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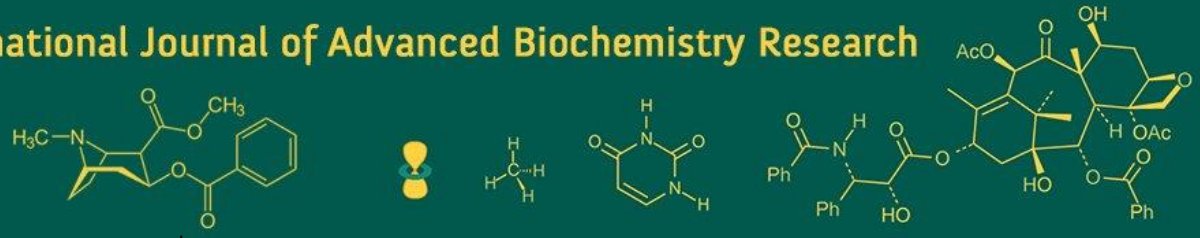

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Utilizing ISSR and DAMD markers and agro-morphological traits for genetic refinement of some elite lemongrass (*Cymbopogon flexuosus* Steud)

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Abstract
This study explores the genetic diversity and potential breeding strategies in Lemongrass (*Cymbopogon flexuosus* Steud) through morphological and molecular analyses. Analysis was conducted in Research Cum Instructional Farm, Department of Genetics and Plant Breeding, and PMBB R.H. Richharia lab, College of Agriculture, IGKV Raipur (C.G.). Morphological assessment of six selected accessions, including two promising lines C.G. CF-2 and C.G. CF-4 and four checks, revealed significant variations in treatment for all the traits except oil density depicts enough variation present in the genotype. High GCV and PCV is reported by herbage yield. Strong positive correlations were observed between herbage yield and parameters like leaf blade width, leaf blade length, tiller number, leaf area index, and culm length. Molecular analyses using DAMD and ISSR markers demonstrated polymorphic patterns within Lemongrass species, indicating genetic variability. C.G. CF-4 explains the uniqueness compare to checks which have high in oil content with 73.38% citral, making it potential standard varieties. The integration of morphological and molecular markers it also facilitates precise selection of lines for further breeding programs for developing promising lines accelerated development of high-yielding and high-oil-content varieties.

Keywords: DNA Fingerprinting, GC analysis, citral, diversity, high oil percentage

Introduction
Lemongrass is an aromatic, tall, clumped perennial grass belonging to the family Poaceae and the genus *Cymbopogon* and species *flexuosus*. It is a tropical plant that yields aromatic oil from leaves. The name "lemongrass" is derived from the typical lemon-like odour of the essential oil present in the leaves and shoot (Tajidin *et al*., 2012) [26]. The name *Cymbopogon* was derived from the Greek words “Kymbe” (boat) and “pogon” (beard), referring to the flower spike arrangement (Khanuja *et al*., 2005) [13]. *Cymbopogon* is a genus comprising about 180 species, subspecies, varieties, and subvarieties. 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) [14]. In India, the East Indian lemongrass grows naturally and is also widely grown in Uttar Pradesh, Kerala, Assam, and Maharashtra. It is distributed in China and Guatemala as well. West Indian lemongrass is thought to have come from Sri Lanka or Malaysia. Grown in the West Indies, Guatemala, Brazil, Congo, Tanzania, India, Thailand, Bangladesh, Madagascar, and China, it is extensively dispersed throughout the tropics. According to Joy *et al*. (2006) [10], Jammu's lemongrass is primarily found in North Indian states including Jammu and Kashmir, Sikkim, Assam, Bengal, Madhya Pradesh, and Chhattisgarh. In India's Western Ghats, near the Chinnor Wildlife Sanctuary, lemongrass is grown extensively. Lemongrass is traditionally grown in semi-arid tropical climates with high rainfall. It is developed under irrigation. In India aromatic and medicinal plant having area and production of 731’000 ha and 626’000 MT respectively [Department of Agriculture & Farmers Welfare, 2023-24]. In Chhattisgarh aromatic and medicinal plants occupy area and production of 6.608’000 ha and 46.173 MT respectively [Department of Agriculture & Farmers Welfare, 2022-23]. Lemongrass has prioritize number one under aromatic crops in India due to its wide application on culinary,

cosmetic and pharmaceutical confectioneries. [ICAR DAMPR AICRP-MAP&B Report 2022-23]. Citral, geraniol, citronellol, citronellal, linalool, elemol, 1,8-cineole, limonene, β-caryophyllene, methyl heptenone, geranyl acetate, and geranyl formate are found in the essential oils of different *Cymbopogon* species. The characteristic aroma, flavour, or essence of the plant is carried by the extremely volatile aroma molecules found in the essential oil extracted from lemongrass shoots and leaves. Lemongrass essential oil is located on the adaxial surface of leaf mesophyll in parenchyma tissue cells (Lewinsohn *et al*., 1998) [11]. 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. The essential oils were extracted by hydro-distillation method and its quality analyzed by GC-MS. The major chemical components identified from the accession are citral (neral+geranial), geraniol, geranial, limonene, linalool, citronella, neral, citronellyl acetate, geranyl acetate. In essential oil of lemongrass anti-funglll activity was reported in in-vitro state and it shows the zero growth in *Sclerotium sp* and *Alterneria sp.* Culture (Ekka *et al.*, 2023) [7]. Genetic diversity is essential for developing appropriate strategies for breeding, germplasm management and the utilization of genetic resources (Paterson, 1996) [16]. Genetic control and phenotypic variability are agro-morphological traits important for identifying stable varieties for developing improve lines for breeding programme Rathia *et al*., 2022) [20]. For evaluating genetic variants among the most economically significant plants, a variety of molecular markers have shown to be effective tools (Amiteye, 2021) [3]. Without having to look at the DNA sequences of the species' genome, a molecular method such as inter simple sequence repeat (ISSR) markers and DAMD markers has been described for evaluating genetic diversity study in plants (Abdelaziz *et al*., 2020) [1]. (Adibah *et al*., 2012) [2].

