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Chemical Analysis of Essential Oil and Genetic Diversity Using ISSR and SSR Markers in Cultivated Lemongrass (*Cymbopogon citratus*) Accessions

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ABSTRACT: Leaves and essential oils for therapeutic purposes are important traits in lemongrass (*Cymbopogon citratus*). In this study, the genetic diversity of ten *C. citratus* accessions collected around Nigeria was investigated using simple sequence repeat (SSR) and inter simple sequence repeat (ISSR) markers. Also, we determined the chemical content of *C. citratus* essential oil. In this study, the essential oil was extracted using hydro-distillation and the constituents of the oil were determined with gas chromatography-mass spectrophotometry (GC-MS). The markers produced a total of 47 alleles with a mean PIC value of 0.73 in the accessions. The cluster analysis grouped the accessions into two major groups showing genetic relatedness between the accessions: LG07, LG03 and LG01 are the closest members, while LG08 is very distant from the other accessions. In addition, the factorial analysis categorized the ten accessions into four quadrants, which have some similarities to the dendrogram grouping. GC-MS analysis resulted in the identification of 18 compounds. Essential oils of LG02 and LG03 had a citral content of 49.38 % and 66.47 %, respectively. These results revealed the genetic diversity among the accessions evaluated that can be used to maximize the use of genetic resources of *C. citratus* varieties.

Keywords: *Cymbopogon,* Essential oil, Genetic diversity, ISSR marker, SSR marker

Introduction

Lemongrass is an aromatic, tall, clumped perennial grass belonging to the family Poaceae and the genus *Cymbopogon*. It is a tropical plant that yields aromatic oil. The name "lemongrass" is derived from the typical lemon-like odour of the essential oil present in the shoot (Tajidin *et al.*, 2012). The name *Cymbopogon* was derived from the Greek words “Kymbe” (boat) and “pogon” (beard), referring to the flower spike arrangement (Khanuja *et al.*, 2005). *Cymbopogon* is a genus comprising about 180 species, subspecies, varieties, and sub-varieties. The plants are up to and above 1 meter tall, with narrow and long leaves that are mostly characterized by the presence of silica thorns aligned on the leaf edges (Mathews *et al.*, 2002).
Lemongrass is distributed in Africa, the Indian Subcontinent, South America, Australia, Europe and North America. In India, they grow wild in all regions, extending from sea level to an altitude of 4200 m. Several species are endemic to India. The east Indian lemongrass grows wild in India and is well cultivated in Kerala, Assam, Maharashtra, and Uttar Pradesh. It is also distributed in Guatemala and China. West Indian lemongrass is believed to have originated either from Malaysia or Sri Lanka. It is widely distributed throughout the tropics and is grown in the West Indies, Guatemala, Brazil, Congo, Tanzania, India, Thailand, Bangladesh, Madagascar

and China. Jammu's lemongrass is mostly confined to North Indian states such as Jammu and Kashmir, Sikkim, Assam, Bengal and Madhya Pradesh (Joy *et al.*, 2006). Lemongrass is cultivated on a large scale at Chinnor wildlife sanctuary in the Western Ghats of India (Nair and Jayakumar, 1999). Traditionally, lemongrass is grown in high rainfall areas, but under semi-arid tropical conditions. It is grown under irrigation (Singh and Kumar, 2017).

The essential oil from lemongrass shoots and leaves contains highly volatile aroma compounds and carries the distinctive scent, flavor, or essence of the plant. Essential oil of lemongrass is found in the parenchyma tissue cells, specifically on the adaxial surface of leaf mesophyll (Lewinsohn *et al.*, 1998). They are formed by varied and complex volatile mixtures of chemical compounds such as terpenes associated with aldehyde, alcohols, ketone phenylpropanoids, and other minor compounds (Calo *et al.*, 2015). The essential oils of various *Cymbopogon* species contain citral, geraniol, citronellol, citronellal, linalool, elemol, 1,8-cineole, limonene, β-caryophyllene, methyl heptenone, geranyl acetate and geranyl formate (Sarma *et al.*, 1999).

The chemical composition of the essential oils obtained from different samples of *Cymbopogon citratus* was evaluated by Barbosa *et al.* (2008). The gas chromatography–mass spectrometry (GC-MS) analyses of the essential oils identified 22 compounds, with neral and geranial as the two major components. The total percentage of these two compounds varied within the investigated sample oils from 40.7 to 75.4 %. In 2017, Archana investigated the constituents of essential oil from different accessions of *Cymbopogon citratus* collected from various locations in Kerala. The essential oils were extracted by hydro-distillation and analyzed by GC-MS. The major chemical components identified from the accession are geraniol, geranial, limonene, linalool, citronella, neral, citronellyl acetate, geranyl acetate, citral (neral+geranial). In Nigeria, lemongrass essential oil is used to treat malaria (Melariri, 2010); diabetes (Ademuyiwa and Grace, 2015); and bacterial diseases resulting from *Salmonella typhi* and *E. coli* (Akin-Osanaiye *et al.*, 2007). Additionally, lemongrass is also used in cosmetics (Kuete, 2017). Despite the widespread distribution and consumption of lemongrass, particularly in the developing countries of Africa, there are limited studies on the chemical compounds of the essential oil (Adeyemo *et al.*, 2018). In this regard, it is important to characterize lemongrass accessions based on their yields of essential oils and phytochemical content, which are strongly associated with their health properties.

