



Genetic variability and physicochemical characterization of essential oil in Lemongrass (*Cymbopogon* spp.)

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Abstract

This research analyses the level of genetic variability, heritability and relationships between important oil properties in lemongrass (*Cymbopogon* spp.) in semi-arid weather. Twelve genotypes were evaluated based on the content of essential oils, bit-carboxyl and geraniol capacity, and morphology. Genetic parameters such as the genotypic (s_2g) and phenotypic variance (s_2p) and the genotypic coefficients of variations (GC-V) and phenotypic coefficients of variations (PC V), broad-sense heritability (H₂) and the genetic advance (GA) were obtained to gain an insight into the possibilities of genetic gain. Hydrodistillation was used to examine the essential oil composition and quantified the product using a spectranalyzer, and the compositional validation and chemotype classifications were by the use of gas chromatography-mass spectrometry (GC-MS). The analysis of correlations and path analyses, found out that there are significant positive relationships between the citral content and the oil yield implying that high citral concentration could be selected with the objective of improving quality and quantity of oils. Principal component as well as cluster analysis distinguished the genotypes into three chemotypic groups. The results are very crucial in future breeding and selection of lemongrass varieties which are high-oiled and high-citral with adaptation to semi-arid ecosystems.

Keywords: Lemongrass, genetic variability, heritability, citral, GC-MS, chemotype, path analysis

Introduction

Lemongrass (*Cymbopogon* spp.) is a perennial, tufted aromatic grass widely cultivated across tropical and subtropical regions for its high-value essential oil, which serves as an important industrial raw material in multiple sectors including perfumery, cosmetics, flavoring, beverages, aromatherapy, and pharmaceuticals (Ashaq *et al.*, 2024) [2]. The essential oil is primarily composed of citral, a natural aldehydic compound consisting of two geometric isomers, geraniol (trans-citral) and neral (cis-citral), which together impart the characteristic strong lemon-like aroma (Gutiérrez-Pacheco *et al.*, 2023) [8]. Beyond its fragrance value, citral exhibits potent antimicrobial, anti-inflammatory, insecticidal, and antioxidant activities, making lemongrass oil a preferred ingredient in herbal therapeutics, natural preservatives, and eco-friendly bioproduct formulations (Gurjar *et al.*, 2024) [7]. With the global market witnessing a rapid transition toward plant-based bioactive compounds and sustainable fragrance ingredients, the demand for high-quality lemongrass oil has increased substantially in recent years. This growing industrial and pharmaceutical relevance has shifted the focus of contemporary *Cymbopogon* breeding programs toward improving essential oil yield and optimizing chemical composition (Mwithiga *et al.*, 2022) [11]. However, achieving consistent genetic gains in oil content and citral proportion requires a thorough understanding of the genetic variability, heritability patterns, and expected genetic advance for the key morphological, physiological, and biochemical traits that govern oil productivity and quality. Genetic variability constitutes the cornerstone of any successful crop improvement program, as it determines the extent of response to selection and the potential for genetic enhancement (Begna & Teressa, 2024) [3]. The trends of dissimilar essential oil yield, composition as well as

agronomic performance found among accessions within *Cymbopogon* are explained by genotypic diversity, environmental factors and large genotype x environment (GxE) interactions (Padalia *et al.*, 2021) [12]. This form of interaction tends to alter the pathway of biosynthesis and storage of the minor product including citral, geraniol, and limonene, thus affecting the quantity and quality of the oil. It is therefore essential to deconstruct the genetic variation and environmental variations in identifying stable and high-performance genotype by a variety of cultivation environments. QG parameters, including the genotypic coefficient of variation (GCV), the phenotypic coefficient of variation (PCV), the broad-sense heritability (H₂), and the genetic advance (GA) are used by them as indispensable quantitative genetic tools (Singh *et al.*, 2019) [14]. Although GCV and PCV help to understand the extent of genetic and total variability, heritability estimates suggest what percentage of the phenotypic variation is explained as the result of genetic factors (Madhuri *et al.*, 2024). High heritability with high genetic progress normally indicates that additive gene action is pre-eminent meaning that direct phenotypic selection would be effective in the enhancement of the genes. On the other hand, low heritability with low genetic advance suggests an absence of additive or epistatic genes effects and high levels of environmental modulation leading to the possibility of the recurrent selective pressures, hybridization or markers assisted breeding being more effective (de Oliveira *et al.*, 2023; Aburab *et al.*, 2020) [15]. Preceding studies have established between moderate and high amounts of heritability of traits which include content of essential oils, plant height, litter number, and citral rate in *Cymbopogon* germplasm, suggesting considerable genetic control and possibilities of selection (Munda *et al.*, 2021) [10]. But most of these studies have only addressed a small range of genotypes only or certain setting of the

