



Diversity analysis and evaluation of lemongrass (*Cymbopogon flexuosus*) accessions for morphological, oil yield and oil composition traits under different fertility levels for Uttarakhand region

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ABSTRACT

Lemongrass (*Cymbopogon flexuosus*) is a highly valuable aromatic and therapeutic herb, well known for its essential oil content. The present study was carried out during 2021–2023 at Centre for Aromatic Plants, Selaqui, Dehradun, Uttarakhand to analyse the diversity of various lemongrass accessions collected from Uttarakhand for their morphological traits, oil yield, and oil composition traits. These various lemongrass accessions were further evaluated in a randomized complete block design (RCBD) with three replications under different fertility levels for their agromorphological performance. Yield components such as herb yield and essential oil yield, including oil composition, were also determined. Statistical analysis and genetic parameters indicated significant variations among lemongrass accessions for the majority of morphological and yield traits. Accessions AC-16 and AC-37 performed superiorly for traits such as plant height, leaf length, and width, whereas accessions AC-51 and AC-37 showed higher herb and oil yields under an increased dose of fertilizer. The accessions showed wide variability with respect to oil composition; from our study, we identified each of the three citral-rich (AC-16, AC-37, and AC-51) and geraniol-rich (AC-20, AC-71, and AC-65) accessions. The better-performing accessions for morphological, yield and oil composition related traits may also be used in breeding programmes as potential genetic stock.

Keywords: *Cymbopogon flexuosus*, Citral, Fertility levels, Geraniol, Morphological traits

Lemongrass (*Cymbopogon flexuosus*) is a perennial grass that belongs to the genus *Cymbopogon* and comes under Poaceae family. Within the genus *Cymbopogon*, there are approximately 102–104 species; among them, 45 species have been reported in India (Verma *et al.* 2018). The most commonly cultivated and economic species of *Cymbopogon* are *C. flexuosus*, *C. citratus*, *C. winterianus*, *C. martini* var. *motia* and *sofia*, and *C. nardus* var. *nardus* (Yogendra *et al.* 2021). Among these, *C. flexuosus* is a popular one, widely grown for its aromatic oil, which contains a substantial amount of citral and other bioactive compounds. *C. flexuosus* is primarily grown in the tropical and subtropical regions of America, Asia, and Africa, ranging from grasslands and mountains to arid areas (Mwithiga *et al.* 2022). Lemongrass stands as a versatile herb, revered for its numerous applications in pharmaceuticals, cosmetics, and culinary practices due to its essential oils (Akhila 2009, Francisco *et al.* 2011). The leaves of lemongrass plant are commonly used in preparing herbal tea and various culinary purposes.

The antimicrobial substances present in lemongrass exhibit significant efficacy in preventing or eliminating pathogenic microorganisms, even at relatively low concentrations (Paramita *et al.* 2014, Bhagobaty and Borkataky 2021). Furthermore, certain sectors utilize lemongrass essential oil as a sustainable option for pesticides.

Growing essential oil-bearing crops, including lemongrass, offers a more useful and sustainable method of obtaining naturally derived raw materials for both domestic and industrial uses (Carrubba and Catalano 2009). Many researchers have been aiming to improve both the quantity and quality of oil in various kinds of soil in response to the increasing demand for lemongrass oil. Lemongrass grows in a variety of soil types and geographical areas, and its productivity is influenced by environmental factors including soil, temperature, rainfall, and light intensity, as well as genetic potential. The application of additional fertilizer can boost lemongrass dry biomass (Zheljazkov *et al.* 2011). The most important feature of every population's characteristic is its variability. The amount, kind, and magnitude of variability must be estimated in order to realize the response to selection. Variability determines the direction of the breeding process, which helps understand the nature

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of the population and the type of breeding activity carried out to improve its genetic potential. Hence, the objective of the current study was to select the best lemongrass accession by comparing and evaluating their morphological characteristics, oil yield, and oil composition under different fertilizer doses in rainfed areas of Uttarakhand.