Genetic diversity is essential for developing appropriate strategies for breeding, germplasm management and the utilization of genetic resources (Paterson, 1996). Various molecular markers have proven to be powerful tools for the assessment of genetic variations among the most economically relevant plants (Amiteye, 2021). A molecular technique, such as simple sequence repeats (SSRs), also known as microsatellites, is one of the most widely preferred methods in molecular studies due to their many known advantages, like their high reproducibility, multi-allelic nature, high polymorphism and extensive genome coverage (Varshney *et al.*, 2005). Also, inter simple sequence repeat (ISSR) markers have been reported to produce multiple DNA fragments (each of which is considered a locus) in a single reaction, and are easily applicable for genetic diversity analysis in plants (Abdelaziz *et al.*, 2020), without the need to first examine the DNA sequences of the genome of the species (Adibah *et al.*, 2012).

Previously, Kumar and Bennetzen (1999) assessed the genetic diversity of 25 accessions of *Cymbopogon* aromatic grasses, including eight species, two hybrids and one mutant strain, using SSR of the rice genome. A total of 151 bands were produced, ranging from 3 to 12 per primer pair. The polymorphic information content (PIC) values varied from 0.14 to 0.92, with an average of 0.72. Jaccard's similarity coefficient ranged from 64 to 87 % among the paired accessions. The pattern of genetic diversity neither matched the known taxonomic classification nor did it always match the distribution of chemical constituents of the essential oils available in this collection.

Furthermore, genotypic and phenotypic variations among 12 genotypes of *C. flexuosus* were assessed by Debajit *et al.* (2015) based on random amplified polymorphic DNA (RAPD) and ISSR markers. It is possible to improve the quality and quantity of essential oils by correlating genotypic and phenotypic data among accessions. Many lemongrass species are widely distributed or adapted to the agro-climatic conditions in Nigeria. However, according to the best of our knowledge, there is no previous report on the characterization of genetic variability among lemongrasses grown in Nigeria, using ISSR and SSR markers. Therefore, the objectives of this research were to: (1) examine genetic diversity using SSR and ISSR markers and assess genetic relationships among *Cymbopogon citratus* accessions collected across Nigeria, and (2) determine the chemical content of the essential oil of *Cymbopogon citratus.*

Materials and methods

Plant materials: Ten (10) lemongrass local accessions were collected from different geographical zones in Nigeria. Table 1 lists the lemongrass accessions along with their geographical origins. A loamy soil

supplemented with organic manure was used to grow these varieties at the University of Lagos botanical garden. The lemongrass stalks were planted on ridges with a planting distance of 3 feet from each other. The plants were watered frequently and weeded to ensure they grow well.

Table 1: List of the lemongrass accessions used in the study from nine states in Nigeria

S/N	Codes	Town, State
1	LG01	Apaola, Kwara
2	LG02	Ejigbo, Osun
3	LG03	Ibafo, Ogun
4	LG04	Ibadan, Oyo
5	LG05	Ikosu, Ekiti
6	LG06	Lagos, Lagos
7	LG07	Ogbomoso, Oyo
8	LG08	Omuaran, Kwara
9	LG09	Eganyi, Kogi
10	LG10	Tunga, Niger

DNA extraction, PCR amplification and genotyping: Young leaves (80–100 mg fresh weight) were collected, freeze-dried, and ground to a fine powder in an extraction tube using stainless steel balls in a Genogrinder-2000 (Thomas Scientific, USA). DNA was isolated following Dellaporta *et al.* (1983). It was dissolved in sterile water and stored at 4 °C. Isolated genomic DNA was separated using 2 % (w/v) agarose gel electrophoresis, and it was then stained with ethidium bromide for quality evaluation. The DNA concentration of all samples was quantified by NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, Delaware, USA). Six ISSR loci were selected based on reported amplifications (Melo *et al.*, 2011) and seven SSR markers developed from *Cymbopogon jwarancusa* (Jones) Schult (Kumar *et al.*, 2007) genome and were used for optimization using a genotyping set. Two ISSR primers generated nonscorable alleles and five SSR primers did not generate amplicons. Finally, only 4 ISSRs and 2 SSRs were used for genotyping the ten accessions. The primers were synthesized by Inqaba Biotec West Africa Ltd., South Africa. Polymerase chain reaction (PCR) mixture for the ISSR analysis was made for a final volume of 25 µL, containing 20 ng of DNA, 1 U of Taq DNA polymerase, 10 mM of Tris-HCL (pH 8.0), 2 mM of $MgCl_2$, 0.25 µM of each DNTP and 0.2 µM of oligonucleotide. DNA amplification was done under the following conditions: 95 °C for 15 min, followed by 40 cycles of 94 °C for 30 sec, annealing temperature, 72 °C for 2 min and final extension for 7 min at 72 °C. The products from the amplifications were separated in 2% agarose gel stained with ethidium bromide, visualized under UV light in the presence of the SafeView™ DNA stains (Applied Biological Materials Inc., Richmond BC, Canada) and images were captured. For the SSR analysis, genomic DNA was amplified in 25 mL reaction mixtures each containing 100 ng genomic DNA, 1 x PCR buffer, 15 mM $MgCl_2$, 200 mM dNTP, 0.2 mM each of the forward and reverse primers and 1 U Taq polymerase. Amplification was done in a thermal cycler. Initial denaturation at 94 °C for 3 min; followed by 40 cycles of 94 °C for 30 sec, 54 to 62 °C for 1 min, 72 °C for 2 min and a final extension at 72 °C for 10 min. The PCR products were separated using 6 % (w/v) non-denaturing polyacrylamide gel electrophoresis in 1 × TBE TM buffer. It was visualized under UV light in the presence of SafeView™ DNA stain (Applied Biological Materials Inc., Richmond BC, Canada) and images were captured.

