quang Tri, Vietnam	16°41′10.7″ N 106°53′46.6″ E
14	HUIB_CC18	QT3	PX699685	Quang Tri, Vietnam	16°50′33.4″ N 107°04′53.6″ E
15	HUIB_CC24	QT9	PX699686	Quang Tri, Vietnam	16°46′14.9″ N 107°08′13.9″ E
16	HUIB_CC25	QT10	PX699687	Quang Tri, Vietnam	16°43′09.0″ N 107°13′34.0″ E
17	HUIB_CC26	BD1	PX699688	Gia Lai, Vietnam	13°33′49.6″ N 109°00′43.0″ E
18	HUIB_CC27	BD2	PX699689	Gia Lai, Vietnam	13°45′53.7″ N 109°09′04.7″ E
19	HUIB_CC28	BD3	PX699690	Gia Lai, Vietnam	13°51′39.2″ N 109°11′09.9″ E
20	HUIB_CC30	BD5	PX699691	Gia Lai, Vietnam	13°47′38.7″ N 109°10′38.3″ E
21	HUIB_CC31	QN1	PX699692	Quang Ngai, Vietnam	15°06′32.7″ N 108°47′23.2″ E
22	HUIB_CC32	QN2	PX699693	Quang Ngai, Vietnam	15°04′52.4″ N 108°50′03.1″ E
23	HUIB_CC33	QNAM1	PX699694	Da Nang, Vietnam	15°52′40.3″ N 108°04′46.0″ E
24	HUIB_CC34	TTH2	PX699695	Hue, Vietnam	16°31′10.3″ N 107°19′35.2″ E
25	HUIB_CC35	NA1	PX699696	Nghe An, Vietnam	18°40′14.2″ N 105°36′42.8″ E
26	HUIB_CC36	NA2	PX699697	Nghe An, Vietnam	18°40′11.8″ N 105°36′39.7″ E
27	HUIB_CC37	TH1	PX699698	Thanh Hoa, Vietnam	20°23′58.5″ N 105°05′46.4″ E
28	HUIB_CW38	TH2	PX699699	Thanh Hoa, Vietnam	20°23′37.0″ N 105°08′48.9″ E
29	HUIB_CC39	TN1	PX699700	Thai Nguyen, Vietnam	21°27′25.4″ N 105°50′35.2″ E
30	HUIB_CC40	BP1	PX699701	Dong Nai, Vietnam	11°33′04.5″ N 106°44′40.8″ E
31	HUIB_CC41	CM1	PX699702	Ca Mau, Vietnam	8°55′04.8″ N 104°56′28.0″ E
32	HUIB_CC42	VL1	PX699703	Vinh Long, Vietnam	10°10′22.0″ N 106°06′02.2″ E
33	HUIB_CC43	QN3	PX699704	Quang Ngai, Vietnam	15°01′00.7″ N 108°49′50.1″ E
34	HUIB_CC44	TTH3	PX699705	Hue, Vietnam	16°24′53.4″ N 107°32′55.2″ E
35	HUIB_CC45	HCM1	PX699706	Ho Chi Minh, Vietnam	11°01′06.7″ N 106°31′32.8″ E

Molecular analysis

Genomic DNA extraction

The genomic DNA of each accession was extracted from young leaves by the CTAB (cetyl trimethyl ammonium bromide) method and purified to achieve a ratio of A260:A280 between 1.8 and 2.0, following the previously described protocol (Rasphone et al. 2023).

ITS characterization

The genomic DNA of 35 accessions were amplified with ITS_{u1-4} primers (Rasphone et al. 2022) in a PCR system (Applied Biosystems,99 USA). The PCR mixture contained 20 ng/μL DNA, 5 μL of 2X MyTaq Mix100 (Meridian Bioscience, US) and 0.5 μM of primer, all in a total volume of 15 μL. The thermocycling program was conducted as follows: 95 °C for 5 min; followed by 35 cycles of 95 °C for 15 s, 56 °C for 15 s, and 72 °C for 30 s; concluding with 72 °C for 5 min. PCR products were then sequenced using the Sanger sequencing method at 1 st BASE (Apical Scientific Sdn. Bhd., Malaysia) and analyzed using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Multiple sequence alignment was performed with MEGA 12 software to construct a phylogenetic tree (Kumar et al. 2024). Additionally, DNAsp v6 software was used to evaluate various parameters of the sequences, including the number of variable (polymorphic) sites, the number of mutations, nucleotide diversity, average number of nucleotide differences, minimum number of recombination events, number of Haplotypes, and Haplotype diversity (Rozas et al. 2017).

RAPD analysis

The genomic DNA of three selected accessions (HUIB_CC2, HUIB_CW3 and HUIB_CC32) was prescreened using 200 UBC (The University of British Columbia) RAPD primers (Bioneer, Korea) (see Supplemental Table) to identify polymorphic primers. The identified polymorphisms were then confirmed using the selected accessions along with three additional accessions (HUIB_CC2, HUIB_CW3, HUIB_CC32, HUIB_CC36 and HUIB_CW38) to screen for polymorphic primers applicable to the entire germplasm. The PCR reactions, with a total volume of 15 μL, included of 20 ng/μL DNA, 5 μL of 2X MyTaq Mix (Meridian Bioscience, US), 1.675 mM MgCl2, 0.67 μM of RAPD primer, and 167.5 μM of each dNTP. PCR amplification was conducted following the previously described thermal cycling protocol (Truong et al. 2013). The resulting PCR products were then separated on a 1.5% agarose gel and visualized under ultraviolet light. A binary matrix was constructed for the analysis of the RAPD data.This matrix was used to calculate various evaluation indicators and genetic similarity coefficients to develop a UPGMA clustering tree using NTSYS software v2.10 m. The evaluation indicators included total number of bands (TB), number of monomorphic bands (MB), percentage of polymorphic bands (PPB), polymorphism information content (PIC), marker index (MI), and resolving power (Rp). Additionally, the matrix was analyzed using R version 4.5.2 and RStudio version 2025.09.2-418 to generate a heatmap of loci with the complexheatmap package and to perform Principal Coordinates Analysis (PCoA) using the ggplot2, ggfortify, and cluster packages.

Phytochemical marker analysis

A total of 100 g of fresh *Cymbopogon* plant material was harvested at 12 weeks after planting and finely ground. From this, 20.0 g of the homogenized sample was transferred into a 250 mL conical flask containing 100 mL of n-hexane. The mixture was sonicated for 15 min, and then macerated for 24 h at room temperature. The resulting extract was first filtered through coarse filter paper and then passed through a 0.45 μm membrane filter to obtain a clear filtrate. The chemical constituents of the *Cymbopogon* extract were analyzed using a GC–MS/MS TQ8040 system (Shimadzu Corporation, Tokyo, Japan). The chromatographic conditions were as follows: helium was used as the carrier gas, a DB-5 capillary column was employed, the injection port temperature was maintained at 250 °C, the flow rate was set at 1.71 mL/min, and the injection volume was 1.0 μL. The oven temperature was set to 50 °C for 1 min, then increased at a rate of 5 °C per minute to 250 °C and held for an additional minute. The mass spectrometer operated over a scan range of 50–500 m/z. The data was analyzed using IBM SPSS Statistics (version 27) and RStudio. Mean values of the data were compared and separated using ANOVA and Duncan's test ($\alpha = 0.05$). Principal Component Analysis (PCA) was applied to the standardized data to explore multivariate patterns and discriminate among lemongrass accessions; PCA results were interpreted based on score and loading structures following standard PCA methodology (Jolliffe 2002). To identify groups among accessions, K-means clustering was conducted (McQueen 1967). The optimal number of clusters (k) was selected using the Gap statistic (Tibshirani et al. 2001), implemented with the "gap_stat" approach (factoextra package) (Kassambara 2020). Heatmaps were generated to visualize the relative accumulation levels of characteristic compounds across the resulting groups, and hierarchical clustering was applied to both accessions and compounds to support pattern visualization and group separation. Heatmaps were produced using the pheatmap package (Kolde 2025).

Results and discussion

Molecular-based genetic diversity analysis

ITS-based analysis

The ITS-based phylogenetic tree effectively organized lemongrass germplasm into well-supported clades that corresponded to the recognized species of *Cymbopogon* (Fig. 1). Most of the accessions, which were collected from Vietnam and Laos, group closely with authenticated reference sequences of *Cymbopogon citratus* (GenBank accessions AF019823 and KT309069). This clade is robustly supported by a bootstrap value of 100, confirming the accurate taxonomic classification of these accessions as *C. citratus* and indicating low interspecific divergence within this group. Within the *C. citratus* clade, the short branch lengths and limited internal resolution suggest a high level of genetic similarity among the accessions. This observation aligns with previous studies that have reported limited genetic diversity in cultivated *C. citratus*. This lack of diversity is primarily attributed to factors such as vegetative propagation, limited sexual reproduction, and a long-term emphasis on selecting uniform essential oil traits (Baruah et al. 2017; Shamsheer et al. 2022).In contrast, only two accessions, TH2 and V2, formed a distinct cluster outside the *C. citratus* clade. Instead, they grouped with reference sequences of *C. winterianus* (KT309076) and *C. flexuosus* (KX828252, KX828250). However, these accessions were more closely related to *C. winterianus* and were identified as such. This separation was supported by moderate to high bootstrap values (51–77), indicating greater genetic divergence among *Cymbopogon* species than within *C. citratus*.