MATERIALS AND METHODS

The present study was carried out during 2021–2023 at Centre for Aromatic Plants, Selaqui (30° 20' 7.69" N and 78° 2' 44.38" E; altitude of 683 m amsl), Dehradun, Uttarakhand. The location received an average rainfall of 1181 mm annually.

Experimental details: Initially, the seeds were collected from different farmer's fields in the Pauri region of Uttarakhand and for initial evaluation total 72 lines were generated. From these 11 accessions were selected and brought to the Centre for Aromatic Plants, Selaqui, Dehradun, Uttarakhand for detailed evaluation. The experiment was laid out in a randomized complete block design (RCBD) under rainfed conditions in three replications, comprised of 11 different lemongrass accessions along with check CIM-Shikhar. The experimental plots measured 3.0 m × 4.5 m, with a spacing of 60 cm × 45 cm between rows and plants, respectively. The accessions were evaluated for various morphological traits, herb yield, oil yield, and composition across three different fertility levels. These levels included the control (F_0) fertilizer dose of 120:80:60 NPK, with F_1 receiving 150% of the control dose and F_2 receiving 75% of the control dose.

Observations: The morphological observations recorded for the traits, viz. plant height (PH), leaf length (LL), leaf width (LW), circumference (CC), and number of slips/plant (NSL). The yield parameters, such as herbage yield (HY) and essential oil yield (OY), were determined by calculating them using the net plot yield and then converting the results to quintals per hectare, whereas oil-related traits such as oil content (OC), geraniol (GL), and citral (CL) components were expressed in percentage. Observations were documented on three randomly chosen plants from each accession.

Analytical methods

Essential oil extraction: Oil extraction was performed using hydro-distillation method with 250 g of fresh leaves from each accession of lemongrass, and this process was replicated three times utilizing the Clevenger apparatus. The condensed essential oil is gathered in the form of droplets in a sealed tube connected to the Clevenger apparatus. The separation of the aqueous layer from the essential oil was achieved using a separating funnel. After being dehydrated with anhydrous Na_2SO_4 , the oil samples were stored in a cold, dark condition until further examination. The oil content was determined as the average of three samples, expressed as a percentage of the total weight. The oil yield was calculated based on the fresh weight of the material, using the ratio of volume to weight (v/w) (Kulkarni *et al.* 2003).

Gas chromatographic (GC) analysis: GC analysis was carried out using an Agilent 7890B gas chromatograph with a flame ionization detector (FID). An Agilent HP-5 column with a 30 m length, 320 μm internal diameter, and 0.25 μm film thickness was utilized for the separation process. The samples were injected into a split/splitless inlet at a temperature of 250°C, employing a split ratio of 1:35. Nitrogen served as the carrier gas with a consistent flow rate of 2 ml/min. The column oven temperature was programmed to start at 60°C and rise at a rate of 3°C/min until reaching 240°C, where it was maintained for two minutes. The temperature of the FID was maintained at 280°C.

Statistical analysis: Calculation of genetic parameters and analysis of variance (ANOVA) was carried out using OPSTAT in data obtained from morphological and agronomical evaluations of germplasms. The principal component analysis (PCA) and dendrogram constructed using R programme. Descriptive statistics such as means, standard deviations, and ranges were calculated for each trait. ANOVA was performed to assess the significance of differences among accessions for each trait. Statistically significant lines with distinct morphological and yield traits were identified.