Materials and Methods

Plant Materials: In present studies two germplasm accessions and four checks is used which consist of landraces collected from Chhattisgarh. They are C.G. CF-2 and C.G. CF-4 and checks are C.G. LG-1, CKP 25, Krishna and CIM- Shikhar. The passport data of two accessions prepared by NBPGR guidelines and are presented Table 11.
The research study was executed out in Research –Cum Instructional Farm of IGKV Raipur (C.G.). All entries of lemongrass was planted in three replications in an RCBD design and content will be planted with a spacing of 30cm X 30cm row to row and plant to plant. In order to fetch a good harvest, all recommended packages of activities was followed. Randomly five plants were selected from each accessions and observation were for following traits; plant height (cm), culm length (cm), culm diameter (cm), leaf blade length (cm), leaf blade width (cm), leaf area index, total number of tillers/plant, number of leaves/culm, herbage yield (g), oil content (%), oil density (g/cm^3) and citral percentage and some qualitative traits; culm colour and leaf blade colour.

Statistical Analysis: To estimate the significance, genetic paramters, correlation and path analysis GRAPES version (1.0.0.) software (Gopinath *et al*. 2020) [8] is operated and to assess molecular diversity and construct dendogram PAST 4.03 version is accessed.

Analysis of citral content by gas chromatography (GC): GC analysis was recorded as per reported method (Kumar *et al.* 2009) temperature of injector and detector was kept at 253 °C. Compound separation was done by using oven temperature programme rate of 7 °C to 253 °C with a hold time of 2 min up to 70°C remaining time period, final run time was 30 min. Injector volume was taken 1µl for the run, detector is FDI and carrier was liquid nitrogen, peaks were identified by standard sample procedure from Sigma – Aldrich in the same GC condition. The essential oil was extracted by the hydro distillation method of lemongrass plant, and oil was compared for citral analysis in each samples.

DNA extraction and PCR amplification: DNA Extraction Total genomic DNA of each genotype has been performed by a CTAB (cetyl tri methyl ammonium bromide) protocol as described by Doyle and Doyle 1987. The concentration of DNA was measured by the DNA fluorimeter and kept at –20°C until use.

DNA quantification: The software will display the concentration of the DNA sample in ng/µL based on the absorbance at 260 nm (A260). A260/A280 and A260/A230 ratios will also be displayed, providing information about the purity of the sample. Ideal A260/A280 ratio for pure DNA is typically around 1.8, and the A260/A230 ratio should be around 2.0-2.2. In quantification process good amount of DNA is quantified which further used for PCR amplification.

DMAD assay: DAMD-PCR Assay four DAMD primers were tested as previously reported in other species [9, 14, 17]. A 25 µl reaction volume containing 1X PCR buffer, 2 mM MgCl2, 0.25 mM dNTPs, 25 pmol primer, 1.5 unit of Taq DNA polymerase, and 50 ng of template DNA was used to conduct the DAMD-PCR amplification reaction. In a gradient thermal cycler (Bio-Rad; T-Gradient), PCR amplification was carried out. It was set up to run 35 cycles, with a 4-minute denaturation cycle at 94 ºC first. Each cycle included an extension step at 72 ºC for 2 min, a denaturation step for 1 min at 94 ºC, an annealing step for 2 min at Tm changing based on each primer evaluated, and an extension cycle for 7 min at 72 ºC in the final cycle.
After that, the PCR products were separated in 0.5X TBE buffer on a 1.8% ethidium bromide-stained agarose gel (Bio-Rad). An ultraviolet trans-illuminator was used to visualise the results of a two-hour electrophoresis run at 100 V. The amplification products of DAMD-PCR were quantified by molecular weight using a standard VC 100bp Plus DNA Ladder.
Same procedure is applied for ISSR marker assay.

DAMD-PCR Five DAMD primers named M-13, F VII e8-C, M-13-2, 33-6-2 and 6.2 H(+) were used for DAMD analysis. A 25 µL PCR reaction mixture included 5.00 µL of genomic DNA, 1× GoTaq® Flexi Buffer, 3.0 mM MgCl2, 200 µM dNTPs, 0.4 µM primer, and 0.5 units of GoTaq® DNA polymerase. The amplification reaction in the thermal cycler began with a five-minute pre-denaturation stage at 94 °C, followed by 35 PCR cycles of denaturation at 94 °C for

30 seconds, annealing at 55 °C for one minute, and extension at 72 °C for one minute. The final step involved polishing the PCR results for seven minutes at 72 °C. In all PCR amplification reactions a sample without template DNA was used as a negative control to check the presence of contaminations.

Table 1: Primer details of DAMD markers

Primer number	Primer name	Primer sequence5'-3'	Ta °C
1.	M-13	GAGGGTGGCGGTTCCT	55
2.	F VII e8-C	CCTGTGTGTGTGCAT	47
3.	M-13-2	GTAAAACGACGGCCAGT	55
4.	33-6-2	AGGGCTGGAGG	56
5.	6.2 H(+)	AGGAGGAGGGGAAGG	56

ISSR-PCR: Five ISSR primers, UBC-840, UBC-841, ISSR-01, ISSR-05, and UBC-834, were employed. To optimize the annealing temperature for each ISSR primer, pilot experiments were conducted using a range of annealing temperatures and varying DNA volumes. The annealing temperature range was set to be 2-10 °C below the denaturation temperature (Td) of each primer. According to Ranade and Farooqui (2002) [19], the Td was calculated by adding 2 °C for each A or T and 4 °C for each G or C in the primer sequence.