The ITS-based genetic diversity indices indicate minimal intraspecific variation within the analyzed

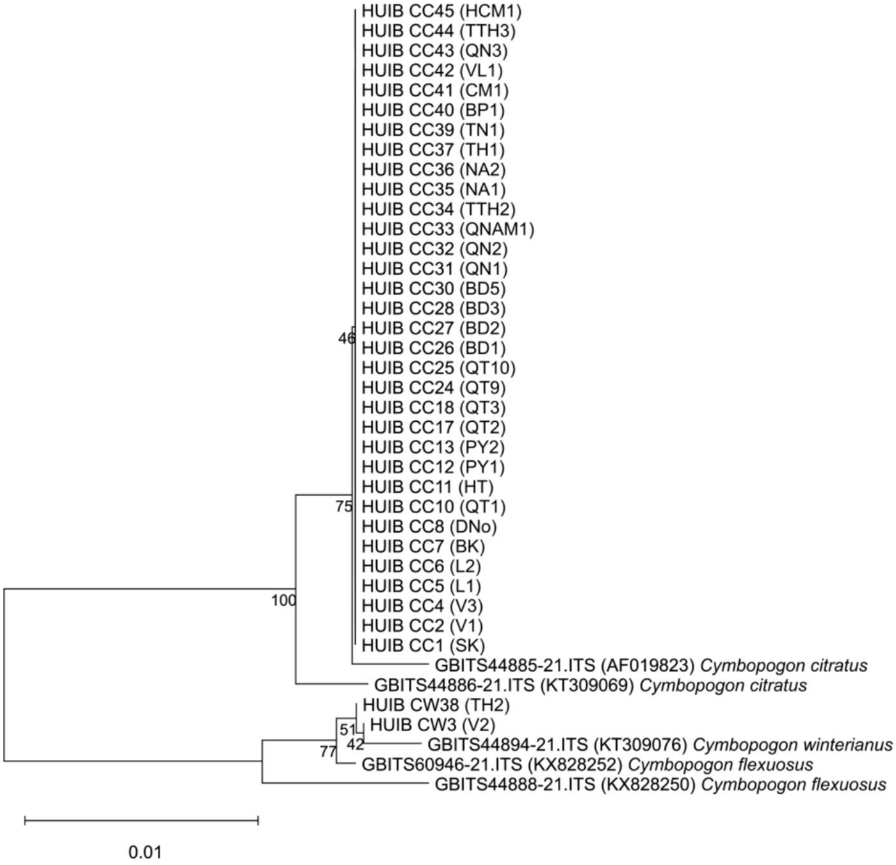


Fig. 1 Evolutionary relationships of taxa based on internal transcribed spacer (ITS) region in the genetic nucleus of *Cymbopogon* population

Cymbopogon population (Table 2). Although 19 polymorphic sites (S) and an equal number of mutations (Eta = 19) were detected, the overall nucleotide diversity remains low at $\pi = 3.24 \times 10$¯3, and the average number of pairwise nucleotide differences was also small at $k = 2.11$. This suggests that the sequence variation is confined to a few substitutions shared among most accessions rather than broadly distributed across the population. Only two haplotypes (h = 2) were identified, with extremely low haplotype diversity (Hd = 0.111). The combination of low Hd and low π is characteristic of populations with restricted genetic variation, which commonly results from clonal or vegetative propagation, directional selection, or historical bottlenecks (Tajima 1983; Nei 1987). In cultivated *Cymbopogon*, particularly *C. citratus*, such patterns are expected because propagation is predominantly vegetative and germplasm selection has focused on maintaining uniform essential oil profiles, exceptionally high citral content. Comparable studies using ITS and other nuclear markers have reported similarly narrow genetic diversity in cultivated *Cymbopogon* compared to its wild relatives. This emphasizes that while ITS regions provide strong resolution at the species level, they have limited discriminatory power at the intraspecific level (Bishoyi et al. 2017). Thus, the findings in this study are consistent with previous study (Bishoyi et al. 2017). The results indicate that the *Cymbopogon* population studied is genetically homogeneous at the ITS locus. This finding underscores the need to include more polymorphic markers, such as RAPD, to better capture subtle genetic variation for conservation and breeding purposes.

Table 2 Polymorphism evaluation indices of *Cymbopogon* population based on internal transcribed spacer (ITS) sequence analysis

Indices	Population
No. of variable (polymorphic) sites, S	19
No. of mutations, Eta	19
Nucleotide diversity (per site), Pi ($\times 10^{-3}$)	324
Average number of nucleotide differences, k	210,756
Number of haplotypes, h	2
Haplotype diversity, Hd	0111

RAPD-based analysis

Out of 200 primers prescreened across three *Cymbopogon* accessions such V1, TH2 and QN2, only ten primers showed polymorphism. These polymorphic primers were then confirmed using the selected accessions, along with two additional ones: V2 and NA2. Eight primers were ultimately chosen because they generated the most distinct, clear, and polymorphic bands (Table 3). These primers were then used to assess genetic diversity through RAPD analysis of 35 *Cymbopogon* accessions (Fig. 2).

RAPD marker analysis reveals moderate genetic polymorphism among the analyzed *Cymbopogon* accessions (Table 3). A total of 86 bands were amplified across eight primers, averaging 10.75 bands per primer, of which 61.28% were polymorphic. This level of polymorphism is comparable to previous RAPD-based studies on *Cymbopogon*, which typically reported polymorphic band percentages ranging from approximately 50 to 70%, depending on the species composition and germplasm origin (Bishoyi et al. 2016; Baruah et al. 2017; Shasany et al. 2000). The PIC (Polymorphism Information Content) values ranged from 0.027 to 0.115, with a mean of 0.082,

Table 3 Characteristics and polymorphism parameters of random amplified polymorphic DNA (RAPD) markers in *Cymbopogon* accessions

No	Primer	Band size (bp)	TB	PB	PPB	PIC	Rp	MI	EMR
1	UBC#317	350–1500	10	5	50.000	0.075	0.857	0.188	2.500
2	UBC#318	350–1400	10	5	50.000	0.068	0.743	0.169	2.500
3	UBC#350	300–1800	13	7	53.846	0.054	0.743	0.204	3.769
4	UBC#399	500–2500	14	12	85.714	0.115	1.771	1.183	10.286
5	UBC#411	500–1350	7	6	85.714	0.106	0.800	0.545	5.143
6	UBC#460	300–2000	15	11	73.333	0.114	1.943	0.920	8.067
7	UBC#465	500–1800	8	2	25.000	0.027	0.229	0.013	0.500
8	UBC#493	450–1800	9	6	66.667	0.097	0.971	0.387	4.000
Mean		300–2500	10.750	6.750	61.284	0.082	1.007	0.451	4.596

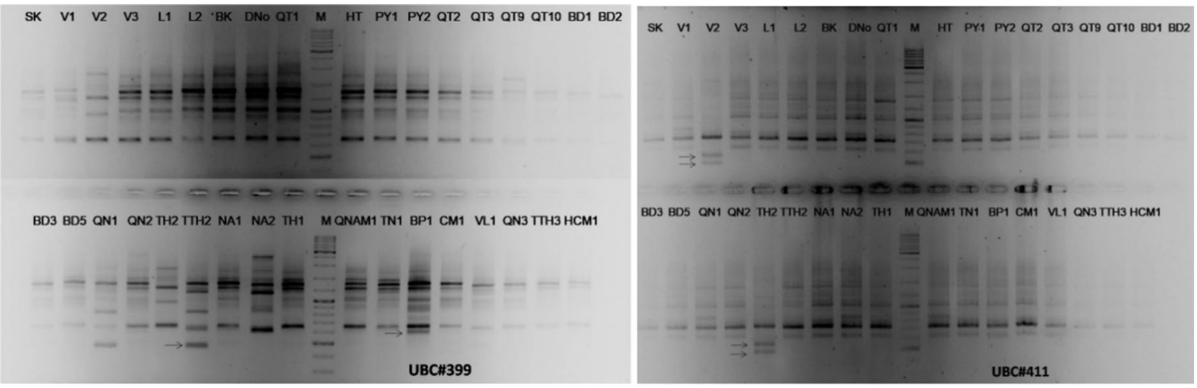
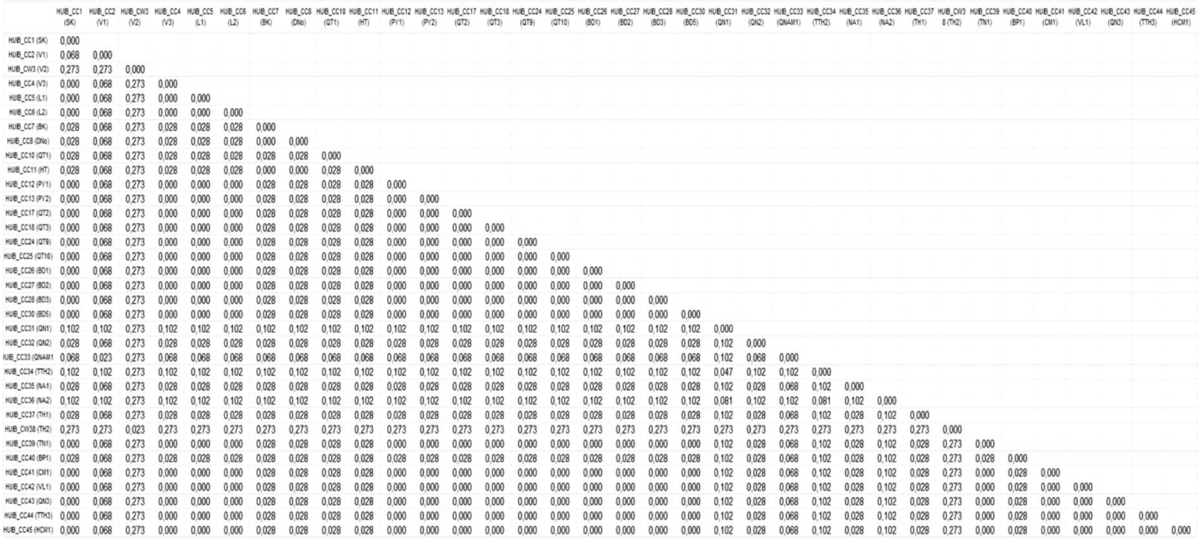