Table 2: List of ISSR and SSR markers and their sequences used in this study

Markers	Primer sequences	Annealing Temp. (°C)	References
ISSR817	CACACACACACACACAA	45	Melo *et al*., 2011
ISSR866	CTCCTCCTCCTCCTCCTC	55	Melo *et al*., 2011
ISSR879	CTTCATTTCACTTCA	34	Melo *et al*., 2011
ISSR881	GGGTGGGGTGGGGTG	53	Melo *et al*., 2011
SSR075	F: TGGCTAAGGAAGAATGCTC	58	Kumar *et al*., 2007
	R: CAGCGACGTCAATGCCACC		
SSR163	F: ACGCGGAGCCGACAGAGGATCT	60	Kumar *et al*., 2007
	R: GGCAGGGATGGGGGCGTCTGG		

Data analysis: Polymorphic ISSR and SSR loci were manually scored using binary number with presence as "1" and absence as "0". Genetic distances were measured based on shared allele frequencies. Parameters of genetic diversity, including the observed number of alleles (Na) per marker, the average number of observed alleles), gene diversity and polymorphic information content (PIC) value for each locus were estimated by Power Marker version 3.25 (Liu and Muse, 2005). Both ISSR and SSR data were pooled together and used to generate the distance matrix with NTSYS-PC software with 1000 bootstrap repetitions which were further processed to

produce the dendrogram based on unweighted pair group method with arithmetic average (UPGMA) cluster procedure and a factorial analysis were performed using the Darwin 6.0 statistical software (Perrier and Jacquemoud-Collet, 2006).

Extraction of essential oil from Cymbopogon citratus: Fresh mature leaves of each accession (780 g of LG02 and 940 g of LG03) were collected and chopped into small pieces and the essential oil was extracted using hydrodistillation. It was performed at 100 °C for 6 hours in an all-glass Dean and Stark apparatus modified to allow for the lowest phase return via Clevenger equipment. The collected oil was dried over anhydrous sodium sulphate and then filtered. Finally, the hexane solution was evaporated using a rotary evaporator at 40 °C to give a yellowish essential oil, which was then stored at 4 °C for gas chromatography–mass spectrometry (GC–MS) analysis (Mehedi *et al.*, 1974).

Percentage yield of essential oil (EO): The yield of the essential oil obtained was calculated using the formula described by Rao *et al.* (2005):

$$\text{Yield of essential oil (\%)} = \frac{\text{amount of essential oil (g) obtained}}{\text{amount of raw materials (g) used}} \times 100\ \%$$

GC–MS analysis: Chemical analysis of the lemongrass EO was performed using the Agilent 7820A gas chromatograph coupled to a 5975C inert mass spectrometer (with triple axis detector) and electron impact source (Agilent Technologies) instrument with an HP-5 capillary column coated with 5 % phenyl methyl siloxane (30 m length × 0.32 mm diameter × 0.25 μm film thickness). The oven was initially programmed at 60 °C (1 min), then ramped at 4 °C/min to 110 °C (3 min), followed by temperature program rates of 8 °C/min to 260 °C (5 min) and 10 °C/min to 300 °C (12 min). Run time was 56.25 min with a 3 min solvent delay. The carrier gas was helium, used at a constant flow rate of 1.573 ml/min, an initial nominal pressure of 1.9514 psi and at an average velocity of 46 cm/s. One microliter of the sample was injected in splitless mode at an injection temperature of 260 °C. Purge flow was 21.5 ml/min at 0.50 min with a total gas flow rate of 23.355 ml/min; gas saver mode was switched on. The resulting compounds were identified by their retention times, mass spectra with those of standards or their retention indices (RI) with published data and their mass spectra with the National Institute of Standards and Technology (NIST) library.

Results

SSR and ISSR polymorphisms: All 6 polymorphic loci (4 ISSR and 2 SSR) markers produced a total of 47 alleles with a mean of alleles per locus, ranging from 8.5 alleles for ISSR to 6.5 alleles for SSR. PIC values ranged from 0.31 (SSR 163) to 0.85 (ISSR 866) with a mean of 0.73. The ISSR and SSR markers showed different percentage polymorphisms; ISSR 817 and ISSR 866 were 88.89 % and 77.78 % respectively while ISSR 879, SSR 163 and SSR 075 were all 100 % polymorphic. The results further showed that the expected heterozygosity (He) varied from 0.34 (SSR 163) to 0.86 (ISSR 866) across the 6 loci. The most polymorphic locus was SSR 075 which generated 10 alleles (Table 3).