RESULTS AND DISCUSSION

Morphological characterization: The morphological characters of lemongrass accessions showed wide variability with respect to traits like plant growth habit, stalk colour, and leaf character. However, traits like leaf sheath colour, leaf blade surface and leaf blade margin remained consistent across all accessions, displaying purplish-green, hairy, and acute features, respectively. On maturity, the elongated leaf sheath appears leathery-purplish near the culm. Accessions AC-10, AC-18, AC-20, AC-37, AC-51, AC-65, and AC-71 exhibited an erect growth habit similar to CIM-Shikhar, while accessions AC-16, AC-26, AC-29, and AC-72 showed a semi-erect habit. These variations among different traits are attributed to genetic segregation and collections from diverse regions of Uttarakhand. The qualitative trait of stalk colour was categorized into three types: yellowish-green (Check, AC-16, AC-26, AC-37, and AC-72), purplish-green (AC-10, AC-18, AC-20, AC-51), and green (AC-29, AC-65, AC-71). Leaf character showed two types of variation: broad leaves (Check, AC-16, AC-26, AC-29, AC-37, and AC-51) and narrow leaves (AC-10, AC-18, AC-20, AC-65, AC-71, and AC-72). These observations align with previous studies that also reported significant morphological diversity among lemongrass accessions due to their varied genetic backgrounds and ecological adaptations (Joy *et al.* 2006, Susilowati and Syukur 2022). The visual characteristics of the lemongrass accessions including the Royal Horticultural Society (RHS) colour codes for precise identification are shown in Table 1.

Selection for traits such as plant height, leaf length, leaf width, and number of tillers is polygenic in nature and shows complex inheritance with relatively low heritability and high environmental influence (Dinkar 2013, Das and

Table 1 Lemongrass accessions characters

Accession	Stem type (Habitat)	RHS colour code (Stalk)	RGB code (Stalk)	Leaf character
Check	Erect	RHS 144A	RGB (77, 119, 42)	Broad leaf
AC-10	Erect	RHS 137A	RGB (77, 119, 42)	Narrow leaf
AC-16	Semi-Erect	RHS 144A	RGB (77, 119, 42)	Broad leaf
AC-18	Erect	RHS 137A	RGB (77, 119, 42)	Narrow leaf
AC-20	Erect	RHS 137A	RGB (77, 119, 42)	Narrow leaf
AC-26	Semi-Erect	RHS 144A	RGB (77, 119, 42)	Broad leaf
AC-29	Semi-Erect	RHS 137B	RGB (77, 119, 42)	Broad leaf
AC-37	Erect	RHS 144A	RGB (77, 119, 42)	Broad leaf
AC-51	Erect	RHS 137A	RGB (77, 119, 42)	Broad leaf
AC-65	Erect	RHS 137B	RGB (77, 119, 42)	Narrow leaf
AC-71	Erect	RHS 137B	RGB (77, 119, 42)	Narrow leaf
AC-72	Semi-Erect	RHS 144A	RGB (77, 119, 42)	Narrow leaf

RHS, Royal Horticultural Society; RGB, Red Green Blue.

Sharma 2023). The phenotypic evaluation of morphological traits was performed to study genetic basis of variation. ANOVA demonstrated significant differences among accessions for various traits, leading to the identification of lines with desirable morphological traits and high yield. The Broad Sense Heritability (BSH) estimates were high for most traits, with values ranging from 79.01% for HY to 92.07% for CL, indicating a strong genetic influence on these traits. The genetic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were also substantial, with GCV ranging from 57.51% for HY to 65.98% for CL, and PCV from 64.70% for HY to 69.25% for CC. The genetic advance (GA) values were consistently high across traits, with the lowest being 79.62 for HY and the highest being 94.98 for OC. These results suggest that

there is significant genetic potential for improving these traits through selection (Table 2).

The dendrogram, based on different morphological and yield-related traits, illustrates the relationships among the lemongrass accessions (Fig. 1). A dendrogram-based analysis of genetic variation derived from morphological characteristics revealed that accessions were split into four groups with a similarity level of roughly 80%. Group I consisted of AC-10, AC-18, and AC-72; Group II included AC-26, Check, and AC-29; Group III included AC-71, AC-20, and AC-65; and Group IV consisted of AC-51, AC-16, and AC-37. Based on the analysis, Group III includes accessions with geraniol-rich compounds, whereas Group IV consists of accessions with citral-rich compounds. These findings are consistent with previous studies that have demonstrated significant genetic diversity and chemical composition variation among lemongrass accessions (Obaleye *et al.* 2023).