Fig. 2 The polymerase chain reaction (PCR) products generated from the selected random amplified polymorphic DNA (RAPD) primers (UBC#399 and UBC#411) exhibit distinct polymorphisms among *Cymbopogon* accessions. M: the 100 bp ladder; Arrows: polymorphic bands

indicating that the RAPD primers provided low to moderate discriminatory power. Such low PIC values are typical for dominant markers, such as RAPD, especially in clonally propagated crops where allelic variation is restricted (Powell et al. 1996). However, primers UBC#399 and UBC#460 exhibited relatively higher PIC values (greater than 0.11), suggesting they were more effective at detecting polymorphisms within the studied population. The resolving power (Rp) varied significantly among the primers, ranging from 0.229 to 1.943, with UBC#460 and UBC#399 demonstrating the highest Rp values. A higher Rp indicates a primer's ability to distinguish among genotypes and is often associated with greater polymorphism and band informativeness (Prevost and Wilkinson 1999). Consistently, these primers also showed higher marker index (MI) and effective multiplex ratio (EMR), confirming their superior performance. The mean marker index (MI) of 0.451 and effective multiplication rate (EMR) of 4.596 indicated a moderate overall efficiency of the RAPD system in capturing genetic variability. Similar MI values have been reported in successfully differentiated accessions of *Cymbopogon* using RAPD markers (Baruah et al. 2017; Shasany et al. 2000). Additionally, the relatively high percentage of polymorphic bands (PPB) observed with specific primers, such as UBC#411 (85.7%) suggesting localized genomic variability.

The RAPD-based genetic distance matrix indicated low overall genetic divergence among *Cymbopogon* accessions. Most genetic distance values fall within the range of 0.000–0.068, with only a few pairs of accessions reaching distances of 0.102 to 0.273 (Fig. 3). The presence of zero genetic distance among many pairs of accessions indicated that they were genetically identical or nearly identical clones. This reflects practices of clonal multiplication and the widespread exchange of planting materials. These findings align with similar patterns reported in other studies on *Cymbopogon* using RAPD and ISSR markers (Baruah et al. 2017; Shasany et al. 2000; Munda et al. 2022). The highest distances, approximately 0.273, were observed between *C. winterianus* accessions and *C. citratus* accessions. This suggests the presence of distinct genotypes that may have originated from different source populations or species. Similar maximum genetic distances based on RAPD analysis have been reported between geographically separated *Cymbopogon* accessions and between cultivated and semi-wild materials (Baruah et al. 2017).

The UPGMA dendrogram constructed from RAPD markers showed high genetic similarity among accessions withineach species, including *C. citratus* and *C. winterianus* (Fig. 4). Most accessions group closely together, without clear geographic separation, reflecting a genetically narrow cultivated gene pool. Such clustering patterns are typical of *C. citratus* and *C. winterianus* that were primarily propagated vegetatively and subjected to strong selection for uniform agronomic and essential oil traits (Shasany et al. 2000; Powell et al. 1996). The two *C. winterianus* accessions, such as TH2 and V2, were placed on more distant branches, suggesting a greater genetic divergence



	HUIB_CC1 (SK)	HUIB_CC2 (V1)	HUIB_CW3 (V2)	HUIB_CC4 (V3)	HUIB_CC5 (L1)	HUIB_CC6 (L2)	HUIB_CC7 (BK)	HUIB_CC8 (DNo)	HUIB_CC10 (QT1)	HUIB_CC11 (HT)	HUIB_CC12 (PY1)	HUIB_CC13 (PY2)	HUIB_CC17 (QT2)	HUIB_CC18 (QT3)	HUIB_CC24 (QT9)	HUIB_CC25 (QT10)	HUIB_CC26 (BD1)	HUIB_CC27 (BD2)	HUIB_CC28 (BD3)	HUIB_CC30 (BD5)	HUIB_CC31 (QN1)	HUIB_CC32 (QN2)	HUIB_CC33 (QNAM1)	HUIB_CC34 (TTH2)	HUIB_CC35 (NA1)	HUIB_CC36 (NA2)	HUIB_CC37 (TH1)	HUIB_CW38 (TH2)	HUIB_CC39 (TN1)	HUIB_CC40 (BP1)	HUIB_CC41 (CM1)	HUIB_CC42 (VL1)	HUIB_CC43 (QN3)	HUIB_CC44 (TTH3)	HUIB_CC45 (HCM1)
HUIB_CC1 (SK)	0,000																																		
HUIB_CC2 (V1)	0,068	0,000																																	
HUIB_CW3 (V2)	0,273	0,273	0,000																																
HUIB_CC4 (V3)	0,000	0,068	0,273	0,000																															
HUIB_CC5 (L1)	0,000	0,068	0,273	0,000	0,000																														
HUIB_CC6 (L2)	0,000	0,068	0,273	0,000	0,000	0,000																													
HUIB_CC7 (BK)	0,028	0,068	0,273	0,028	0,028	0,028	0,000																												
HUIB_CC8 (DNo)	0,028	0,068	0,273	0,028	0,028	0,028	0,000	0,000																											
HUIB_CC10 (QT1)	0,028	0,068	0,273	0,028	0,028	0,028	0,028	0,028	0,000																										
HUIB_CC11 (HT)	0,028	0,068	0,273	0,028	0,028	0,028	0,000	0,000	0,028	0,000																									
HUIB_CC12 (PY1)	0,000	0,068	0,273	0,000	0,000	0,000	0,028	0,028	0,028	0,028	0,000																								
HUIB_CC13 (PY2)	0,000	0,068	0,273	0,000	0,000	0,000	0,028	0,028	0,028	0,028	0,000	0,000																							
HUIB_CC17 (QT2)	0,000	0,068	0,273	0,000	0,000	0,000	0,028	0,028	0,028	0,028	0,000	0,000	0,000																						
HUIB_CC18 (QT3)	0,000	0,068	0,273	0,000	0,000	0,000	0,028	0,028	0,028	0,028	0,000	0,000	0,000	0,000																					
HUIB_CC24 (QT9)	0,000	0,068	0,273	0,000	0,000	0,000	0,028	0,028	0,028	0,028	0,000	0,000	0,000	0,000	0,000																				
HUIB_CC25 (QT10)	0,000	0,068	0,273	0,000	0,000	0,000	0,028	0,028	0,028	0,028	0,000	0,000	0,000	0,000	0,000	0,000																			
HUIB_CC26 (BD1)	0,000	0,068	0,273	0,000	0,000	0,000	0,028	0,028	0,028	0,028	0,000	0,000	0,000	0,000	0,000	0,000	0,000																		
HUIB_CC27 (BD2)	0,000	0,068	0,273	0,000	0,000	0,000	0,028	0,028	0,028	0,028	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000																	
HUIB_CC28 (BD3)	0,000	0,068	0,273	0,000	0,000	0,000	0,028	0,028	0,028	0,028	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000																
HUIB_CC30 (BD5)	0,000	0,068	0,273	0,000	0,000	0,000	0,028	0,028	0,028	0,028	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000															
HUIB_CC31 (QN1)	0,102	0,102	0,273	0,102	0,102	0,102	0,102	0,102	0,102	0,102	0,102	0,102	0,102	0,102	0,102	0,102	0,102	0,102	0,102	0,102	0,000														
HUIB_CC32 (QN2)	0,028	0,068	0,273	0,028	0,028	0,028	0,028	0,028	0,028	0,028	0,028	0,028	0,028	0,028	0,028	0,028	0,028	0,028	0,028	0,028	0,102	0,000													
HUIB_CC33 (QNAM1	0,068	0,023	0,273	0,068	0,068	0,068	0,068	0,068	0,068	0,068	0,068	0,068	0,068	0,068	0,068	0,068	0,068	0,068	0,068	0,068	0,102	0,068	0,000												
HUIB_CC34 (TTH2)	0,102	0,102	0,273	0,102	0,102	0,102	0,102	0,102	0,102	0,102	0,102	0,102	0,102	0,102	0,102	0,102	0,102	0,102	0,102	0,102	0,047	0,102	0,102	0,000											
HUIB_CC35 (NA1)	0,028	0,068	0,273	0,028	0,028	0,028	0,028	0,028	0,028	0,028	0,028	0,028	0,028	0,028	0,028	0,028	0,028	0,028	0,028	0,028	0,102	0,028	0,068	0,102	0,000										
HUIB_CC36 (NA2)	0,102	0,102	0,273	0,102	0,102	0,102	0,102	0,102	0,102	0,102	0,102	0,102	0,102	0,102	0,102	0,102	0,102	0,102	0,102	0,102	0,081	0,102	0,102	0,081	0,102	0,000									
HUIB_CC37 (TH1)	0,028	0,068	0,273	0,028	0,028	0,028	0,028	0,028	0,028	0,028	0,028	0,028	0,028	0,028	0,028	0,028	0,028	0,028	0,028	0,028	0,102	0,028	0,068	0,102	0,028	0,102	0,000								
HUIB_CW38 (TH2)	0,273	0,273	0,023	0,273	0,273	0,273	0,273	0,273	0,273	0,273	0,273	0,273	0,273	0,273	0,273	0,273	0,273	0,273	0,273	0,273	0,273	0,273	0,273	0,273	0,273	0,273	0,273	0,000							
HUIB_CC39 (TN1)	0,000	0,068	0,273	0,000	0,000	0,000	0,028	0,028	0,028	0,028	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,102	0,028	0,068	0,102	0,028	0,102	0,028	0,273	0,000						
HUIB_CC40 (BP1)	0,028	0,068	0,273	0,028	0,028	0,028	0,028	0,028	0,028	0,028	0,028	0,028	0,028	0,028	0,028	0,028	0,028	0,028	0,028	0,028	0,102	0,028	0,068	0,102	0,028	0,102	0,028	0,273	0,028	0,000					
HUIB_CC41 (CM1)	0,000	0,068	0,273	0,000	0,000	0,000	0,028	0,028	0,028	0,028	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,102	0,028	0,068	0,102	0,028	0,102	0,028	0,273	0,000	0,028	0,000				
HUIB_CC42 (VL1)	0,000	0,068	0,273	0,000	0,000	0,000	0,028	0,028	0,028	0,028	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,102	0,028	0,068	0,102	0,028	0,102	0,028	0,273	0,000	0,028	0,000	0,000			
HUIB_CC43 (QN3)	0,000	0,068	0,273	0,000	0,000	0,000	0,028	0,028	0,028	0,028	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,102	0,028	0,068	0,102	0,028	0,102	0,028	0,273	0,000	0,028	0,000	0,000	0,000		
HUIB_CC44 (TTH3)	0,000	0,068	0,273	0,000	0,000	0,000	0,028	0,028	0,028	0,028	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,102	0,028	0,068	0,102	0,028	0,102	0,028	0,273	0,000	0,028	0,000	0,000	0,000	0,000	
HUIB_CC45 (HCM1)	0,000	0,068	0,273	0,000	0,000	0,000	0,028	0,028	0,028	0,028	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,102	0,028	0,068	0,102	0,028	0,102	0,028	0,273	0,000	0,028	0,000	0,000	0,000	0,000	0,000