Table 3: Genetic diversity estimations by 6 loci

Primers	**Na**	**PIC**	**(He)**
ISSR817	9	0.79	0.82
ISSR866	9	0.85	0.86
ISSR879	9	0.82	0.84
ISSR881	7	0.77	0.8
SSR163	3	0.31	0.34
SSR075	10	0.84	0.86
Mean	**7.83**	**0.73**	**0.75**

Na: number of alleles per locus; PIC: polymorphism information content,
He: expected heterozygosity

Cluster analysis: The dendrogram (Figure 1) divided the 10 accessions into two major groups. Group I contained 6 accessions, while group II contained 4 lemongrass accessions (LG10, LG09, LG04 and LG08). Accessions obtained from the southwestern part of Nigeria were mostly clustered together in group I except for LG01, which is from a north central state (Kwara), indicating their genetic similarity and being distinct from the accessions collected from the north central part which clustered in group II. We used the genotypic data to

perform a factorial analysis based on Jaccard's to characterize the diversity among the 10 accessions of *C. citratus* (Figure 2). It showed the equal distribution of the 10 accessions across the axes. The first axis accounted for accessions from Niger, Kogi, Osun, Kwara and Ogun states, while accessions from Lagos, Ekiti, Oyo, and Kwara were grouped together on the second axis. However, within the axis, there are three clusters that are distinct from one another. Cluster A has accessions from Kwara, Osun, Ekiti, Lagos and Oyo which is very similar to the G-I cluster seen in the dendrogram. Cluster B contained accessions from Oyo and Kwara while cluster C had accessions from Ogun, Kogi and Niger.

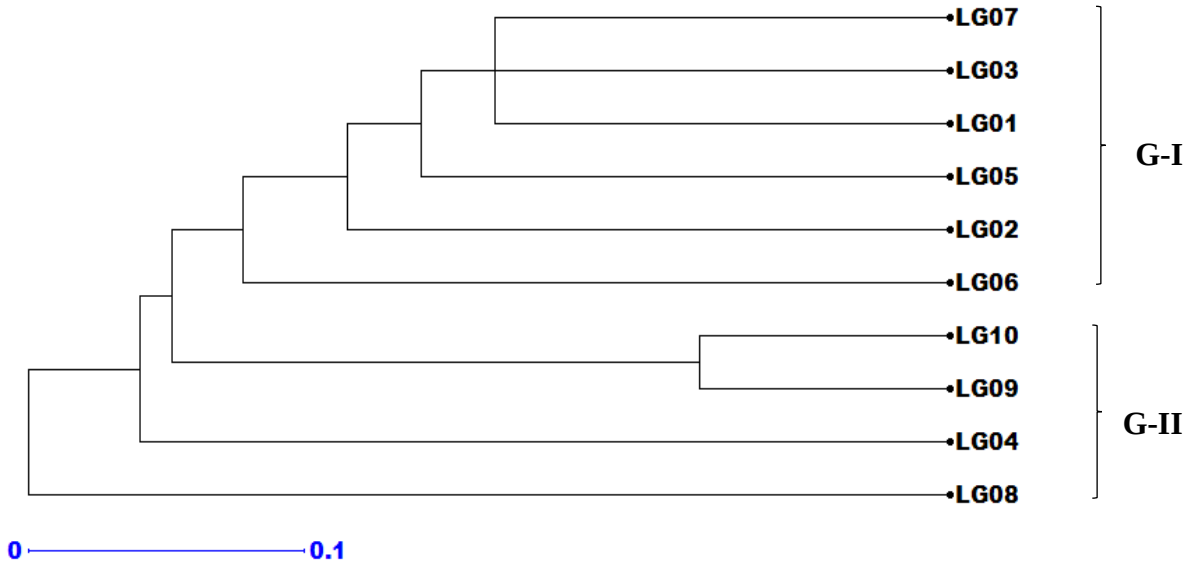

Figure 1: Dendrogram of the 4 ISSR and 2 SSR markers across the 10 lemongrass accessions

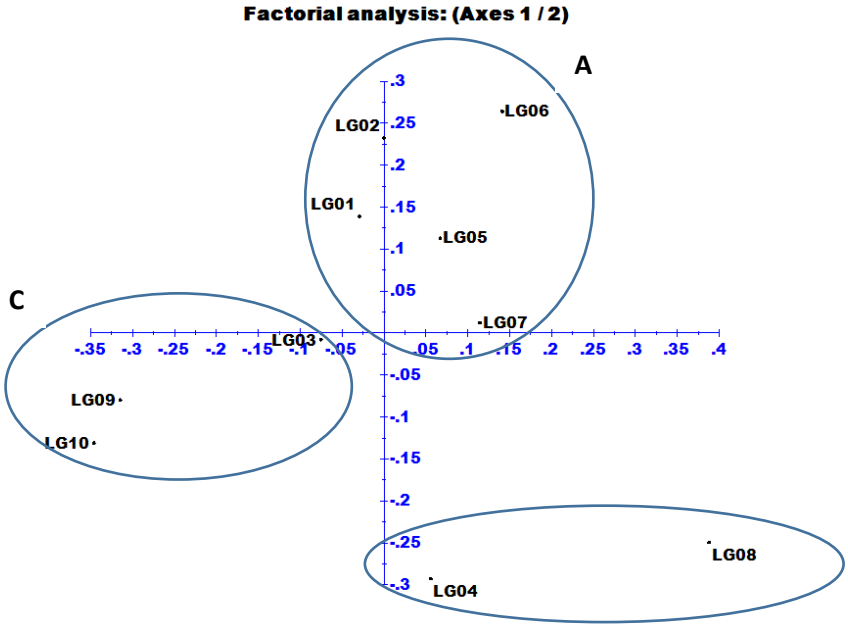

Figure 2: Factorial plot of the 4 ISSR and 2 SSR markers across 10 lemongrass accessions of lemongrass.

Percentage of total essential oil yield: The percentage oil yield of LG02 was observed to be 0.34 %, whereas LG03 accession's yield was 0.36 % as given in Table 4. The extracted essential oils exhibited a pale yellowish oil with a strong pleasant citrus aroma.

Table 4: Essential oil yield from 2 accessions of *C. citratus*.