environment hence giving to that full picture of the existing genetic variety. Global analyses of morphological, agronomic, and biochemical variability among multiple *Cymbopogon* accessions are necessary in terms of outlining the genetic structure and inter-relationship data behind the oil yield and quality (Gupta *et al.*, 2018; Jat *et al.*, 2025)^[6, 9]. In addition, the use of gas chromatography-mass spectrometry (GC-MS) in the profile of essential oils has created additional regimes in chemotype differentiation and classification on the basis of metabolites (Pankaj *et al.*, 2017)^[13]. However, there is little literature which correlates morphological variability, inter-trait correlations and oil compositional diversity in response to different agro-ecological conditions, especially semi-arid and stress-prone environment (Tabanca *et al.*, 2006)^[16]. Temperature, soil wetness, as well as nutrient availability has been shown to be an influential factor on the secondary metabolite production, which ultimately results in changes in the levels of citral and geraniol content (Dagar *et al.*, 2013; Skaria *et al.*, 2006)^[4, 15].

Therefore, identifying genotypes with stable performance and desirable chemotypic expression across environments remains a critical step toward the development of elite, high-oil-yielding cultivars suitable for industrial exploitation.

Additionally, despite the wide distribution of *Cymbopogon* species, the genetic basis of correlations among morphological descriptors (plant height, tiller density, leaf area), biomass production, and oil quality traits is not fully understood. Such knowledge is vital for developing selection indices that combine agronomic vigor with chemical excellence. The current study has hence been formulated to fill these gaps by incorporating the use of an integrated exploration comprising of genetic, biochemical, and chemometric techniques. The significant aims of this work are the following:

1. To measure the degree of genetic variation, heritability (broad sense) and expected genetic progress of the essential oil content and its main constituents especially citral and geraniol.
2. To determine the genotypic and phenotypic correlation of significant morphological, yield-related, and biochemical traits in an attempt to determine the selection indices in genetic enhancement.
3. To chemically profile the composition of the essential oils using GC-MS profiling and to distinguish the analyzed genotypes by using multivariate statistical techniques including cluster and principal component analysis basing on the proportions of citral and geraniol.

This detailed characterization should be useful in determining insights on genetic architecture of the essential oil traits and develops the identification of promising lemongrass genotypes to use in breeding programs in an effort to produce high-yield and quality-improved phenotypes capable of surviving in a wide range of growing conditions.

Materials and Methods

1. Plant Material and Experimental Design

Twelve genetically distinct lemongrass (*Cymbopogon* spp.) genotypes, designated as LG-1 to LG-12, were evaluated in this study. The experimental trial location was a Aromatic