Fig. 3 Pairwise genetic distance matrix of *Cymbopogon* accessions based on random amplified polymorphic DNA (RAPD) markers

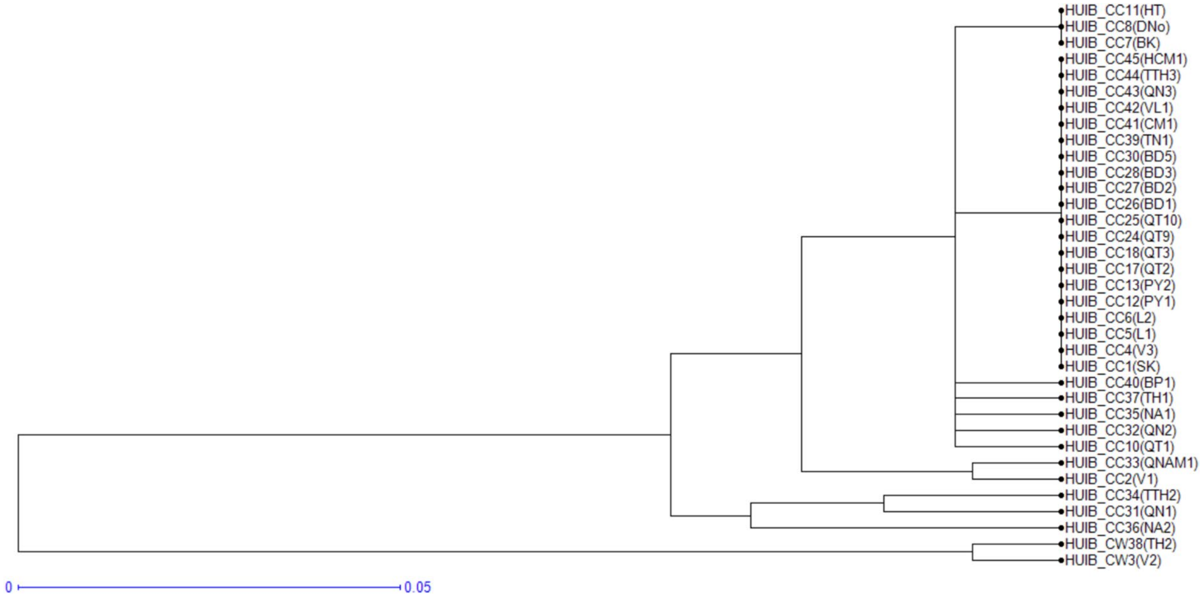


Fig. 4 Dendrogram showing genetic relationship among of *Cymbopogon* accessions based on random amplified polymorphic DNA (RAPD) marker analysis

from the main cluster containing all the *C. citratus* accessions. These two accessions represented distinct genotypes that were introduced from different species. The result is consistent with previous studies indicating that a few divergent accessions in *C. citratus* and related species were observed using RAPD and ISSR markers (Bishoyi et al. 2016; Baruah et al. 2017). The lack of strong geographic structure in the dendrogram suggested extensive exchange of planting materials across regions, leading to the homogenization of cultivated germplasm.

Regarding molecular polymorphism, although additional marker systems such as ISSR or SSR could further enhance resolution, the present study employed *ITS* for species-level discrimination combined with extensive RAPD prescreening of 200 primers, from which eight highly informative markers were selected. This integrated strategy proved sufficient to resolve genetic structure relevant to germplasm identification and selection, and provides a reliable molecular framework for subsequent chemotype-based evaluation and breeding.

Phytochemical diversity analysis

The chemical profiles of the *Cymbopogon* germplasm revealed 13 dominant components such β-myrcene, Cis-ocimene, linalool L, citronella, β-citronellol, Z-citral, geraniol, E-citral, geranyl acetate, Trans-caryophyllene, α-bergamotene, germacrene D-4-ol, and juniper camphor. Additionally, there were 14 minor constituents identified such α-pinene,camphene, dl-limonene, 1,8-cineole, citronellyl acetate, 3-allyl-guaiacol, α-humulene, germacrene-D, gamma-cadinene, α-patchoulene, β-ionol, α-longipinene, elemol, and (-)-caryophyllene oxide. Each of these components played significant biological roles, commercial applications, and showedstatistically significant differences among accessions (Table 4; Fig. 5). Among these constituents, E-citral (geranial) and Z-citral (neral) were consistently the most abundant compounds (Fig. 6). Accessions such as PY1, TN1, QNAM1, and TTH3 are located near the Z-Citral and E-Citral vectors (Fig. 7).E-citral concentration ranged from 22.480 to 67.577%. Sample SK had the highest concentration of E-citral (67.577%) among all the samples studied. This composition is characteristic of high-quality lemongrass oils, as citrals contribute to their distinct lemon scent and antimicrobial characteristics (Barbosa et al. 2008). Interestingly, some samples, such as V2, showed a notable deviation with a much lower E-citral content (22.480%) and significantly higher levels of geraniol (41.240%). These concentrations was lower than those found in fresh plant leaves of Brazilian commercial *Cymbopogon citratus*, which exhibited a citral content of 81.7% (Barbosa et al. 2008).This suggests that botanical or geographical variations may influence the biosynthetic pathways (Wang et al. 2024; De Martino et al. 2009), and that the extraction method can affect the yield and final composition of the essential oil (Schaneberg & Khan, 2002). The Z-citral content showed significant variation across different samples. For example, sample BD1 and QNAM1 had the highest Z-citral content at 25.107% and 25.027%, respectively, slightly surpassing sample TTH3, which had 24.447%. In contrast, sample TH2 exhibited a relatively lowest Z-citral level at 9.787%. The phytochemical profiles in this study were obtained using n-hexane-assisted extraction with sonication, which results in a volatile extract rather than a hydrodistilled essential oil. This extraction method may influence the relative abundance of certain constituents. However, the major monoterpenoids found in *Cymbopogon citratus*, particularly the citral isomers and geraniol, have been consistently detected across various extraction methods. This consistency allows for reliable comparative interpretations of chemotypic variation. Therefore, the term "essential oil composition" is retained in this study to facilitate comparison with previous research (Avoseh et al. 2015; Fekri et al. 2021).

The dominant groups of geraniol and β-citronellol included accessions V2, which was clearly separated on PCA, and QT3 and QN2, both of which showed superior accumulation of geraniol and β-citronellol (Fig. 6).Accessions V2 and HCM1 gained high geraniol concentrations of 41.240% and 36.967%, respectively. In contrast, sample SK, while high in citrals, contained only 4.973% geraniol, indicating a specific chemotypic specialization. This finding aligns with the results of previous studies (Dangol et al. 2023; Munda et al. 2022). This is particularly interesting because the biosynthesis of geraniol competes with that of citral, both originating from the same precursor, geranyl pyrophosphate (GPP). This competition highlights the plant's metabolic prioritization (De Martino et al. 2009).