Accession no	Location	Oil yield (%)
LG02	Osun	0.34
LG03	Ogun	0.36

Chemical composition of the extracted essential oil: The analysis of the GC-MS chromatographs (Figure 3) of the essential oil of LG02 and LG03 accessions revealed 17 major chemical components which include: -β-myrcene, β-pinene, neral (citral b), geranial (citral a), geranyl propionate, bicyclo[3.1.1]hept-2-en-6-one, 2,7,7-trimethyl, beta.-bisabolene, farnesene, 2-tridecanone, caryophyllene oxide, longifolene, trans-geranylgeraniol, geranyl linalool, geranyl butyrate, and farnesol in different proportions in LG02 and LG03 (Table 6). In the accessions, the most prominent phytochemical compounds are citral (neral and geranial) and linalool. Among aldehydes, LG02 (Ejigbo, Osun) was rich in only neral (49.38 %) and not geranial (0 %) which are collectively known as citral while LG03 (Ibafo, Ogun) had neral (35.87 %) and geranial (30.60 %). For terpenoids, LG02 had 3.38 % and 8.97 % of trans-geranylgeraniol and geranyl linalool respectively while LG03 had 10.39 % and 6.97 % of the same constituents. Apart from geranial, geranyl propionate and bicyclo[3.1.1]hept-2-en-6-one, 2,7,7-trimethyl were not detected in LG02 whereas linalool oxide and bicyclo[3.1.1]hept-2-ene,2,6-dimethyl-6-(4-methyl-3-pentenyl) were not found in LG03.

Table 6: Concentration of the essential oil compound and percentage composition in 2 accessions of *C. citratus*

Compounds	**Molecular Formula**	**Molecular weight (g/mol)**	**LG02 (%)**	**LG03 (%)**
Beta-Myrcene	$C_{10}H_{16}$	136.23	0.53	0.32
Beta-Pinene	$C_{10}H_{16}$	136.23	1.38	0.9
Neral (β-citral)	$C_{10}H_{16}O$	152.24	49.38	35.87
Geranial (α-citral)	$C_{10}H_{16}O$	152.24	0	30.6
Geranyl propionate	$C_{13}H_{22}O_2$	210.31	0	3.73
Bicyclo[3.1.1]hept-2-en-6-one, 2,7,7-trimethyl	$C_{10}H_{14}O$	150.22	0	1.53
Beta.-Bisabolene	$C_{15}H_{24}$	204.35	2.12	1.97
Farnesene	$C_{15}H_{24}$	204.36	2.71	0.07
2-Tridecanone	$C_{13}H_{26}O$	198.34	1.81	0.96
Caryophyllene oxide	$C_{15}H_{24}O$	220.35	1.31	0.94
Longifolene	$C_{15}H_{24}$	204.36	0	1.87
Trans-Geranylgeraniol	$C_{20}H_{34}O$	290.5	3.38	10.39
Geranyl linalool	$C_{20}H_{34}O$	290.5	8.97	6.97
Geranyl butyrate	$C_{14}H_{24}O_2$	224.34	2.19	0.28
Farnesol	$C_{15}H_{26}O$	222.37	7	0.62
Linalool oxide	$C_{10}H_{18}O_2$	170.25	3.18	0
Bicyclo[3.1.1]hept-2-ene,2,6-dimethyl-6-(4-methyl-3-pentenyl)	$C_{15}H_{24}$	204.35	1.39	0