Crop Research Farm, Bundelkhand, which is located in a semi-arid agroclimatic environment with hot summers, moderate winters with a rainfall of about 800-1000 mm mainly in the monsoon season. The experimental site had a sandy loam soil, slightly alkaline (pH 7.5-8.0) in its reaction, and moderately fertile possessing sufficient organic carbon and available nutrients. Land preparation before the start of planting, deep-ploughing was carried followed by harrowing and blanketing to ensure that the field was in good till and even. The experiment was planned as an experiment with the type of complete block design (RCBD) and was carried out three times in order to minimize the influence of the environment and give more indicative information about the performance of the genotype. Each experimental plot had 4 rows spaced 60 cm apart and delimit one another with 45 cm spacing of the plants covered with healthy uniform slips which had been transplanted just prior to the commencement of the season. There was an equal amount of recommended agronomic practices (e.g. irrigation schedule, weeding, earthing-up and pest management) in the plots. Parameters of growth (i.e., e.g. height of the plant, the nb. of tillers and nb. of leaf area was measured periodically with the interval of assessment of the genotypes and compared with genotypes to estimate the difference in morphology in the genotypes. The measurements of the environmental data of the site of trial in terms of temperature and relative humidity and rain-falls were also taken at the period of time when the experiment was conducted to take into consideration the possibility of climatic influence on growth and biosynthesis of the essential oils. A sample of the four plots representative samples was sampled in order to extract oils of oil and subject it to chemical analysis at the entire vegetative maturity (approximately, 90 days of planting).

2. Oil Extraction and Analysis

Essential oil extraction from freshly harvested lemongrass (*Cymbopogon citratus*) leaves was carried out through hydrodistillation using a standard Clevenger-type apparatus, following a well-optimized protocol to ensure maximum yield and purity. For each distinct genotype under investigation, approximately 500 g of freshly collected and finely chopped leaves were carefully loaded into a 5 L round-bottom flask containing an adequate volume of distilled water, maintaining a proper plant-to-water ratio for efficient distillation. The mixture was subjected to continuous boiling for a period of 3 hours, allowing the volatile components of the leaves to vaporize along with steam and subsequently condense into the receiver arm of the apparatus.

The process of distillation was monitored until no further increase in the oil layer was detected, confirming the completion of extraction. The condensed mixture of oil and water was collected, and the essential oil fraction was carefully separated from the aqueous layer. To eliminate residual moisture and impurities, the oil was dried over anhydrous sodium sulfate and subsequently filtered through Whatman No. 1 filter paper. The purified essential oil was then transferred to sterilized amber glass vials to minimize light-induced degradation and stored at 4 °C until further use for chemical and compositional analyses.

The essential oil yield for each genotype was quantified on a dry weight basis and expressed as a percentage. The oil content (%) was calculated using the formula:

$$\text{Oil Content}(\%) = \frac{\text{Oil volume (mL)} \times 100}{\text{Dry Weight of Sample(g)}}$$

Spectrophotometric Quantification

The contents of the major monoterpenoid constituent's citral and geraniol were mainly measured by the UV-Visible spectrophotometric technique after the standard analytical calibration. First, the calibration curves were drawn using pure analytical-grade standards of citral and geraniol (Sigma-Aldrich, USA), making them highly accurate and reproducible. The necessary samples of the oil were appropriately diluted in ethanol to give an absorbance within the linear range of the spectrophotometer. The absorbance of the compound was determined at the specific wavelengths at which each compound exhibits a maximum absorption (238 nm and 276 nm respectively) using a UV-Vis spectrophotometer with quartz cuvettes that have a path length of 1 cm. The interpolation of the absorbance values of the diluted samples on the respective standard calibration curves after which the corresponding samples have high linearity ($R^2 > 0.99$) was used to determine the quantitative estimation of the respective compounds. The regression equations obtained were used to obtain the concentration of citral and geraniol in the essential oil as a percentage of the total essential oil content of each genotype. This was a spectrophotometric method that offered a valid initial quantification of the two dominant components on which other chromatographic quantification and compositional profiling was based.