The highest concentration of β-citronellol was observed in sample QN2 at 7.43%. High levels of citronellol often occured alongside increased geraniol content, as seen in samples V2 and TH2. This relationship reflects the interconnected and co-regulated branches of the monoterpenoid/isoprenoid biosynthetic pathway (Bergman et al. 2020).The ratio of citronellol to geraniol varied depending on the developmental stage of the leaves and the region where they are harvested (Fekri et al. 2021) as well as climatic and geographical conditions (Zolotilov et al. 2022).β-Myrcene is a monoterpene hydrocarbon

Table 4 Major phytochemical components in percentage observed in *Cymbopogon* germplasm

No	Accession name	Accession code	Beta-myrcene	Cis-ocimene	Linalool L	Citronella	Beta-citronellol	Z-citral	Geraniol
1	SK	HUIB_CC1	2.953 m ± 0.075	0.507i ± 0.015	0.357op ± 0.025	0.000 l ± 0.000	0.437v ± 0.006	18.953u ± 0.015	4.973ff ± 0.006
2	V1	HUIB_CC2	1.510 s ± 0.017	0.393 k ± 0.040	0.467i-l ± 0.012	0.000 l ± 0.000	0.723rs ± 0.023	20.537p ± 0.006	5.477ee ± 0.098
3	V2	HUIB_CW3	0.000t ± 0.000	0.000r ± 0.000	0.330o-q ± 0.010	3.620b ± 0.026	4.083e ± 0.029	10.027x ± 0.006	41.240a ± 0.036
4	V3	HUIB_CC4	1.576 s ± 0.040	0.280no ± 0.036	0.293q ± 0.015	0.000 l ± 0.000	0.547tu ± 0.015	19.910qr ± 0.030	6.150dd ± 0.056
5	L1	HUIB_CC5	2.970 m ± 0.040	0.373kl ± 0.021	0.427 k-m ± 0.015	0.120j ± 0.000	1.340p ± 0.010	21.793ij ± 0.061	21.843 l ± 0.045
6	L2	HUIB_CC6	1.940r ± 0.044	0.627 h ± 0.012	0.440j-m ± 0.020	0.087 k ± 0.006	1.120q ± 0.020	21.483 m ± 0.038	12.680u ± 0.030
7	BK	HUIB_CC7	2.937 m ± 0.100	0.793f ± 0.021	0.323pq ± 0.021	0.000 l ± 0.000	0.507uv ± 0.015	22.450 g ± 0.078	4.253gg ± 0.060
8	DNo	HUIB_CC8	2.563o ± 0.096	0.197pq ± 0.006	0.437j-m ± 0.015	0.000 l ± 0.000	0.720rs ± 0.026	22.797f ± 0.045	10.807w ± 0.077
9	QT1	HUIB_CC10	2.737n ± 0.045	1.000c ± 0.010	0.477 h-j ± 0.015	0.157 h ± 0.006	0.783r ± 0.012	19.213t ± 0.045	9.020z ± 0.020
10	HT	HUIB_CC11	1.660 s ± 0.040	0.267o ± 0.006	0.370no ± 0.010	0.147hi ± 0.012	0.440v ± 0.010	17.303w ± 0.015	8.070 cc ± 0.036
11	PY1	HUIB_CC12	3.683 h ± 0.107	0.403jk ± 0.031	0.320pq ± 0.046	0.000 l ± 0.000	0.740r ± 0.026	21.880i ± 0.070	8.567aa ± 0.023
12	PY2	HUIB_CC13	2.227q ± 0.023	0.217p ± 0.015	0.197r ± 0.012	0.000 l ± 0.000	2.117 lm ± 0.068	21.103n ± 0.040	22.277 k ± 0.061
13	QT2	HUIB_CC17	2.417op ± 0.040	0.443j ± 0.021	0.520e-h ± 0.020	0.183 g ± 0.015	2.420j ± 0.010	20.020q ± 0.010	27.970 g ± 0.046
14	QT3	HUIB_CC18	3.183kl ± 0.083	0.000r ± 0.000	0.000 s ± 0.000	0.000 l ± 0.000	5.533b ± 0.116	17.523v ± 0.061	30.730d ± 0.062
15	QT9	HUIB_CC24	3.557hi ± 0.049	0.910d ± 0.020	0.527e-g ± 0.015	0.130ij ± 0.000	0.793r ± 0.021	21.083n ± 0.046	10.220x ± 0.026
16	QT10	HUIB_CC25	3.100 lm ± 0.030	0.510i ± 0.010	0.407 mn ± 0.015	0.000 l ± 0.000	0.637st ± 0.006	19.900r ± 0.026	9.590y ± 0.017
17	BD1	HUIB_CC26	2.317pq ± 0.055	0.173q ± 0.006	0.423 lm ± 0.006	0.000 l ± 0.000	0.820r ± 0.030	25.107a ± 0.045	11.060v ± 0.020
18	BD2	HUIB_CC27	3.177kl ± 0.046	0.653 h ± 0.006	0.630b ± 0.017	0.303e ± 0.006	3.120 h ± 0.000	21.760jk ± 0.017	22.517j ± 0.006
19	BD3	HUIB_CC28	3.280jk ± 0.040	0.370kl ± 0.017	0.507e-i ± 0.023	0.327d ± 0.015	3.517 g ± 0.021	23.073e ± 0.064	30.490e ± 0.070
20	BD5	HUIB_CC30	2.747n ± 0.147	0.000r ± 0.000	0.463i-l ± 0.045	0.000 l ± 0.000	1.603n ± 0.015	19.860rs ± 0.056	18.863r ± 0.006
21	QN1	HUIB_CC31	5.990b ± 0.207	0.000r ± 0.000	0.353op ± 0.042	0.000 l ± 0.000	3.927f ± 0.055	23.543c ± 0.225	25.513i ± 0.0379
22	QN2	HUIB_CC32	3.407ij ± 0.278	0.627 h ± 0.023	0.303q ± 0.021	0.000 l ± 0.000	7.437a ± 0.237	19.750 s ± 0.139	38.843b ± 0.263
23	QNAM1	HUIB_CC33	4.617f ± 0.029	1.043b ± 0.065	0.747a ± 0.055	0.000 l ± 0.000	2.207kl ± 0.031	25.027a ± 0.085	18.520 s ± 0.017
24	TTH2	HUIB_CC34	3.990 g ± 0.111	0.660 h ± 0.017	0.493 g-i ± 0.047	0.000 l ± 0.000	2.363j ± 0.093	23.393d ± 0.065	19.837o ± 0.058
25	NA1	HUIB_CC35	2.210q ± 0.108	0.717 g ± 0.040	0.543d-f ± 0.025	0.123j ± 0.015	3.423 g ± 0.012	21.553 lm ± 0.102	27.173 h ± 0.093
26	NA2	HUIB_CC36	5.397 cd ± 0.188	0.850e ± 0.036	0.500f-i ± 0.010	0.000 l ± 0.000	3.007i ± 0.055	23.147e ± 0.064	20.913n ± 0.040
27	TH1	HUIB_CC37	6.420a ± 0.085	1.053ab ± 0.040	0.617bc ± 0.015	0.390c ± 0.020	1.360op ± 0.030	22.687f ± 0.076	19.057q ± 0.015
28	TH2	HUIB_CW38	0.020t ± 0.000	0.000r ± 0.000	0.547de ± 0.006	5.723a ± 0.012	2.093 m ± 0.012	9.787y ± 0.025	27.203 h ± 0.070
29	TN1	HUIB_CC39	3.640 h ± 0.106	0.437j ± 0.015	0.487 g-i ± 0.016	0.000 l ± 0.000	0.563tu ± 0.025	21.653kl ± 0.042	8.337bb ± 0.021
30	BP1	HUIB_CC40	3.050 lm ± 0.026	0.317 mn ± 0.015	0.470i-k ± 0.020	0.253f ± 0.025	1.447o ± 0.025	17.360w ± 0.026	21.473 m ± 0.040
31	CM1	HUIB_CC41	5.25d ± 0.079	0.820ef ± 0.020	0.600bc ± 0.020	0.303e ± 0.006	2.253 k ± 0.049	23.333d ± 0.040	21.947 l ± 0.038
32	VL1	HUIB_CC42	5.533c ± 0.107	0.827ef ± 0.023	0.577 cd ± 0.015	0.000 l ± 0.000	5.233d ± 0.076	20.947o ± 0.075	29.213f ± 0.085

Table 4 (continued)

No	Accession name	Accession code	Beta-myrcene	Cis-ocimene	Linalool L	Citronella	Beta-citronellol	Z-citral	Geraniol
33	QN3	HUIB_CC43	4.930e ± 0.075	0.403jk ± 0.015	0.300q ± 0.017	0.190 g ± 0.026	2.207kl ± 0.021	22.147 h ± 0.015	19.667p ± 0.031
34	TTH3	HUIB_CC44	3.663 h ± 0.081	0.337 lm ± 0.031	0.487 g-i ± 0.015	0.270f ± 0.026	1.147q ± 0.055	24.447b ± 0.076	12.950t ± 0.035
35	HCM1	HUIB_CC45	4.857e ± 0.032	1.087a ± 0.025	0.427 k-m ± 0.040	0.190 g ± 0.030	5.373c ± 0.074	19.937qr ± 0.051	36.967c ± 0.158