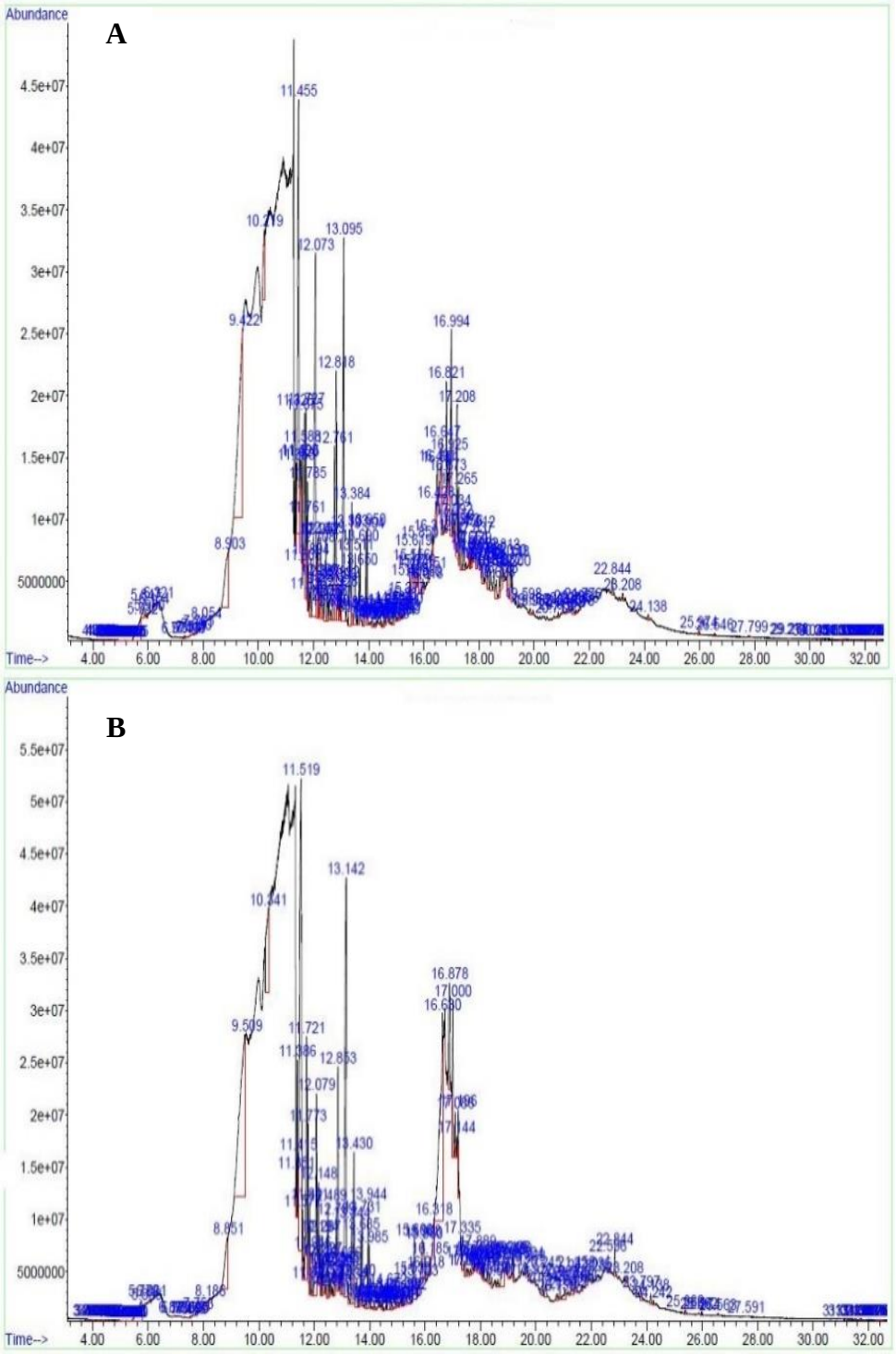

Figure 3: GC-MS chromatograph of essential oil components of *Cymbopogon citratus* in (A) LG02 and (B) LG03

Discussion

DNA-based markers can be utilized to identify similarities and differences between accessions when morphological descriptions are limited. Cultivar identification and genetic diversity studies are extremely beneficial in protecting and conserving genetic resources. Most commonly, SSR and ISSR markers are used to examine the genetic relationships among accessions of a species. In this study, four ISSR markers (ISSR817 and ISSR886, ISSR879 and ISSR881) and two SSR markers (SSR163 and SSR075) were used to determine the genetic relationship between ten accessions of *C. citratus*. In the 10 accessions of *C. citratus*, 4 ISSR markers produced a total of 34 scorable alleles, which is comparable to 37 loci from the same set of 4 ISSR markers used for molecular characterization among the ten coriander accessions (Melo *et al.*, 2011). ISSR markers detect genomic polymorphisms with high sensitivity and have the potential to determine intra- and interspecific levels of variation (Zietkiewicz *et al*., 1994). The two SSR markers in this study generated 13 scoreable loci compared to Kumar *et al.* (2007), which generated 18 loci from the same primers and a total of 95 loci from 18 SSR markers used in that study. Though a total of 7 SSRs were optimized many times, only 2 of them (SSR 075 and SSR163) were amplified with expected allele sizes across the ten lemongrass accessions and were highly reproducible. The inability of the SSR markers (SSR001, SSR125, SSR126, SSR133, and SSR114) to amplify the *C. citratus* accessions studied here, could be because the primers were designed from the *C. jwarancusa* genome (Kumar *et al*., 2007). The number of alleles per primer pair ranged from 3 (SSR 163) to 10 (SSR 075), indicating the efficiency of these primers to be informative and useful in analyzing genetic diversity of the ten accessions. The mean percentage polymorphism of the four ISSR markers used in the present study is 88.8 9% is comparable to 93.22 % reported by Debajit *et al.* (2015) among 12 genotypes of *Cymbopogon flexuosus* based on 4 ISSR markers and 42.69 % mean percentage polymorphism observed by Bhattacharya *et al.* (2010) among the 11 accessions of *Cymbopogon winterianus*. The mean percentage polymorphism of SSR is 100 % compared to 81 % observed by Kumar *et al.* (2009) in 25 accessions of three *Cymbopogon* species (Citrati, Rusae and Schoenanthi) using 20 SSR primer pairs. Both markers provided differentiation and diversity of closely related accessions, as evidenced by the SSR's 88.89 % polymorphism compared to its 100 % polymorphism. The PIC values observed from the six markers ranged from 0.31 to 0.85. Five out of the 6 primer pairs gave a PIC value of more than 0.5. Hence, they could be classified as very informative. The observed high proportion of polymorphic loci suggested that there was profound genetic heterogeneity at the inter-species and intra-species levels, as also previously found (Sangwan *et al.*, 2001). Comparison of PIC values for marker systems (a parameter associated with the discriminating power of markers) indicated that the mean PIC value for ISSR markers was 0.81, while that of SSR was 0.58 indicating a better resolving power of ISSR markers over SSR (Guo and Elston, 1999).

In the dendrogram constructed to estimate genetic diversity among ten accessions, the accessions were classified into two major clusters based on their genetic relatedness and diversity. The accessions in this study exhibit moderate genetic diversity. The factorial plot shows some correlation with the dendrogram, distributing the *Cymbopogon* accessions around the 4 quadrants. The ability to resolve genetic variation among different genotypes may be more directly related to the number of polymorphisms detected with each marker technique (Bhattacharya *et al.*, 2010). Based on this study, it can be concluded that ISSR and SSR markers are powerful tools as potential diagnostic markers for *Cymbopogon* cultivars. Molecular markers can be exploited for the generation of potential hybrid lines with controlled breeding and hybridization strategies in the *Cymbopogon species* (Sharma *et al.*, 2000).