Table 2: Primer details of ISSR markers

Primer number	Primer name	Primer sequence5'-3'	Ta °C
1.	UBC-840	GAGAGAGAGAGAGAGACT	52
2.	UBC-841	GAGAGAGAGAGAGAGACTC	54
3.	ISSR-01	ACACACACACACACACG	52
4.	ISSR-05	AGAGAGAGAGAGAGAGT	57
5.	UBC-834	AGAGAGAGAGAGAGAGCT	52

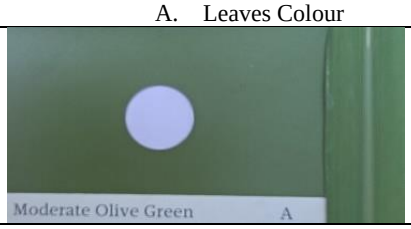
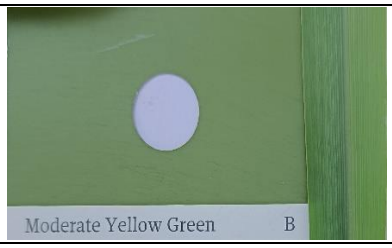
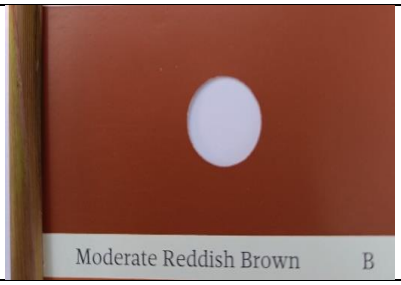
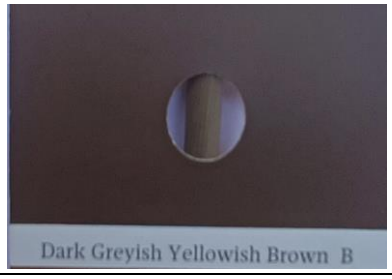
Results

Analysis of morphological profile

The qualitative traits for culm colour and leaf colour was recorded for all the genotype using R.H.S. colour chart, which depicts that C.G. CF-4 moderate reddish brown A, C.G. CF-2 is dark greyish yellow brown B, C.G. LG-1 is moderate reddish brown B, CKP-25 is light olive grey A, Krishna is Greyish Brown A and CIM Shikhar is moderate brown D for the culm colour same observation is taken for leaves colour (Table 3).

Analysis of variance for quantitative traits show significant for all traits except oil density, which depicts enough variability present among the germplasm.

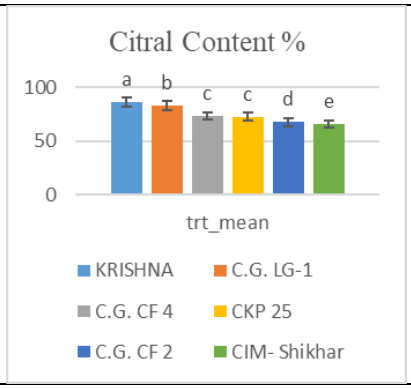
Table 3: RHS Chart comparison

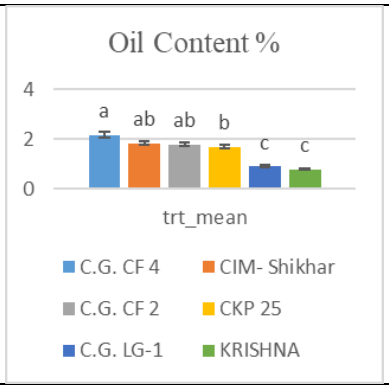
A. Leaves Colour		
		
C.G. LG-1	C.G. CF-2	C.G. CF-4
		
CKP-25	Krishna	CIM-Shikhar
B. Culm Colour		
		
C.G. LG-1	C.G. CF-2	C.G. CF-4

		
CKP-25	Krishna	CIM-Shikhar

Mean Table: All quantitative traits are epitomized in graphical form with mean values traits are categorized in a-e groups. (Table 4)

Table 4: Mean table of traits with graphical representation





Genotypic and Phenotypic Co-efficient of variation: In Analysis of genetic parameters high GCV stated by herbage yield 49.13% after that culm length 43.94% and total number of tillers/plant 39.31%. For PCV it was perceived in herbage yield 62.77 subsequently 46.66% for total number of tillers/plant and culm length 45.32% (*Fig*. 7 Table 7).

Heritability and Genetic Advance as percent of mean: Citral content percentage with a very high heritability (98.22%), Citral content percentage stands out as a trait with substantial genetic control, its high heritability suggests that selection based on this trait could lead to Considerable genetic advances. Leaf area index also demonstrates high heritability (89.56%) along with significant phenotypic and genotypic variability. The genetic advance (GAM 5%) for LAI is substantial (74.29%), indicating that selection for this trait could result in significant improvements in leaf area. Leaf blade length high heritability (90.05%) with genetic advance (GAM 5%) is also notable (57.51%), suggesting that selection for this trait could lead to substantial improvements in final yield (*Fig*. 7 Table 7).

Correlation analysis: In analysis among selected elite lines genotypic correlation for herbage yield is positive strong correlated with maximum traits like leaf blade width, leaf blade length, plant height, culm diameter, culm length, total number of tillers/plant, leaf area index, oil content (%) and citral content, it shows negative correlation with oil density. Phenotypic correlation for herbage yield is found significant positive correlated with leaf blade width prior to that leaf area index and culm diameter (Table 8a and 8b).