The principal component 1 explained 55.40% of the total proportion of variance, while the principal component 2 explained 24.01% of the total proportion of variance. The PC1 and PC2 together explained 79.41 % of the variance, while the remaining 8 PCs explained 20.59% (Fig. 2A). The SDs of PC1 and PC2 are 2.35 and 1.55, respectively, which is greater than one and hence significant. The genotypes were scattered around the biplot, depicting the variation and diversity between individuals. The acute angle between two vectors indicates a positive correlation, while the obtuse angle indicates a negative correlation between the traits (Fig. 2B). The traits PH, LL, NSL, OY, and OC contribute most to PC1, while GL contributes most to PC2 (Fig. 2C). These findings are consistent with previous studies on lemongrass, which also highlighted the significant impact of these traits on overall variance and their contribution to genetic diversity (Yogendra *et al.* 2021).

Further, these 11 different accessions were grown under different fertility levels to check performance analysis and the observed data for different traits was analysed statistically. The statistical analysis of data between different accessions indicates that a significant effect on morphological traits is mainly due to the genetic potential of accessions and can also be affected by environmental factors like soil fertility, nutrient uptake during the cropping period, temperature, water, and sunlight (Joy *et al.* 2006, Dagar *et al.* 2013). Analysis of variance for all 10 traits showed the accessions had significant variation among the accessions as well as

Table 2 Genetic parameters of lemongrass accessions

	PH	LL	LW	CC	NSL	HY	OY	OC	GL	CL
BSH	89.75	88.89	89.26	89.99	80.12	79.01	91.48	91.08	90.52	92.07
GCV	64.96	64.82	65.23	65.69	58.19	57.51	65.95	65.94	65.80	65.98
PCV	68.57	68.75	69.04	69.25	65.01	64.70	68.95	69.10	69.16	68.76
GA	90.83	91.33	93.76	94.15	81.89	79.62	94.98	94.50	92.44	92.25

PH, Plant height; LL, Leaf length; LW, Leaf width; CC, Circumference; NSL, Number of slips/plant; HY, Herbage yield; OY, Essential oil yield; OC, Oil content; GL, Geraniol; CL, Citral; BSH, Broad sense heritability (%); GCV, Genetic coefficient of variation (%); PCV, Phenotypic coefficient of variation (%); GA, Genetic advance.

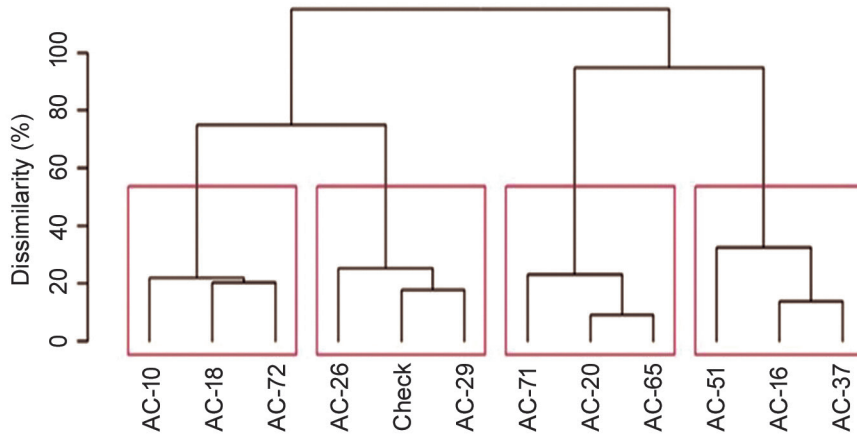


Fig. 1 Dendrogram of 12 lemongrass accessions based on morphology and oil related traits. Legends: In X-axis Accessions and in Y-axis Dissimilarity (%).