The essential oils extracted from the leaves of two lemongrass accessions (LG02 and LG03) by hydro-distillation produced a pale yellow colour with a yield of 0.34 and 0.40 %, respectively, compared to 0.21 % revealed in a recent study (Rodrigues *et al.* (2022). The yield obtained from fresh leaves agrees with the study of Hanaa *et al.* (2012), based on the result, the oil content yields ranged from 0.2 to 0.50 %. Hanaa *et al.* (2012) further stated that with proper management and an effectively selected strain, oil content could yield up to 0.66-0.90 %. In this study, the chromatographic and mass spectrometry analysis of the volatile oils resulted in the identification of 13 major compounds in LG02 and 15 in LG03. A few other components present in the sample in trace amounts were also identified. However, they are not listed in the study because their presence was not considered of any relevance. Citral imparts a characteristic lemon-like aroma to the EOs of *Cymbopogon spp*. (Husain, 1994). According to Negrelle and Gomes (2007), independent of their origin, *Cymbopogon* essential oils are composed of about 30 to 93.74 % citral: neral (β-citral) and Geranial (α-citral) with the general predominance of geranial. In this study, the total citral content of LG02 is 49.38 %, which constitutes only neral (β-citral), while LG03 had 66.47 % total citral content of which neral (β-citral) is more abundant (35.87 %) than geranial (α-citral; 30.60 %). The present study is in agreement with Tajidin *et al.* (2012). They reported that the percentage citral content (geranial and neral) was highest in *Cymbopogon* species. The differences in oil yield, citral percentage and essential composition between LG02 and LG03 might be due to varying genetic backgrounds. This is because the accessions were seen to differ from each other in the dendrogram and factorial

plot. There is a possibility that the LG03 accession with 66.47 % of citral will be selected for breeding in the future (Sharma *et al*., 2000).

Conclusion

This study assessed the genetic diversity of ten *C. citratus* accessions regardless of their geographical distribution using ISSR and SSR markers. In addition, the two lemongrass accessions studied contained elevated levels of citral in their essential oils. The development of SSR markers that are specific for *C. citratus* is needed for the extensive assessment of genetic diversity. Also, it will promote the breeding and utilization of *C. citratus* resources.

References

Abdelaziz SM, Medraoui L, Alami M, Pakhrou O, Makkaoui M, Ould Mohamed Salem Boukhary A, Filali-Maltouf A: Inter simple sequence repeat markers to assess genetic diversity of the desert date (*Balanites aegyptiaca* Del.) for Sahelian ecosystem restoration. Sci Rep, 10(1). 2020. https://doi.org/10.1038/s41598-020-71835-9.

Ademuyiwa AJ, Grace OK: The effects of *Cymbopogon citratus* (Lemongrass) on the antioxidant profiles Wistar albino rats. Merit Res J, 3: 51-58. 2015.

Adeyemo OA, Osibote E, Adedugba A, Bhadmus OA, Adeoshun AA, Allison MO: Antioxidant activity, total phenolic contents and functional group identification of leaf extracts among lemongrass (*Cymbopogon citratus*) accessions. NISEB J, 18: 1595-6938. 2018.

Adibah A, Ling LP, Tan S, Faridah Q, Christianus A: Development of single-locus DNA microsatellite markers using 5′ anchored ISSR-PCR method for the mangrove horseshoe crab, *Carcinoscorpius rotundicauda* (Latreille, 1802) in Peninsular Malaysia. Mol Biol Rep, 39: 3815-3820. 2012.

Akin-Osanaiye BC, Agbaji AS, Dakare MA: Antimicrobial activity of oils and extracts of *Cymbopogon citratus* (Lemon Grass), Eucalyptus citriodora and Eucalyptus camaldulensis. J Med Sci, 7(4). 2007.

Amiteye S: Basic concepts and methodologies of DNA marker systems in plant molecular breeding. Heliyon, 7(10): e08093. 2021. https://doi.org/10.1016/j.heliyon.2021.e08093

Barbosa LCA, Pereira UA, Martinazzo AP, Maltha CRÁ, Teixeira RR, Melo EdC: Evaluation of the chemical composition of Brazilian commercial (DC) Stapf samples. Molecules, 13(8): 1864-1874. 2008.

Bhattacharya S, Bandopadhyay TK, Ghosh PD: Efficiency of RAPD and ISSR markers in assessment of molecular diversity in elite germplasms of *Cymbopogon winterianus* across West Bengal, India. Emir J Food Agric, 22(1): 13-24. 2017. doi:10.9755/ejfa.v22i1.4903.

Calo JR, Crandall PG, O'Bryan CA, Ricke SC: Essential oils as antimicrobials in food systems - A review. Food Control, 54: 111-119. 2015.

Debajit S, Sukriti D, Sneha G, Mohan L, Singh BB: RAPD and ISSR based Intra-specific molecular genetic diversity analysis of *Cymbopogon flexuosus* L. Stapf with a distinct correlation of morpho-chemical observations. Res.J Biotechnol, 10: 7. 2015.

Dellaporta SL, Wood J, Hicks JB: A plant DNA minipreparation: version II. Plant Mol Biol Rep, 1: 19-21. 1983.

Guo X, Elston R: Linkage information content of polymorphic genetic markers. Hum Hered, 49(2): 112-118. 1999.