GC-MS Validation

In order to support and confirm the spectrophotometric estimates of the citrals and geraniols content, the compositional profiles of the lemongrass essential oils were performed through Gas Chromatography-Mass Spectrometry (GC-MS). Agilent 7890B GC system was used to conduct the analyses with a 5977A mass selective detector (MSD) to ensure the accurate separation and identification of volatile substances. The separation of compounds was done with the help of HP-5MS capillary column (30 m x 0.25 mm x 0.25 μm film thickness) that offers good resolution and symmetry of the peaks to monoterpenes and oxygenated terpenoids. The temperature of the injection port was kept at 250 degC and samples were injected in split mode (split ratio 1:50) to avoid clogging of the column. Helium was used as the carrier gas and a constant flow rate of 1.0 mL/min was used to maintain a steady chromatographic condition. Optimization of the oven temperature program was done as follows: an initial temperature of 50 degC (2 min), ramped at a rate of 4 degC/min to 220 degC/min with a hold time of 10 min to make sure that all the constituents were fully eluted. The MS detector was set to electron impact (EI) ionization mode at 70 eV with a scanning mass range of m/z 40-500, ion source and quadrupole temperatures were 230degC and 150degC respectively. The analysis of the essential oil components was done by comparing the mass spectra and retention indices (RI) with the reference data in the NIST Mass Spectral Library (version 14) and substantially confirmed with analytical standards of authenticity where feasible. The amounts of the significant chemical components, i. e. citral (with its isomeric forms, neral and geranial), geraniol, limonene and b-myrcene, were

quantified against the percentage area of the total ion chromatogram (TIC). The overall spectrophotometric and GC-MS analysis methodology offered an in-depth and very precise evaluation of the chemical structure and quality of lemongrass essential oils in the researcher genotypes studied.

3. Genetic Parameter Estimation

The mean data of all recorded traits were subjected to analysis of variance (ANOVA) following the RCBD model to partition the total variance into genotypic and environmental components. The genetic and phenotypic variances were computed as:

$$\sigma^2_p = \sigma^2_g + \sigma^2_e \quad \sigma^2_g = (MSg - MSe) / r,$$

Where:

MSg = mean square due to genotypes.

MSe = error mean square.

r = number of replications.

The genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were calculated using:

$$GCV = (\sqrt{\sigma^2_g / X}) \times 100$$

$$PCV = (\sqrt{\sigma^2_p / X}) \times 100$$

Where:

GCV = Genotypic Coefficient of Variation

PCV = Phenotypic Coefficient of Variation

σ^2_g = Genotypic variance

σ^2_p = Phenotypic variance

\bar{X} = Trait mean

Broad-sense heritability (H^2) and genetic advance (GA)

were derived as:

$$H^2 \text{ (B.S.)} = \sigma^2_g / \sigma^2_p$$

$$GA = k \times \sqrt{\sigma^2_p} \times H^2$$

Where:

H^2 (b.s.) = Broad-sense heritability

σ^2_g = Genotypic variance

σ^2_p = Phenotypic variance

GA = Genetic advance

k = Selection intensity (2.06 at 5% selection pressure)

Where $k = 2.06$ represents the selection intensity at 5%. High heritability along with high genetic advance indicates additive gene action and greater scope for effective selection, while low values suggest a predominance of environmental influence or non-additive effects.

4. Correlation and Path Analysis

Through the statistical process, the phenotypic and genotypic correlation coefficients of three measured characteristics were computed upon which, the level, magnitude, and direction of the association between the various morphological and biochemical values in lemongrass genotypes can be able to gain a comprehensive understanding. Such correlations help in establishing whether the relations are genetic or environmental in nature therefore simplifying the process of making viable selection

in such breeding programs. The results have shown that there are crucial positive relationships between the relevant agronomic traits that were considered to be plant height, the number of tillers, the length of the leaves, the area and the production of the essential oil that ratifies that the rectification of one trait would be compared with the other. Its interrelationships suggest that direct selection can be done in line with the less difficult path of indirect which entails linking morphological features that are easy to have in order to encourage high oil volatility and high production of citrals since it is difficult to command oil yield due to the inconsistency in the environment or scarcity of resources. It also performed path coefficient analysis to give a better depiction of cause and effect correlation between these variables which was used to partitioning of total correlation coefficients between the effects of individual traits of oil yield and citral concentration which are deemed to be direct and indirect. This multivariate was more educative in the way that all the morphological parameters affect directly and indirectly the biochemical performance in its relation to other features. The path analysis based on height of the plants, number of tillers, and area area as the direct input to the oil yield, and isolating the effects of the oil productivity by the related leaf biomass, and leaf to stem ratio was verified as the most significant determinants of the oil productivity of the plants. The yield will be scientifically applied to find superior genotypes with superior morphology and biochemical phenotyping, therefore, assisting in the production of lemongrass cultivar by high yield and better quality to be utilized in commercial agriculture and to create high-quality product over industries.