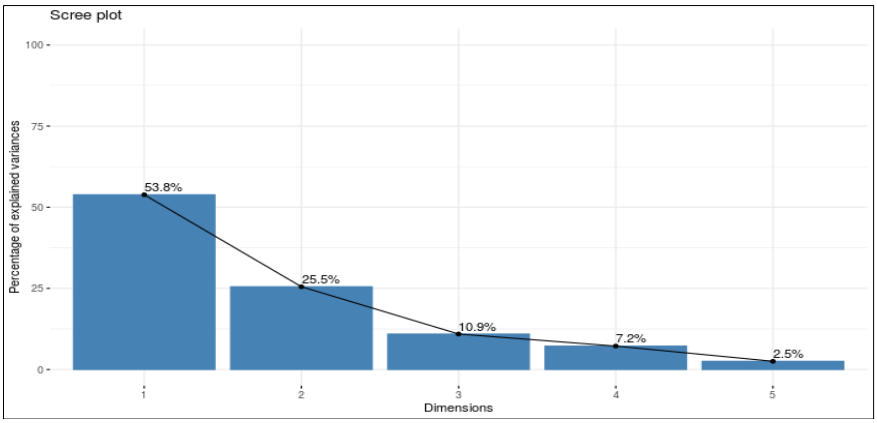


Fig 1: Scree plot of PCA analysis

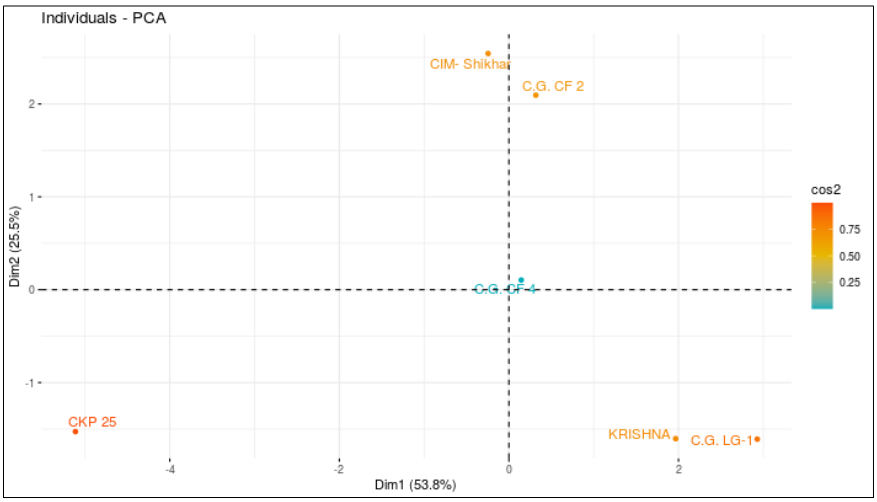


Fig 2: Biplot of PCA analysis of elite genotypes

Principal component analysis (PCA): Principal component with Eigen values greater than 1 explained more than 70% of the variation in the data for the PCA of yield traits. The PC_1 explicated 53% of the variation in the data and PC_1, PC_2 and PC_3 clarified 90.27% of the variation in the data [*Fig* 1; Table 9].

Principal component one (PC_1) loaded plant height, leaf blade length, leaf blade width herbage yield, leaf area index, culm diameter, culm length, citral content %, total number of tiller/plant and number of leaves/culm traits, which contribute maximum in the variation generation and in PC_2 oil content % and oil density contributed for the variation generation (*Fig* 2).

PC Bi-plot for elite traits in C.G. CF-4 positioned near to axis suggesting that the genotype performed similarly and least amount of variation tested and very less stimulates by the environment [*Fig* 3; Table10].

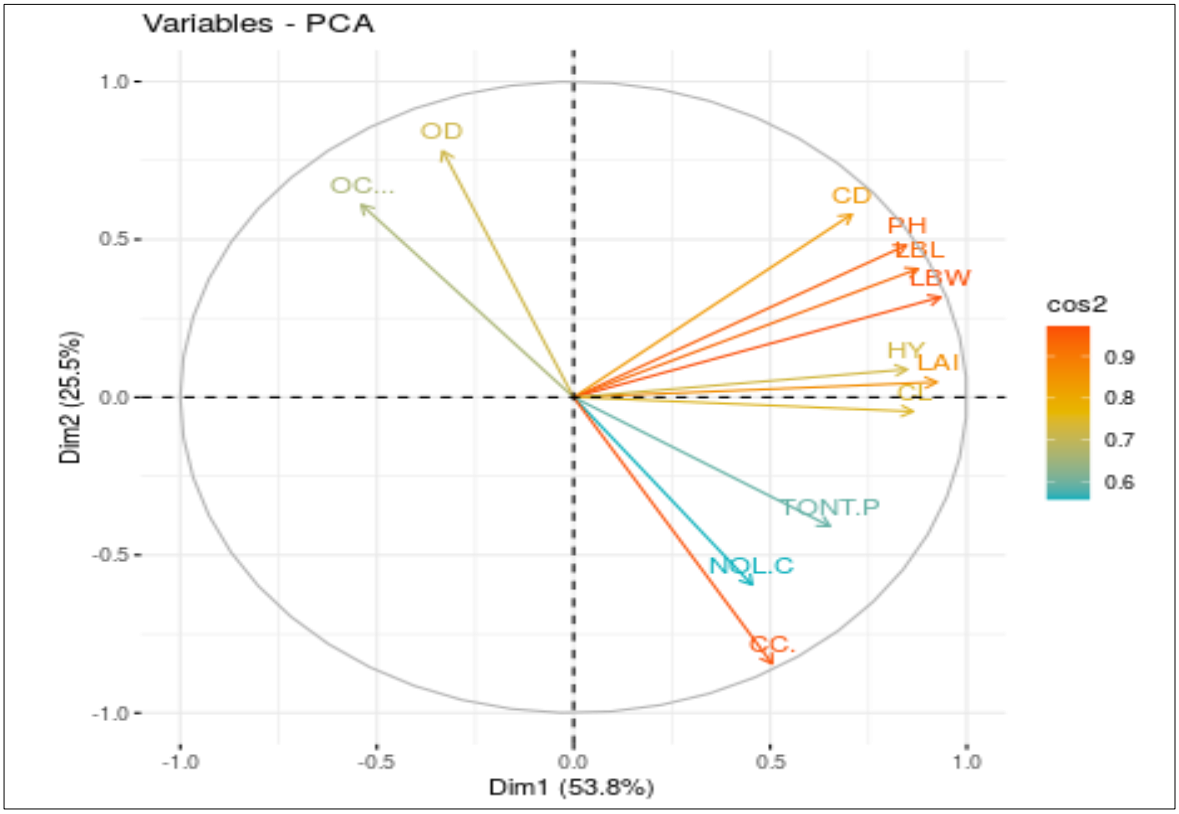


Fig 3: Variables biplot of PCA analysis for traits

Table 5: Details of oil content, oil density, citral percentage and herbage yield data

S. No.	Entries	Oil content (%)	Oil density (g/cm^3)	citral percentage	Herbage Yield (g)
1.	C.G. LG-1	1.01	1.603	83.28	425
2.	C.G. CF-2	1.85	1.608	67.58	325
3.	C.G. CF-4	2.18	1.602	73.38	231.25
4.	CKP-25	1.77	1.595	72.59	37.50
5.	Krishna	0.84	1.601	86.02	313.75
6.	CIM-Shikhar	1.84	1.605	65.76	187.50

Analysis of DAMD profile: The size of DAMD-PCR amplification procedure ranged between 300bp - 3000bp FIG (1). Shows polymorphic patterns within *Cymbopogon* species produced by M-13, F-VII e8-C, M-13-2, 33-6-2 and 6.2H (+). The DAMD marker applied in the molecular study with lemongrass elite entries.