No	Accession name	Accession code	E-citral	Geranyl acetate	Trans-caryophyllene	α-bergamotene	Germacrene D-4-ol	Juniper camphor
1	SK	HUIB_CC1	67.577a ± 0.172	1.880c ± 0.040	0.193pr ± 0.012	0.157kl ± 0.006	0.633 s ± 0.012	1.377x ± 0.006
2	V1	HUIB_CC2	63.420c ± 0.242	0.590q ± 0.017	0.583gh ± 0.006	0.313c ± 0.006	1.547j ± 0.012	4.440e ± 0.017
3	V2	HUIB_CW3	22.480ee ± 0.078	11.850b ± 0.061	1.443b ± 0.006	0.000q ± 0.000	2.170e ± 0.017	0.000 cc ± 0.000
4	V3	HUIB_CC4	63.827b ± 0.170	1.107ij ± 0.015	0.637ef ± 0.006	0.313c ± 0.006	1.240 k ± 0.010	4.117f ± 0.015
5	L1	HUIB_CC5	44.910q ± 0.120	1.090ij ± 0.030	0.533ij ± 0.006	0.290d ± 0.017	0.980no ± 0.020	3.327 k ± 0.006
6	L2	HUIB_CC6	54.247 k ± 0.093	0.897 l ± 0.015	0.560hi ± 0.000	0.373b ± 0.006	1.143 l ± 0.012	4.403e ± 0.006
7	BK	HUIB_CC7	61.840d ± 0.262	1.477f ± 0.057	0.347 mn ± 0.025	0.273de ± 0.012	1.683i ± 0.012	3.120 m ± 0.030
8	DNo	HUIB_CC8	57.190 h ± 0.265	0.887 l ± 0.042	0.407 l ± 0.023	0.233 g ± 0.012	1.107 l ± 0.038	2.663p ± 0.051
9	QT1	HUIB_CC10	56.297i ± 0.100	0.717p ± 0.015	0.743d ± 0.015	0.430a ± 0.000	3.183a ± 0.006	5.243c ± 0.012
10	HT	HUIB_CC11	61.743d ± 0.120	0.770n-p ± 0.020	0.663e ± 0.006	0.373b ± 0.006	2.790c ± 0.010	5.403b ± 0.006
11	PY1	HUIB_CC12	60.933e ± 0.187	1.113ij ± 0.006	0.223p ± 0.006	0.127no ± 0.006	0.563t ± 0.006	1.457w ± 0.006
12	PY2	HUIB_CC13	42.903t ± 0.117	1.397 g ± 0.015	0.447 k ± 0.012	0.283d ± 0.012	2.343d ± 0.015	4.487d ± 0.015
13	QT2	HUIB_CC17	39.323u ± 0.080	0.783no ± 0.015	0.610 fg ± 0.010	0.270de ± 0.010	1.253 k ± 0.015	3.797i ± 0.025
14	QT3	HUIB_CC18	35.257y ± 0.107	0.787no ± 0.015	0.000 s ± 0.000	0.000q ± 0.000	3.030b ± 0.108	4.007 g ± 0.012
15	QT9	HUIB_CC24	57.643 g ± 0.090	0.867 lm ± 0.025	0.353 m ± 0.012	0.253ef ± 0.006	0.927pq ± 0.006	2.740o ± 0.010
16	QT10	HUIB_CC25	60.767e ± 0.103	1.103ij ± 0.006	0.287o ± 0.006	0.160j-l ± 0.000	1.257 k ± 0.006	2.223t ± 0.107
17	BD1	HUIB_CC26	55.353j ± 0.135	1.343 g ± 0.021	0.450 k ± 0.010	0.000q ± 0.000	0.677 s ± 0.012	2.293 s ± 0.006
18	BD2	HUIB_CC27	36.967x ± 0.075	1.227 h ± 0.011	0.837c ± 0.029	0.357b ± 0.006	1.867 g ± 0.015	6.547a ± 0.023
19	BD3	HUIB_CC28	33.907z ± 0.064	1.543e ± 0.015	0.353 m ± 0.006	0.147 lm ± 0.012	0.527t ± 0.021	1.953u ± 0.015
20	BD5	HUIB_CC30	52.410 m ± 0.252	0.643q ± 0.035	0.363 m ± 0.006	0.177i-k ± 0.023	0.883q ± 0.038	1.987u ± 0.015
21	QN1	HUIB_CC31	33.763z ± 0.211	0.877 lm ± 0.051	0.350 mn ± 0.040	0.270de ± 0.010	1.663i ± 0.021	3.747j ± 0.031
22	QN2	HUIB_CC32	24.520 cc ± 0.201	0.903 l ± 0.090	0.360 m ± 0.046	0.257ef ± 0.012	1.050 m ± 0.020	2.540q ± 0.017
23	QNAM1	HUIB_CC33	42.957t ± 0.180	0.750op ± 0.020	0.517j ± 0.015	0.160j-l ± 0.026	0.790r ± 0.017	2.660p ± 0.026
24	TTH2	HUIB_CC34	45.670p ± 0.144	1.073j ± 0.031	0.270o ± 0.010	0.137 mn ± 0.006	0.533t ± 0.006	1.563v ± 0.012
25	NA1	HUIB_CC35	38.907v ± 0.112	0.817 mn ± 0.015	0.400 l ± 0.010	0.280d ± 0.017	1.023 mn ± 0.021	2.833n ± 0.040
26	NA2	HUIB_CC36	43.370 s ± 0.197	0.867 lm ± 0.031	0.270o ± 0.026	0.180ij ± 0.000	0.350v ± 0.017	1.147z ± 0.015
27	TH1	HUIB_CC37	44.597r ± 0.176	1.823 cd ± 0.057	0.200pq ± 0.010	0.193hi ± 0.006	0.340v ± 0.010	1.257y ± 0.015

Table 4 (continued)

No	Accession name	Accession code	E-citral	Geranyl acetate	Trans-caryophyllene	α-bergamotene	Germacrene D-4-ol	Juniper camphor
28	TH2	HUIB_CW38	23.330dd ± 0.040	21.163a ± 0.075	2.780a ± 0.017	0.163j-l ± 0.021	1.973f ± 0.012	0.000 cc ± 0.000
29	TN1	HUIB_CC39	60.387f ± 0.181	1.507ef ± 0.025	0.367 m ± 0.006	0.253ef ± 0.012	0.423u ± 0.006	1.960u ± 0.010
30	BP1	HUIB_CC40	50.610n ± 0.082	1.093ij ± 0.015	0.437 k ± 0.006	0.177i-k ± 0.006	0.953op ± 0.012	2.343r ± 0.006
31	CM1	HUIB_CC41	37.980w ± 0.155	1.147i ± 0.021	0.450 k ± 0.010	0.240 fg ± 0.010	1.783 h ± 0.021	3.890 h ± 0.020
32	VL1	HUIB_CC42	31.513aa ± 0.122	0.973 k ± 0.047	0.530j ± 0.020	0.270de ± 0.017	1.133 l ± 0.029	3.247 l ± 0.042
33	QN3	HUIB_CC43	46.880o ± 0.141	1.793d ± 0.012	0.173pr ± 0.012	0.113op ± 0.006	0.270w ± 0.000	0.917bb ± 0.015
34	TTH3	HUIB_CC44	53.693 l ± 0.196	1.387 g ± 0.006	0.170r ± 0.017	0.100p ± 0.010	0.337v ± 0.021	1.023aa ± 0.012
35	HCM1	HUIB_CC45	25.950bb ± 0.106	0.847 lm ± 0.032	0.323n ± 0.006	0.207 h ± 0.015	1.057 m ± 0.065	2.770o ± 0.035

known for its analgesic, sedative, and muscle-relaxing properties. Additionally, β-myrcene serves as a precursor in the biosynthesis of other terpenes, making it crucial in the formulation of cannabis-derived therapeutics and flavors (Lorenzetti et al. 1991). Sample TH1 contained a notably high concentration of β-myrcene at 6.41%, which is significantly above average, while sample TH2 exhibited much lowest levels at 0.02%. This difference may indicate either a genetic predisposition or regional factors influencing terpene synthesis. Since β-myrcene is also a precursor to various sesquiterpenes and other volatile compounds, its abundance could impact the formation of downstream metabolites (Lorenzetti et al. 1991). Other minor but bioactive compounds included linalool, geranyl acetate, and juniper camphor. Linalool has proven to be an effective topical antimicrobial, making it a valuable ingredient in dermatological formulations (Peana et al. 2003; Phuong et al. 2021). Sample QNAM1 had the highest concentration of linalool at 0.747%, which may enhance its sensory appeal in perfumery applications. In contrast, the other samples contained less than 0.07% linalool, and linalool was not detected in the QT3 sample. Geranyl acetate, an ester derivative of geraniol, is known for its strong antimicrobial properties (Sharmeen et al. 2021). Its captivating fragrance has made it a key ingredient in the perfume industry (Abate et al. 2021). There was significant variation in its concentration among the samples, ranging from 0.643% in sample BD5 to 21.163% in sample TH2. Notably, samples TH2 and V2 had the highest concentrations, with values of 21.163% and 11.850%, respectively. The remaining samples contained less than 2% of geranyl acetate.Juniper camphor concentration varied significantly, ranging from 0% in the TH2 and V2 samples to 6.547% in the BD2 sample. Although less studied in lemongrass, similar findings have been reported in *Juniperus communis* (Sytykiewicz et al. 2025) and *Juniperus foetidissima* (Kurtca et al. 2021).