under different fertility levels of application. ANOVA of all 10 traits for two factors, such as accessions as well as fertilizer doses, is presented in Supplementary Table 1. The accession AC-16 showed higher plant height under control and increased fertilizer level (127.5 and 130.1 cm) among all other accessions; however, the same accession showed a reduced plant height of 101.5cm at reduced fertilizer level (F₂). All accessions recorded higher plant heights compared to check variety CIM-Shikhar plant heights of 102.3 cm and 107.8 cm under recommended and increased fertilizer levels. Similarly, in the case of leaf length, the average length was about 96 cm, 101 cm, and 94.78 cm across the 3 fertility levels, F₀, F₁, and F₂, respectively. The accession AC-37 recorded higher leaf lengths of 100.1 and 107.5 cm at F₀ and F₁, respectively. The mean leaf width of the accessions

at different fertility levels is 1.15, 1.27, and 1.33 cm, respectively, and among these, AC-37 showed the highest leaf width among all the accessions. The leaf width is the quantitative trait that showed higher variation among the accessions; however, accessions do not show any significant variation between different fertility levels. The accessions AC-51 and AC-16 recorded higher herbage yield under control and F₁ fertility levels. For traits like circumference, the AC-20 and AC-51 showed a higher circumference of 112 and 110cm at a higher than recommended dose of fertilizer. The number of slips per accessions varied

among different accessions as well as fertilizer doses; however, the accessions AC-65 and AC-16 showed a higher number compared to other accessions at the F₁ dose of fertilizer (Table 3 and Supplementary Table 2). The variation in the number of slips primarily arises from differences in the inherited traits of the genotypes and fluctuations in environmental conditions (Singh and Singh 1999, Sharma *et al.* 2005 and Yogendra *et al.* 2021). Similarly, the observed results align with the findings of Lal *et al.* (2001), which reported that plant height ranged from 100–160 cm and the number of tillers/plant ranged from 45–65 in four superior clones of lemongrass.

The variation in lemongrass accessions and their specific yield-related traits resulted in a significant difference in both herbage yield and oil production. The yield of herbage varies