Data showed that all markers exhibit polymorphism in low degree which indicates that species are closely related. For DAMD all five markers show polymorphism its avareage PIC ranges from 0.081-0.132 and all DMAD assay successfully produced unique markes and can be further useful for characterization in *Cymbopogon* sp (*Fig* 4)

Analysis of ISSR profile: The size of ISSR-PCR amplification assay ranged between 300bp - 3000bp FIG (2). Shows polymorphic patterns within four markers UBC-840, UBC-841. ISSR-05 and UBC-834. One marker ISSR-01 doesn't show polymorphism in selected lines.

Data showed that four markers showing polymorphism in which UBC-840 expressed high polymorphism and rest three markers show less polymorphism in *Cymbopogon* species. Average PIC for ISSR marker ranges from 0 - 0.312 (*Fig* 5).

Prior to combining both analysis a dendogram had been constructed to dpict the diversity among genotypes (*Fig* 6).

Individual Plant Image

C.G. LG-1

CIM-Shikhar

C.G. CF-4

C.G. CF-2

CKP-25

Krishna

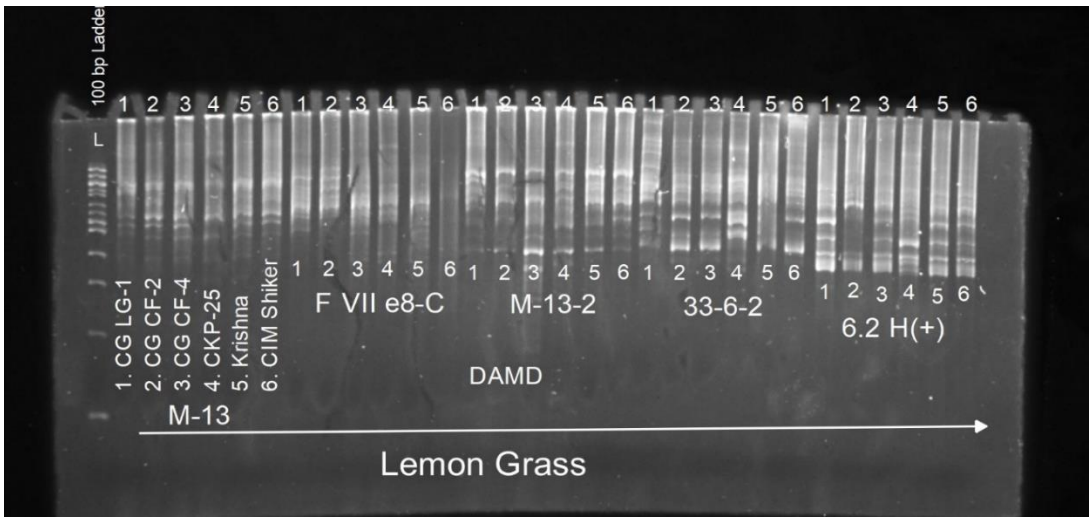


Fig 4: DAMD Profile for fingerprinting analysis in Lemongrass

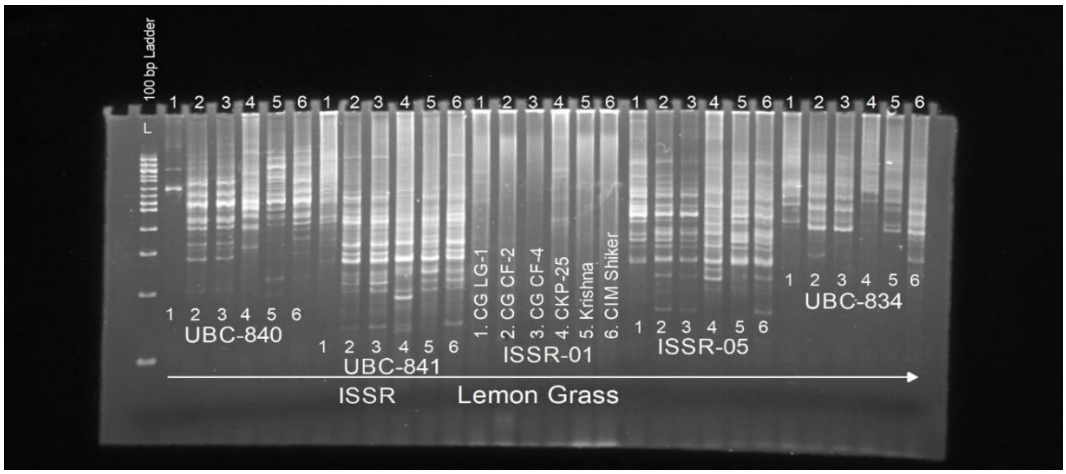


Fig 5: ISSR Profile for fingerprinting analysis in Lemongrass

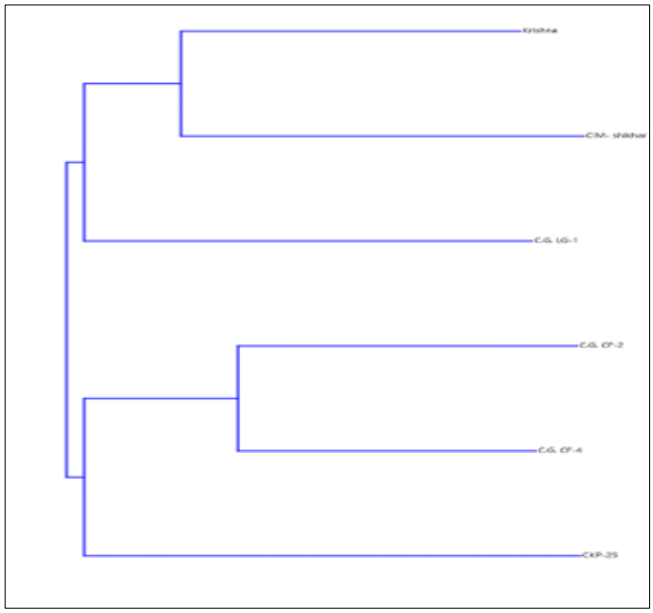


Fig 6: Dendogram of fingerprinting analysis of elite entries

Discussion

The ANOVA for all the traits found significant for most of the trait except for oil density. Analysis shows that enough variability is present in. the entries under study.

Apart from quantitative characters, qualitative parameters discriminates with each other in culm colour and Leaf colour. Maximum oil content (%) was reported in C.G. CF-4 (2.18%) followed by C.G. CF-2 (1.85) and CIM Shikhar (1.84). High oil density was acquired in C.G. CF-2 afterward CIM Shikhar and C.G. LG-1. In Gas Chromatography analysis highest citral percentage was recorded in Krishna subsequently in C.G. LG-1 and C.G. CF-4 (Table).

High PCV and GCV is reported by herbage yield, PCV is considerably higher than GCV which portrayed that environment prejudiced on the phenotypic traits of genotypes. Leaf blade length high heritability (90.05%) with genetic advance (GAM 5%) is also notable (57.51%), suggesting that selection for this trait could lead to substantial improvements in final yield. Due to their combination of high heritability, significant genetic variability, and substantial potential for genetic advance, these traits were indicating their importance in genetic improvement efforts.

Genotypic correlation refers to the statistical association between the genotypes of individuals for two or more traits. It indicates the degree to which the genes controlling one trait also control another. Phenotypic correlation measures the statistical association between the observable traits (phenotypes) of individuals for two or more traits. It reflects the degree to which variation in one trait is related to variation in another trait, regardless of the genetic or environmental factors influencing them.

Principal component with Eigen values superior than 1 expounded beyond 70% of the variation in the data for the PCA of yield traits. The PC_1 explicated 53% of the variation in the data and PC_1, PC_2 and PC_3 elucidated 90.27% of the variant in the data. Principal component one (PC_1) almost all traits contribute maximum in the variation peers and in PC_2 oil content % and oil density contributed for the variation created. Bi-plot for elite traits in C.G. CF-4 positioned near to axis suggesting that the genotype performed similarly and least amount of variation tested and very less stimulates by the environment.