Hanaa AM, Sallam Y, El-Leithy A, Aly SE: Lemongrass (*Cymbopogon citratus*) essential oil as affected by drying methods. Ann Agric Sci, 57(2): 113-116. 2012.

Husain, A. Essential oil plants and their cultivation. Central Institute of Medicinal and Aromatic Plants, Lucknow 226015, Uttar Pradesh, India. 300 pp, 1994.

Joy P, Mathew S, Skaria B, Mathew G, Joseph A, Sreevidya P: Lemongrass Aromatic and Medicinal Plants Research Station. In: Odakkali, Asamannoor, Kerala, India. 32 pp. 2006.

Khanuja SP, Shasany AK, Pawar A, Lal R, Darokar M, Naqvi A, Rajkumar S, Sundaresan V, Lal N, Kumar S: Essential oil constituents and RAPD markers to establish species relationship in *Cymbopogon* Spreng. (Poaceae). Biochem Syst Ecol, 33(2): 171-186. 2005.

Kuete V: Medicinal spices and vegetables from Africa: therapeutic potential against metabolic, inflammatory, infectious and systemic diseases. ISBN 978-0-12-809286-6. Academic Press. 673 pp, 2017.

Kumar A, Bennetzen JL: Plant retrotransposons. Annu Rev Genet, 33(1): 479-532. 1999.

Kumar J, Verma V, Goyal A, Shahi A, Sparoo R, Sangwan R, Qazi G: Genetic diversity analysis in *Cymbopogon* species using DNA markers. Plant Omics, 2(1): 20. 2009.

Kumar J, Verma V, Shahi A, Qazi G, Balyan H: Development of simple sequence repeat markers in *Cymbopogon* species. Planta Med, 73(3): 262-266. 2007. https://doi.org/10.1055/s-2007-967121

Lewinsohn E, Dudai N, Tadmor Y, Katzir I, Ravid U, Putievsky E, Joel DM: Histochemical localization of citral accumulation in lemongrass leaves (*Cymbopogon citratus* (DC.) Stapf., Poaceae). Ann Bot, 81(1): 35-39. 1998.

Liu K, Muse S. PowerMarker: an integrated analysis environment for genetic marker analysis. Bioinformatics, 21: 2128-9. 2005.

Mathews S, Spangler RE, Mason-Gamer RJ, Kellogg EA: Phylogeny of Andropogoneae inferred from phytochrome B, GBSSI, and ndhF. Int J Plant Sci, 163(3): 441-450. 2002.

Mehedi MNH, Bepari NC, Robbani M, Akter KT, Hasan M, Hasan F: Lemon grass oil: effect on physico-chemical properties and postharvest life of banana cv. Amritasagor. Asian J Crop Sci, 3(01): 114-124. 1974.

Melariri PE: The therapeutic effectiveness of some local Nigerian plants used in the treatment of malaria. PhD. Thesis University of Cape Town. 2010

Melo RdA, Resende LV, Menezes D, Beck APA, Costa JCd, Coutinho AE, do Nascimento AVS: Genetic similarity between coriander genotypes using ISSR markers. Hortic Bras, 29: 526-530. 2011.

Nair K, Jayakumar R: Ethnobotany of Hill-Pulaya tribe in the context of biodiversity rehabilitation at Chinnar wildlife sanctuary, Western Ghats of India. J Econ Taxon Bot, 23(2): 431-449. 1999.

Negrelle R, Gomes E: Cymbopogon citratus (DC.) Stapf: chemical composition and biological activities. Rev Bras Plantas Med, 9(1): 80-92. 2007.

Paterson AH: Genome mapping in plants. Academic Press.330 pp, 1996.

Perrier X, Jacquemoud-Collet J: DARwin software: http://darwin.cirad.fr/darwin. 2006

Rodrigues L, Coelho E, Madeira R, Teixeira P, Henriques I, Coimbra MA: Food ingredients derived from lemongrass byproduct hydrodistillation: essential oil, hydrolate, and decoction. Molecules, 27(8): 2493. 2022. https://doi.org/10.3390/molecules27082493

Sangwan N, Yadav U, Sangwan R: Molecular analysis of genetic diversity in elite Indian cultivars of essential oil trade types of aromatic grasses (*Cymbopogon* species). Plant Cell Rep, 20: 437-444. 2001.

Sarma KK, Nath SC, Leclercq PA: The essential oil of a variant of *Cymbopogon flexuosus* (Nees ex Steud) Wats. from northeast India. J Essent Oil Res, 11(3): 381-385. 1999.

Sharma S, Shahi A, Srivastava T: Taxonomic relationship of *Cymbopogon* species based on volatile leaf oil chemical constituents. J Econ Taxon Bot, 24(1): 115-122. 2000.

Singh A, Kumar A: Lemongrass (*Cymbopogon flexuosus*) cultivation, oil Extraction and chemical Composition in Kannauj Region. Int J Innov Res Sc i Eng, 6: 13989-13993. 2017.

Tajidin N, Ahmad S, Rosenani A, Azimah H, Munirah M: Chemical composition and citral content in lemongrass (*Cymbopogon citratus*) essential oil at three maturity stages. Afr J Biotechnol, 11(11): 2685-2693. 2012.

Varshney RK, Graner A, Sorrells ME: Genic microsatellite markers in plants: features and applications. Trends Biotechnol, 23(1): 48-55. 2005.

Zietkiewicz E, Rafalski A, Labuda D: Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics, 20(2): 176-183. 1994.