The chromatographic analysis revealed significant variability among lemongrass accessions regarding the relative abundance of key terpenoid constituents, including cis-ocimene, citronella, trans-caryophyllene, α-bergamotene, and germacrene D-4-ol. Accessions TH2 and V2 displayed notably higher levels of trans-caryophyllene, at 2.780% and 1.443%, respectively. In contrast, the other accessions contained less than 1% of this compound. Conversely, accessions like HCM1, TH1, QNAM1, and QT1 were found to be enriched in cis-ocimene, with concentrations exceeding 1.0%, while the remained samples had concentrations below 1% or showed no presence of this compound, such as BD5, BD1, QN1, QT3, V2, and TH2. This significant chemical variation is likely attributed to genetic diversity and environmental factors influencing secondary metabolite production. These findings align with research indicating that interactions between genotype and environment substantially impact essential oil yield and composition stability in *Cymbopogon* species (Lal et al. 2025). Citronellal is a monoterpenoid aldehyde and is the major oxygenated compound found in the essential oil of *Cymbopogon citratus*, imparting its characteristic lemon scent (Burt et al. 2004). The citronellal content in lemongrass essential oil varies significantly, ranging from 0.087% in accession L2 to 5.723% in TH2. TH2 has the highest citronellal content at 5.723%, followed by accession V2 with 3.620%. The remaining accessions

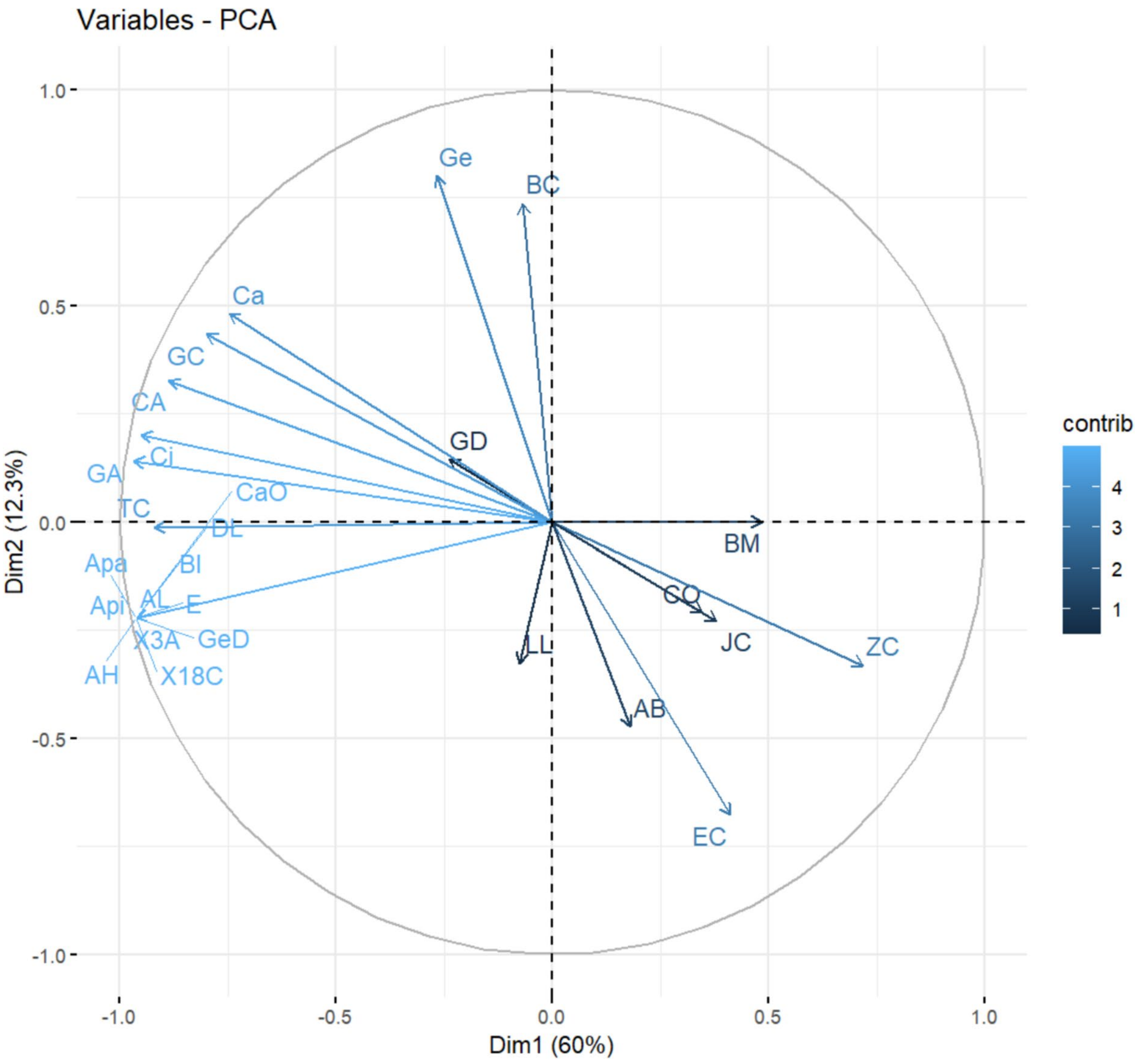


Fig. 5 Correlation plot of phytochemical component variables among lemongrass germplasm derived from Principal Component Analysis (PCA)

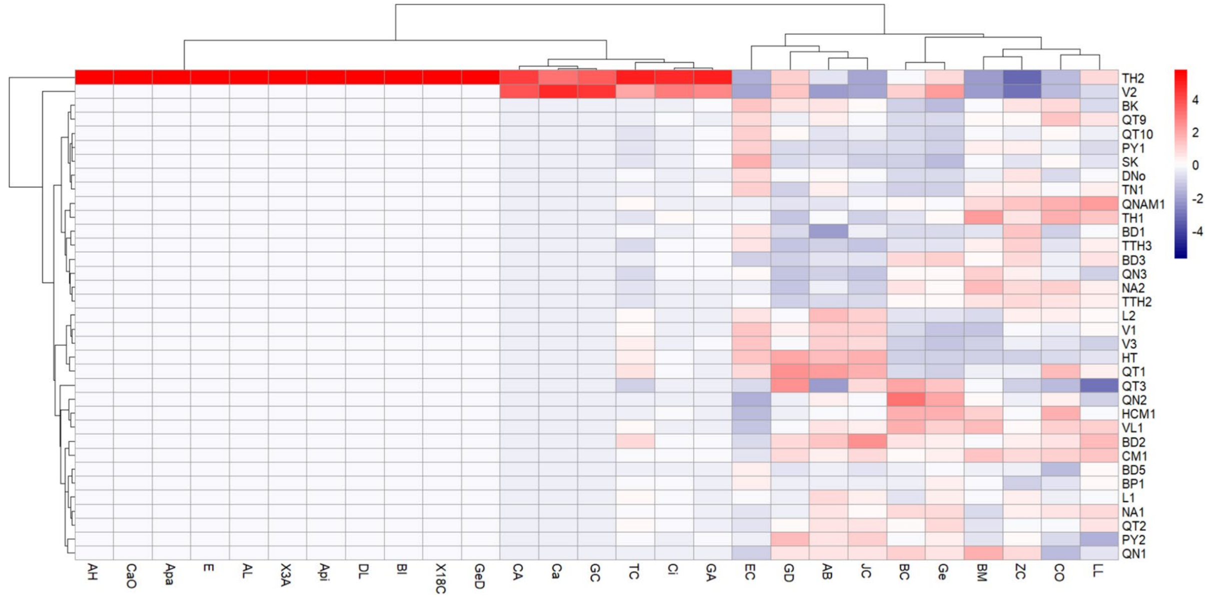


Fig. 6 The heatmap displayed similarity levels based on phytochemical components among lemongrass germplasm. BM, β-myrcene; CO, Cis-ocimene; LL, linaloolL; Ci, citronella; BC, β-citronellol; ZC, Z-citral; Ge, geraniol; EC, E-citral; GA, geranyl acetate; TC, rans-caryophyllene; AB, α-bergamotene; GD, germacrene D-4-ol; JC, juniper camphor; Api, α-pinene; Ca, amphene; DL, dl-limonene; 18 C, 1,8-cineole; CA, citronellyl acetate; 3 A, 3-allylguaiacol; AH, α-humulene; GeD, germacrene-D; GC, gamma-cadinene; Apa, α-patchoulene; BI, β-ionol; AL, α-longipinene; E, elemol; CaO, (-)-caryophyllene oxide