Molecular analysis, DAMD and ISSR profiles revealed polymorphic patterns within the Lemongrass species, indicating genetic variability. The unique markers generated through DAMD and ISSR assays contribute to the understanding of genetic relationships and can aid in the selection of diverse breeding lines for improvement programs.

Conclusion

The analysis conducted on the morphological, DAMD, and ISSR profiles in Lemongrass presents significant insights into the genetic diversity and potential breeding strategies for this plant species.

Firstly, the morphological analysis revealed substantial variations among the treatments, indicating a significant impact of genetic and breeding factors on various parameters. In all genetic parameters, association and path analysis implies that oil yield is very important for aromatic plant. In conducted research C.G. CF-4 is the best outcomes for obtaining maximum oil percentage and justify with selected checks hence it holds the potential to become standard variety.

Future reference: In the future, the insights gained from this research hold promise for the advancement of Lemongrass breeding programs. Integration of morphological and molecular markers will enable more

precise selection of parental lines, accelerating the development of high-yielding and high-oil-content varieties. Further exploration of additional molecular markers and advanced genotyping technologies could enhance the resolution and efficiency of genetic characterization in Lemongrass.

Author Contribution

- **Amita Ekka:** Conceptualization, Methodology, Data Analysis, Writing - Original Draft.
- **Alice Tirkey:** Methodology, Supervision, Visualization, Project Administration.
- **Vivek Kumar Kujur:** Review & Editing.
- **Sunik Kumar Verma:** Methodolgy, molecular work analysis.

Conflict of interest: Author shows no conflict of interest.

Acknowledgement: The author acknowledge the Dr. M.K. Tiwari from SICART for Citral content analysis by conducting GC methodology for research work.

Table 6: Analysis of variance

Source	DF	Plamt height	Culm length	Culm diameter	Leaf blade length	Leaf blade width	Leaf area index	Total number of tillers per plant	Number of leaves per culm	Herbage yield	Oil content(%)	Oil density (g/cm^3)	Citral content (%)
Replication	3	159.21	6.94	0.01	162.79	0.01	0.01	51.67	1.26	15008.33	0.52	0.02	3.12
Treatment	5	4810.43**	342.32**	0.242**	3135.89**	0.84**	0.78**	1045.16**	2.0417**	71729.17**	1.17**	0.0004	271.70**
Error	15	215.45	5.36	0.02	84.32	0.02	0.02	96.83	0.33	9767.50	0.05	0.0010	0.60

Table 7: Genetic Parameters for quantitative trait

Traits	Mean	GCV	PCV	h^2	GAM 5%
PH	125.19	27.07	29.5	84.21	51.18%
CL	20.89	43.94	45.32	94.02	87.77%
CD	1.17	20.19	23.6	73.02	35.59%
LBL	93.88	29.42	31	90.05	57.51%
LBW	1.46	31.04	32.23	92.77	61.59%
LAI	1.15	38.11	40.27	89.56	74.29%
TONT/P	39.17	39.31	46.66	71	68.24
NOL/C	4.71	13.89	18.5	56.41	21.49
HY	253.33	49.13	62.73	61.33	79.26
OC (%)	1.53	34.54	37.69	83.98	65.2
OD	1.6	1.08	1.84	15.11	0.57
CC (%)	74.56	11.01	11.06	99.12	22.59