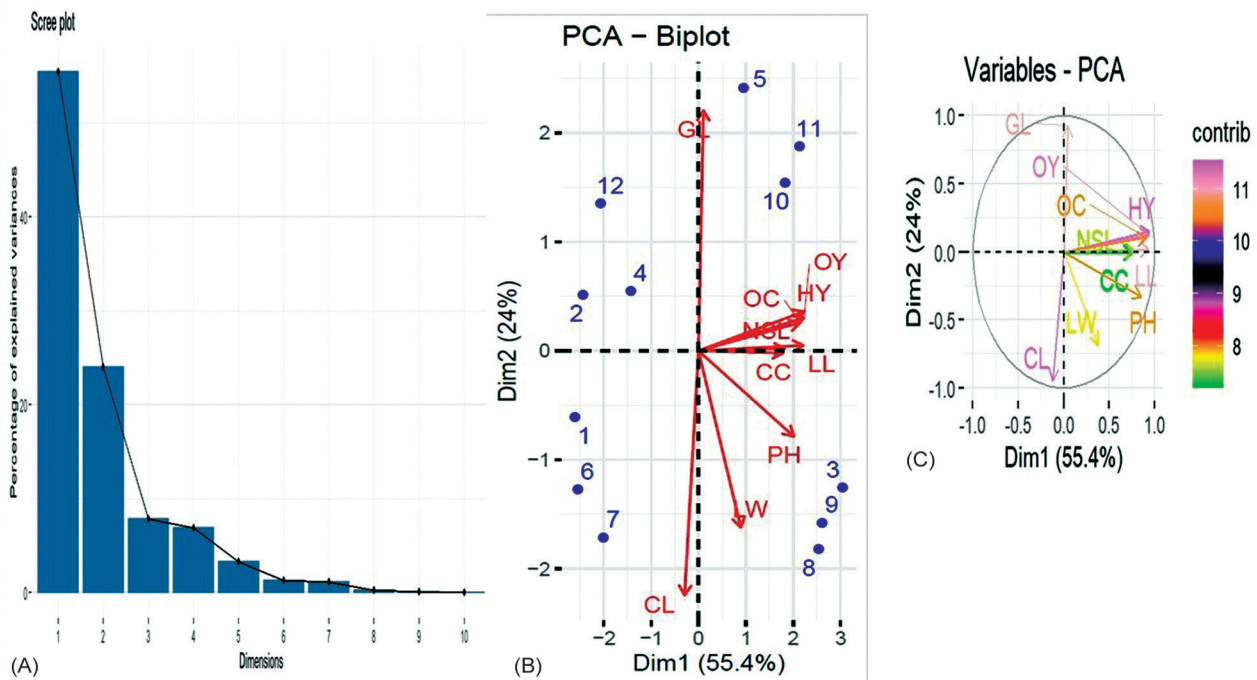


Fig. 2 Scree plot, Biplot and variables to explain morphological diversity using PCA. CL, Citral; LW, Leaf width; PH, Plant height; CC, Circumference; LL, Leaf length; NSL, Number of slips/plant; OC, Oil content; HY, Herbage yield; OY, Essential oil yield; GL, Geraniol.

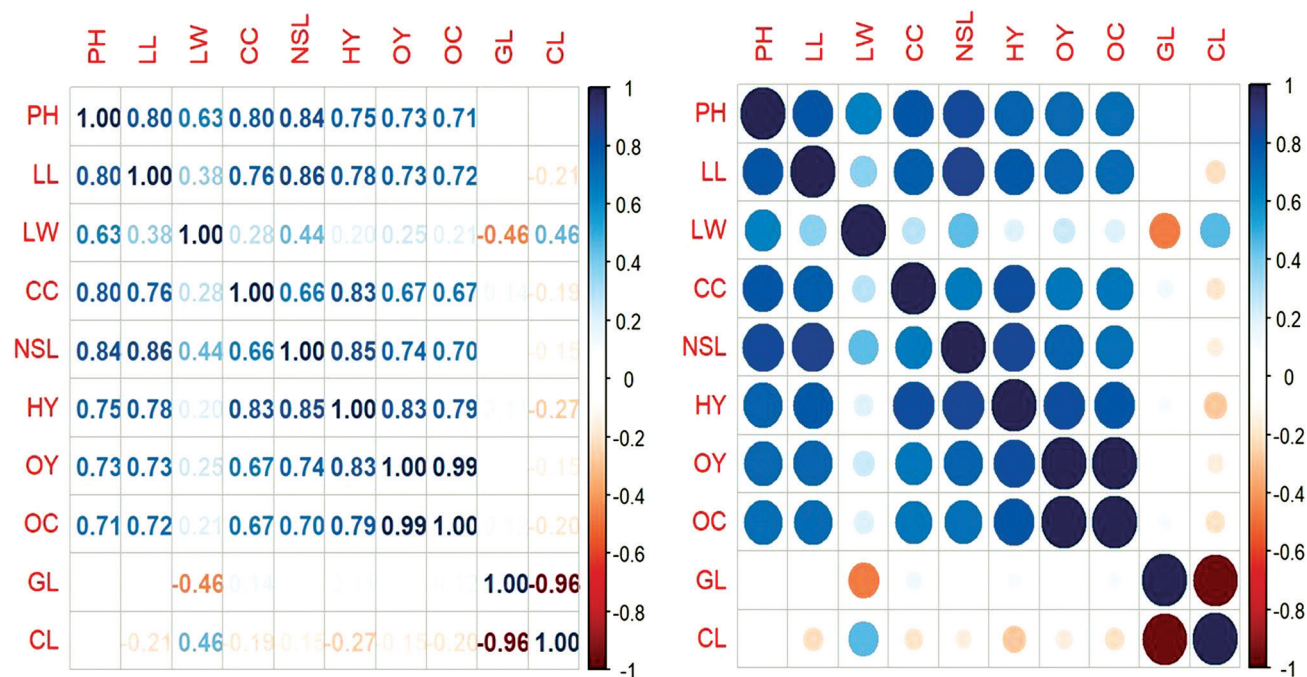


Fig. 3 Correlation study of 12 lemongrass accessions based on morphological characters. PH, Plant height; LL, Leaf length; LW, Leaf width; CC, Circumference; NSL, Number of slips/plant; HY, Herbage yield; OY, Essential oil yield; OC, Oil content; GL, Geraniol; CL, Citral.