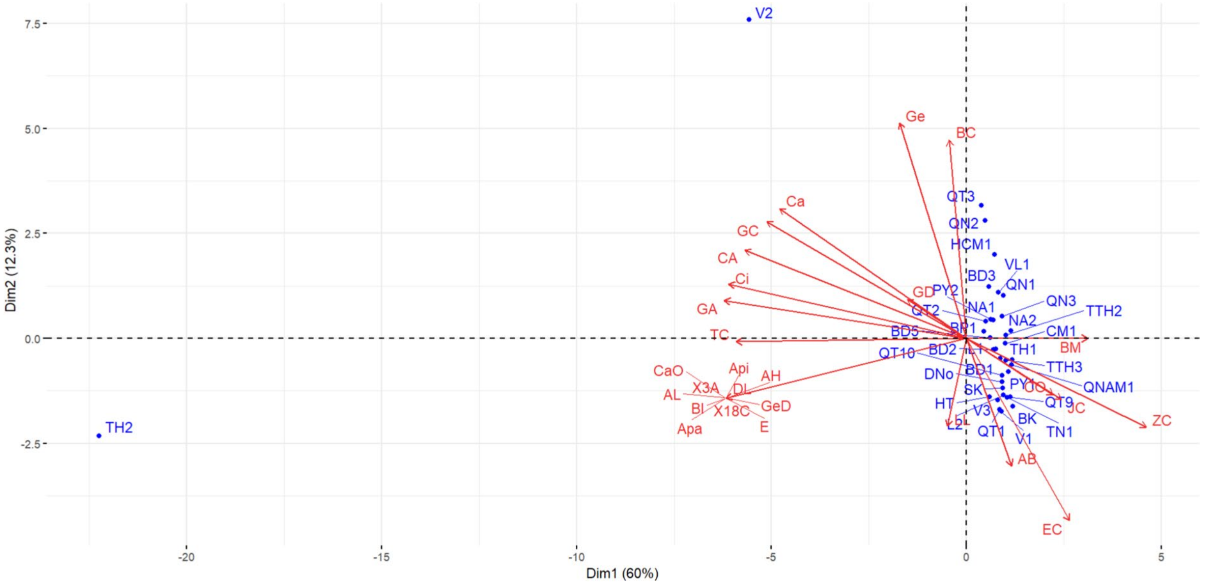


Fig. 7 Correlations among lemongrass germplasm based on their phytochemical components through Principal Component Analysis (PCA). BM, β-myrcene; CO, Cis-ocimene; LL, linalool L; Ci, citronella; BC, β-citronellol; ZC, Z-citral; Ge, geraniol; EC, E-citral; GA, geranyl acetate; TC, Trans-caryophyllene; AB, α-bergamotene; GD, germacrene D-4-ol; JC, juniper camphor; Api, α-pinene; Ca, camphene; DL, dl-limonene; 18 C, 1,8-cineole; CA, citronellyl acetate; 3 A, 3-allylguaiacol; AH, α-humulene; GeD, germacrene-D; GC, gamma-cadinene; Apa, α-patchoulene; BI, β-ionol; AL, α-longipinene; E, elemol; CaO, (-)-caryophyllene oxide

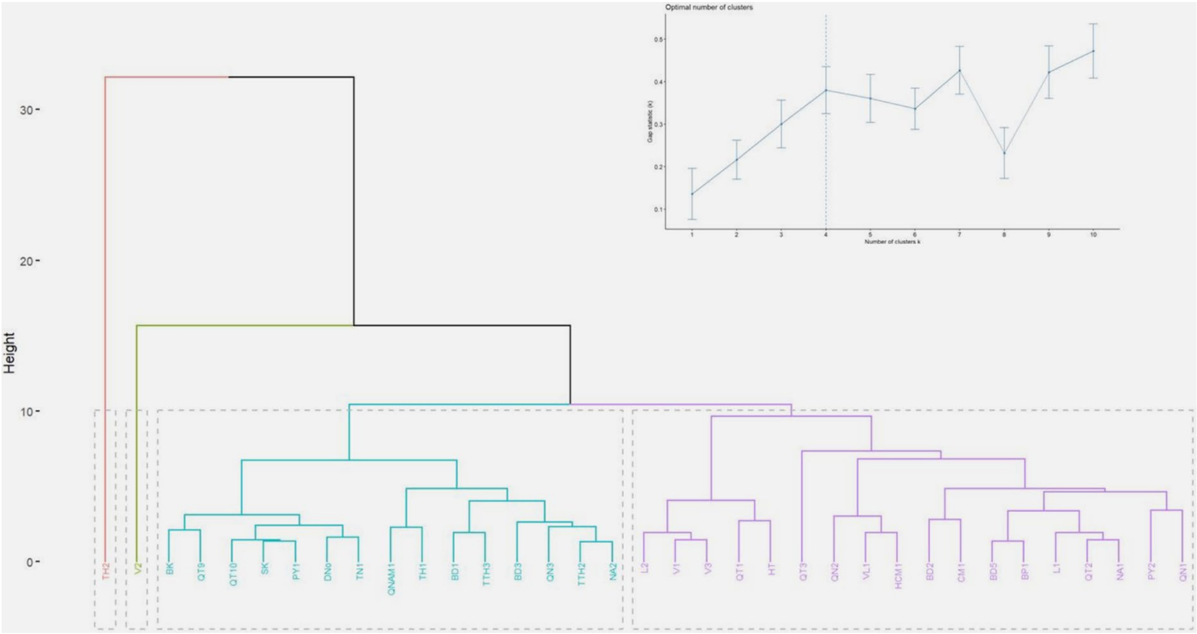


Fig. 8 Hierarchical clustering dendrogram based on phytochemical components of lemongrass germplasm with optimal cluster determination. BM, β-myrcene; CO, Cis-ocimene; LL, linalool L; Ci, citronella; BC, β-citronellol; ZC, Z-citral; Ge, geraniol; EC, E-citral; GA, geranyl acetate; TC, Trans-caryophyllene; AB, α-bergamotene; GD, germacrene D-4-ol; JC, juniper camphor; Api, α-pinene; Ca, camphene; DL, dl-limonene; 18 C, 1,8-cineole; CA, citronellyl acetate; 3 A, 3-allylguaiacol; AH, α-humulene; GeD, germacrene-D; GC, gamma-cadinene; Apa, α-patchoulene; BI, β-ionol; AL, α-longipinene; E, elemol; CaO, (-)-caryophyllene oxide

contain less than 1% of this compound, and citronellal was absent in the following accessions: SK, V1, V3, BK, DNo, PY1, PY2, QT3, QT10, BD1, BD5, QN1, QN2, QNAM1, TTH2, NA2, TN1, and VL1. This variation indicated strong chemotypic differentiation, likely driven by genetic factor. Previous reports have shown significant differences in citronellal (citronella) synthesis among *Cymbopogoncitratus* due to factors such as genotype (Lal et al. 2025), harvesting season (Lahmar et al. 2025), and extraction methods (Hamzah et al. 2013), while the effects of environment and the genotype-by-environment interaction were not significant for the*C. winterianus* and *C. citratus* (Lal et al. 2025). This indicates that the ability to regulate citronellal biosynthesis is specific to certain chemotypes.Germacrene D-4-ol is an oxygenated sesquiterpene alcohol that demonstrates strong antimicrobial, antioxidant, and anti-inflammatory properties (Maldonado et al. 2025). The concentration of germacrene D-4-ol varied across samples, ranging from 0.270% in QN3 to 3.183% in QT1. α-bergamotene was also detected in the samples but at very low percentages, from 0.100% in sample TTH3 to 0.430% in sample QT1, and α-bergamotene was absent in the following accessions: V2, QT3, and BD1. Alpha-pinene, camphene, citronellyl acetate, 3-allylguaiacol, α-humulene, germacrene-D, gamma-cadinene, and α-patchoulene were only observed in sample TH2 at low levels.

Therefore, the diversity of phytochemicals within the lemongrass germplasm was observed and illustrated in Fig. 8. The Gap statistic graph indicated that the optimal number of clusters (k) was 4. Consequently, the lemongrass germplasm was categorized into four distinct groups suchcluster 1 (red) consisted solely of TH2, which was significantly different from the other accessions and can be considered an outlier due to its unique phytochemical characteristics; cluster 2 (green) composed exclusively of V2, which was also markedly different from the majority of the population; cluster 3 (cyan): included samples such as BK, QT9, QT10, SK, PY1, DN0, TN1, QNAM1, TH1, BD1, TTH3, BD3, QN3, TTH2, and NA2; and cluster 4 (purple) containedthe remained accessions.

Thus, the genetic and phytochemical diversities found among and within *Cymbopogon* species align with previous studies (Dangol et al. 2023; Munda et al. 2022; Adhikari et al. 2022; Bassolé et al. 2011; Aly et al. 2025; Sharma et al. 2023; Andrade et al. 2009). Thus, among the germplasm, asccessions SK, BK, V1, V3, TN1, and PY1 demonstrated excellent essential oil quality, characterized by high levels of citral (> 82%) and geraniol (> 4%). Among them, SK contained the highest citral content at 86.530%, making it ideal for industrial extraction. In contrast, PY1 was rich in geraniol (8.567%), making it suitable for pharmaceutical applications. These accessions are recommended for breeding, production, and stabilization of chemotypes.

Conclusion

The integration of molecular and phytochemical analyses revealed low genetic variation but significant chemical diversity among *Cymbopogon* accessions from Vietnam and Laos. ITS-based phylogenetic analysis confirmed the presence of two major species*:* *C. citratus* and *C. winterianus*. Additionally, RAPD markers showed moderate polymorphism, suggesting limited genetic differentiation among the accessions. Despite the observed genetic uniformity, GC–MS profiling revealed substantial variation in the essential oil composition, particularly in the levels of citral, geraniol, and geranyl acetate. These differences likely result from genetic influence on the biosynthesis of secondary metabolites. Thus, accessions SK, BK, V1, V3, TN1, and PY1 showed high citral and geraniol, which are recommended for breeding, production, and chemotype stabilization. The identification of accession SK (which has the highest citral at 86.530%) and PY1 (noted for its high geraniol at 8.567%) represents a major contribution of this study. This research provides valuable insights into the genetic homogeneity and chemical diversity of *Cymbopogon*, thereby supporting the sustainable breeding and conservation of lemongrass genetic resources in Southeast Asia.

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Data availability Data included in the article/Supplementary Tables are referenced in the article. Additionally, the sequences that related to the article were submitted in the NCBI and available at https://submit.ncbi.nlm.nih.gov/subs/?search=SUB15836494

Declarations

Conflict of interest The authors declare no conflicts of interest.

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