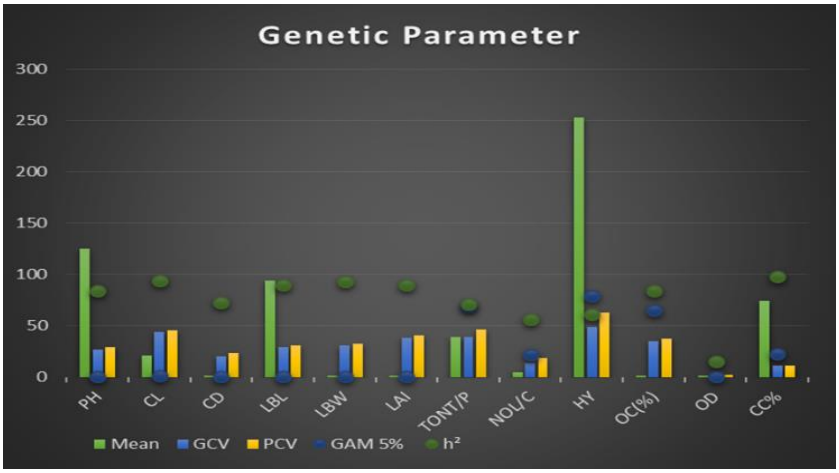


Fig 7: Graphic illustration of genetic variables

Table 8a: Genotypic Correlation for herbage yield

	PH	CL	CD	LBL	LBW	LAI	TONT/P	NOL/C	OC(%)	OD	CC(%)	HY
PH	1	0.727 **	0.7096 **	0.8098 **	0.8521 **	0.8004 **	0.1769	0.1353	-0.1578	-0.1752	0.1104	0.6574 **
CL		1	0.4299 *	0.7436 **	0.71 **	0.8743 **	0.4985 *	0.2878	-0.5279 **	-0.0941	0.5251 **	0.5748 **
CD			1	0.6666 **	0.8021 **	0.5268 **	0.3179	0.2837	-0.0378	-0.2157	-0.0901	0.6507 **
LBL				1	0.9197 **	0.8252 **	0.4516 *	0.1776	-0.203	-0.1244	0.1449	0.755 **
LBW					1	0.7534 **	0.5186 **	0.2364	-0.2837	-0.252	0.1756	0.8129 **
LAI						1	0.3549	0.3877	-0.3343	-0.1927	0.4216 *	0.593 **
TONT/P							1	0.3535	-0.7442 **	-0.0877	0.5701 **	0.5831 **
NOL/C								1	-0.3149	-0.2045	0.6124 **	0.3727
OC (%)									1	0	-0.8191 **	0.5258 **
OD										1	-0.0826	-0.1687
CC (%)											1	0.4152 *
HY												1

Table 8b: Phenotypic Correlation of herbage yield and its attributing traits

	PH	CL	CD	LBL	LBW	LAI	TONT/P	NOL/C	OC(%)	OD	CC(%)	HY
PH	1	0.8267 *	0.9 *	0.8771 *	0.891 *	0.9547 **	0.2996	0.3144	-0.179	-0.5016	0.1151	0.7401
CL		1	0.4613	0.7964	0.7634	0.9496 **	0.6026	0.2968	-0.6182	-0.2002	0.5469	0.72
CD			1	0.818 *	0.9073 *	0.6922	0.2836	0.2473	-0.0269	-0.6947	-0.1057	0.8454 *
LBL				1	0.9906 **	0.8867 *	0.5698	0.2717	-0.206	-0.4843	0.1534	0.8281 *
LBW					1	0.8831 *	0.6195	0.3458	-0.283	-0.4981	0.1793	0.9351 **
LAI						1	0.4734	0.509	-0.4224	-0.4869	0.4561	0.8464 *
TONT/P							1	0.3313	-0.8446 *	-0.0671	0.6546	0.8466 *
NOL/C								1	-0.5222	-0.8846 *	0.7945	0.7558
OC (%)									1	0.0193	-0.8999 *	0.5762
OD										1	-0.2619	-0.7174
CC (%)											1	0.5294
HY												1

Table 9: Eigen Values

PC group	eigenvalue	percentage of variance	cumulative percentage of variance
PC1	6.461	53.84	53.84
PC2	3.06	25.502	79.342
PC3	1.311	10.929	90.271
PC4	0.865	7.205	97.476
PC5	0.303	2.524	100

Table 10: Percent contribution of variables on PCs

Variables	PC1	PC2	PC3	PC4	PC5
PH	11.085	7.59	0.419	3.737	4.53
CL	11.542	0.067	3.998	22.476	1.802
CD	7.743	10.977	2.203	7.17	24.073
LBL	11.878	5.414	0.53	1.563	15.335
LBW	13.481	3.287	0.087	2.218	2.689
LAI	13.218	0.075	2.08	13.462	0.012
TONT.P	6.575	5.46	19.227	14.756	9.372
NOL.C	3.186	11.529	31.574	0.889	6.463
HY	11.133	0.255	0.019	31.475	0.171
OC…	4.504	12.157	21.25	0.228	18.602
OD	1.713	19.891	18.237	0.262	12.938
CC.	3.942	23.297	0.377	1.763	4.013

Table 11: Passport Data of collected germplasm

S. No.	Collector No.	Sample type	Sampling method	Habitat	Site of collection				Latitude (N)	Longitude (E)	Altitude (m)	Ethnobotanical information/ Ethnic group	Remarks (Trait-specific characters)
					Village	Mandal/Taluk / Tehsil	District	State					
1	C.G. CF-2	Population	Random	Partially distributed	Savitri Nagar	Raigarh	Raigarh	C.G.	21.53.83	83.23.55	215	Lemongrass	average height,good oil yielding, tall stature
2	C.G. CF-4	Single	Random	Single	Basmuda	Kharsia Road	Raigarh	C.G.	22.0.46	83.7.13	248	Lemongrass	Tall stature, High oil yielding