from 145.5 q/ha–182.9 q/ha, with an average yield of 162.48, 170.53, and 162.63 q/ha at 3 different fertilizer doses (F_0 , F_1 , and F_2), respectively. Among these, accessions AC-51 and AC-16 showed higher herbage yields of 182.9 q/ha and 180 q/ha, respectively, at the F_1 fertilizer dose. However, the accessions AC-26, AC-29, and AC-10 showed a lower herbage yield compared to the check yield of 164.9 q/ha. The correlation study depicted that trait such as plant height, leaf width, leaf length, and number of slips/plant are positively correlated with herb and oil yield; however, the morphological traits showed a positive correlation with herb yield and oil yield, whereas there was a negative correlation with oil content under increased fertilizer doses (Tripathi *et al.* 2017). The higher herbage yields in these accessions were attributed to improvements in correlated traits such as plant height, circumference, and number of slips (Fig. 3). Similar results observed in the experiment for higher herb yield with higher fertilizer levels might be due to better nutrient uptake and promoting vegetative growth (Kumar *et al.* 2023).

The oil yield of lemongrass is a complex trait that showed significant variation due to the to the study of different accessions and yield-contributing traits. The average oil yields were 189.23 kg/ha for F_0 , 214.76 kg/ha for F_1 and 173.78 kg/ha for F_2 . The corresponding variances were 10.756, 10.393, and 6.270, respectively. The corresponding variances were 10.756, 10.393, and 6.270. The highest average oil yield was observed under increased fertility (F_1), which surpassed the control (F_0) and the reduced fertility (F_2). Additionally, the variation in oil yield was least under high fertility (F_2). These findings suggest that increasing the fertility level leads to higher oil

yields, whereas reducing fertility results in lower yields. The increase in both herbage yield and essential oil yield may be attributed to the production of more slits per plant, increased plant height, and other yield-contributing traits. These results correlated with study conducted by Tripathi *et al.* (2017) on effect of fertility levels on yield of lemongrass under irrigated farming system. Similar findings observed in the study comprise a comparative morphological assessment of lemongrass cultivars for oil yield in the southern region of India (Yogendra *et al.* 2021).

The oil content in lemongrass accessions ranges from 0.8–1.4%, with the primary challenge being to achieve higher oil quality. The major components of lemongrass oil are citral and geraniol (Chauhan *et al.* 2017). Significant variability is observed in the essential oil content among accessions, particularly in geraniol and citral. Geraniol content ranges from 1.9–68.5%, with accessions AC-65, AC-20, and AC-71 showing higher levels; notably, AC-65 exhibited 61.7%, 68.5%, and 67.3% geraniol content at F_0 , F_1 , and F_2 fertility levels, respectively. Citral content ranges from 13–85.2%, with accessions AC-51, AC-29, and AC-26 demonstrating significant improvements; AC-26 achieved the highest citral content of 84.5% and 84.9% at F_1 and F_2 fertility levels, respectively. The GC profiles of citral and geraniol accessions were presented in Supplementary Fig. 1A and B, respectively. Genetic variations and environmental factors, or their combination, contribute to this variability (Allard 1960, Poehlman and Sleper 1995). Accessions with high citral content typically have lower geraniol levels, and vice versa, suggesting distinct production pathways for these compounds. This is consistent with findings by Kumari *et al.* (2009), who reported citral content of 72–75% in Indian

Table 3 Morphological traits of lemongrass accessions under different fertility levels