References

1. Abdelaziz SM, Medraoui L, Alami M, Pakhrou O, Makkaoui M, Ould Mohamed Salem Boukhary A, *et al*. Inter simple sequence repeat markers to assess genetic diversity of the desert date (*Balanites aegyptiaca* Del.) for Sahelian ecosystem restoration. Scientific Reports, 2020, 10(1). Available from: https://doi.org/10.1038/s41598-020-71835-9.
2. 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. Molecular Biology Reports. 2012;39:3815-3820.
3. Amiteye S. Basic concepts and methodologies of DNA marker systems in plant molecular breeding. Heliyon, 2021, 7(10). Available from: https://doi.org/10.1016/j.heliyon.2021.e08093
4. Anand KK, Srivastava RK, Chaudhary LB, Singh AK. Delimitation of species of the Astragalus rhizanthus complex (Fabaceae) using molecular markers RAPD, ISSR and DAMD. Taiwania. 2010;55(3):197-207. Available from: http://dx.doi.org/10.6165/tai.2010.55(3).197
5. Bhattacharya E, Ranade SA. Molecular distinction amongst varieties of mulberry using RAPD and DAMD profiles. BMC Plant Biology. 2001;1(3):3.
6. Doyle JJ, Doyle JL. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin. 1987;19:11-15.
7. Ekka A, Tirkey A, Rathia KS, Kaushal A, Ameen G, Kukur VK, *et al*. Variability assessment and response of water stress and intensity of anti-fungal activity in selected accessions of Lemongrass (*Cymbopogon*

flexuosus). In: National Seminar on Spices and Aromatic Crops, 2023, 77.
8. Gopinath PP, Parsad R, Joseph B, Adarsh VS. GRAPES: General Rshiny Based Analysis Platform Empowered by Statistics; c2020. Available from: 10.5281/zenodo.4923220
9. Husain A. Essential oil plants and their cultivation. Journal of Medicinal and Aromatic Plants. 1994;25:5-7.
10. Joy P, Mathew S, Skaria B, Mathew G, Joseph A, Sreevidya P, *et al*. Lemongrass Aromatic and Medicinal Plants Research Station. Odakkali, Asamannoor, Kerala, India, 2006, 32.
11. Lewinsohn E, Dudai N, Tadmor Y, Katzir I, Ravid U, Putievsky E, *et al*. Histochemical localization of citral accumulation in lemongrass leaves (*Cymbopogon* citratus (DC) stapf. Poaceae). Annals of Botany. 1998;81:35-39.
12. Kasaian J, Behravan J, Hassany M, Emami SA, Shahriari F, Khayyat MH, *et al*. Molecular characterization and RAPD analysis of Juniperus species from Iran. Genetics and Molecular Research. 2011;10:1069-1074.
13. Khanuja SPS, Shasany AK, Pawar A, Lal RK, Darokar MP, Naqvi AA, *et al*. Essential oil constituents and RAPD markers to establish species relationship in *Cymbopogon* Spreng, (Poaceae). Biochemical Systematics and Ecology. 2005;33:171-186.
14. Mathews S, Spangler RE, Mason-Gamer RJ, Kellogg EA. Phylogeny of Andropogoneae inferred from phytochrome B, GBSSI, and ndhF. International Journal of Plant Sciences. 2002;163(3):441-450.
15. Obaleye OE, Adeyemo OA, Osibote EA, Ibrahim OM. Chemical analysis of essential oil and genetic diversity using ISSR and SSR markers in cultivated lemongrass (*Cymbopogon citratus*) accessions. African Scientist. 2023;24(1):63-72.
16. Paterson AH. Genome mapping in plants. Academic Press, 1996, 330.
17. Pomper KW, Crabtree SB, Brown SP, Jones SC, Bonney TM. Assessment of genetic diversity of pawpaw (*Asimina triloba*) cultivars with inter simple sequence repeat markers. Journal of the American Society for Horticultural Science. 2003;128:521-525.
18. Prasad MP, Shekhar S. Antimicrobial activity of *Cymbopogon* species against human pathogens and molecular characterization by RAPD markers. Asian Journal of Biology and Life Sciences. 2013;2:13-18.
19. Ranade SA, Farooqui N. Assessment of profile variations amongst provenances of Neem using single-primer-amplification-reaction (SPAR) techniques. Molecular Biology Today. 2002;3(1):1-10.
20. Rathia SK, Tirkey A, Kashyap D, Ameen G. Genetic control and phenotypic variability in morphological characters in *Cymbopogon flexuosus*. The Pharma Innovation Journal. 2022;11(7):50-54.
21. Saikia D, Dutta S, Ghosh S, Lal M, Bhau BS. RAPD and ISSR based intra-specific molecular genetic diversity analysis of *Cymbopogon flexuosus* L. Stapf with a distinct correlation of morpho-chemical observations. Research Journal of Biotechnology. 2015;10(7):105-113.
22. Sarma A, Sarma H, Sarma TC, Handique K. Screening of essential oil obtained from inflorescence of lemongrass [*Cymbopogon flexuosus* (Nees ex Steud.) Wats] accessions. Indian Journal of Natural Products and Resources. 2011;2:236-241.
23. Seyedimoradi H, Talebi R, Hassani D, Karami F. Comparative genetic diversity analysis in Iranian local grapevine cultivars using ISSR and DAMD molecular markers. Environmental and Experimental Biology. 2012;10:125-132.
24. Singh OP, Singh TP. Genetic variability among some genotypes for morphological characters in lemongrass (*Cymbopogon flexuosus* L. Stapf). Indian Perfumer. 1999;43:35-36.
25. Singh SP, Singh HP, Singh AK, Tiwari RK. Genetic variability and character association in lemongrass (*Cymbopogon flexuosus* L. Stapf). Journal of Spices and Aromatic Crops. 2005;14:155-157.
26. Tajidin NE. Chemical composition and citral content in lemongrass (*Cymbopogon citratus*) essential oil at three maturity stages. African Journal of Biotechnology, 2012, 11.