Acc. No.	Plant height (cm)			Leaf length (cm)			Leaf width (cm)			Circumference (cm)			No. of slips/plant		
	F ₀	F ₁	F ₂	F ₀	F ₁	F ₂	F ₀	F ₁	F ₂	F ₀	F ₁	F ₂	F ₀	F ₁	F ₂
Check	102.3	107.8	100.2	95.5	97.9	91.6	0.9	1.1	1.0	85.45	89.18	83.78	26	35	34
AC-10	115.3	115.2	102.3	92.5	96.4	92.3	1.4	1.2	1.4	83.9	100.6	78.5	32	34	29
AC-16	127.5	130.1	101.5	98.2	106.8	100.5	1.6	1.5	1.4	101.2	105.3	102.0	35	39	30
AC-18	106.9	114.9	100.9	96.2	96.8	93.7	0.9	1.2	1.3	98.6	100.2	94.2	28	38	27
AC-20	116.0	109.9	101.5	98.7	103.0	95.5	0.8	1.1	0.8	95.27	112.1	90.8	32	34	30
AC-26	111.2	116.4	109.6	90.2	91.56	93.1	1.2	1.2	1.6	90.6	109.3	89.6	27	30	24
AC-29	104.6	116.5	100.2	95.5	101.0	93.6	1.4	1.1	1.4	92.15	105.6	90.1	30	32	28
AC-37	119.5	129.8	111.9	100.1	107.5	97.36	1.6	1.6	1.4	98.02	104.6	89.9	31	35	33
AC-51	115.3	116.8	113.1	99.25	100.6	98.5	1.2	1.5	1.4	95.6	110.1	98.56	32	37	29
AC-65	118.5	124.2	108.4	99.96	106.6	94.44	0.8	1.4	1.6	97.3	104.6	96.5	30	40	27
AC-71	120.5	125.2	116.4	98.8	105.5	95.5	1.2	0.9	1.4	101.8	103.9	89.74	34	34	30
AC-72	106.6	109.9	100.5	95.6	99.4	91.2	0.8	1.4	1.2	94.6	98.2	92.6	29	32	25
Mean	113.68	116.39	110.79	96.71	101.09	94.78	1.15	1.27	1.33	94.54	103.64	91.36	30.50	35.0	28.83
CD (P=0.05)	4.743	4.484	5.727	4.190	3.534	2.995	0.058	0.061	0.059	3.987	5.233	3.698	1.768	1.651	1.072

lemongrass collections, and Lal *et al.* (2001), who observed 89% citral content in the SEG 49 clone. Ganjewala *et al.* (2008) also reported citral levels ranging from 82–88% in West Indian lemongrass, supporting the observed variability in citral content across different accessions.

The significant variation observed between different lemongrass accessions for different morphological, yield, and oil composition-related traits. The accessions that performed better over check and showed a higher mean for the majority of the traits were selected. Overall, five accessions (AC-51, AC-29, AC-26, AC-16 and AC-37) were rich in citral component, among these accessions AC-16, AC-37, and AC-51 recorded higher plant height, leaf width, and herb yield, along with being rich in citral oil, which was further evaluated under the citral rich trail. Similarly, the six accessions (AC-10, AC-18, AC-20, AC-71 and AC-72) were belonging to geraniol oil group, among these AC-20, AC-71, and AC-65 exhibit higher geraniol content and were selected for further evaluation in the geraniol-rich trail.

In the present study we evaluated 11 lemongrass accessions for morphological traits such as plant height, number of slips, and oil parameters such as oil yield and oil composition such as citral and geraniol. Among these, accessions AC-16 and AC-37 performed better for traits such as plant height, leaf length, and width, whereas accessions AC-51 and AC-37 showed higher herb and oil yields. The accessions AC-51, AC-29, and AC-26 recorded significant improvement in citral oil content. The accessions showed wide variability with respect to oil compositions such as citral and geraniol. In overall from our study, selected three citral rich accessions AC-16, AC-37 and AC-51 also showing improvement in other traits like herb and oil yield. In similar way identified three accessions AC-20, AC-71, and AC-65 showed geraniol rich compounds. These selected geraniol and citral-rich accessions can be further improved

and evaluated for agronomical superiority, adoptability, and oil parameters at different agro-climatic conditions. The better-performing accessions can be used as a potential genetic stock in an upcoming breeding program.

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