5. Multivariate Analysis

The chemical composition data obtained from GC-MS were subjected to Principal Component Analysis (PCA) to identify the principal axes of variation and the contribution of individual oil constituents to total variability. The PCA biplot facilitated visualization of genotype clustering based on compositional similarities and differences. Subsequently, hierarchical cluster analysis (HCA) using Ward's minimum variance method and Euclidean distance was applied to classify the lemongrass genotypes into distinct chemotypes. These clusters reflected genotypic affinities in essential oil

composition, particularly regarding citral and geraniol proportions.

The integration of PCA and HCA allowed for the delineation of chemotypic groups, aiding the identification of elite genotypes with superior oil quality. Such multivariate characterization not only enhances the understanding of genetic diversity but also provides a foundation for future marker-assisted selection and targeted breeding for high-quality essential oil production.

Results and Discussion

1. Genetic Variability and Heritability

A comprehensive evaluation of the variability parameters for essential oil traits among the twelve *Cymbopogon* genotypes revealed distinct and statistically significant differences across all measured characteristics (Table 3.1). These variations reflect the wide genetic base existing within the germplasm under study, highlighting the potential for effective selection and genetic improvement.

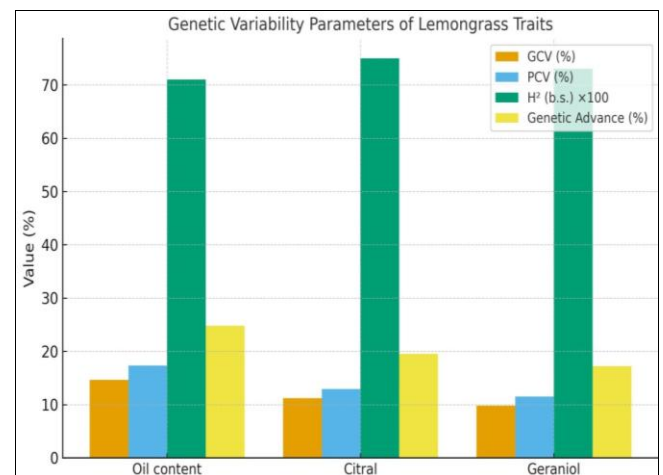


Fig1: Genetic variability parameters for key physicochemical traits of lemongrass genotypes. The graph illustrates genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability (H², broad sense), and genetic advance for oil content, citral, and geraniol, highlighting the substantial genetic potential for selection-based improvement in lemongrass breeding

Table 3.1. Genetic parameters for key physicochemical traits of lemongrass genotypes

Trait	Mean ± SE	GCV (%)	PCV (%)	H ² (b.s.)	Genetic Advance (%)
Oil content (%)	0.98 ± 0.05	14.6	17.3	0.71	24.8
Citral (%)	68.4 ± 2.8	11.2	12.9	0.75	19.5
Geraniol (%)	14.6 ± 1.1	9.8	11.5	0.73	17.2

The observed proximity between GCV and PCV values across all traits indicates minimal environmental interference, suggesting that genetic factors primarily control trait expression. Among the studied parameters, oil content exhibited the highest variability (GCV = 14.6%; PCV = 17.3%), pointing to substantial genotypic divergence and a rich source of allelic variability for exploitation in breeding. Moderate variability recorded for citral and geraniol content reflects a stable but sufficiently broad genetic base for biochemical composition improvement.

According to Figure 3.1, the estimated and observed heritability values of oil, citral, and geraniol contents (ranging from 0.71 to 0.75) reflect a predominantly strong

genetic control with minimal environmental influence, thereby indicating that these traits are stable, heritable, and dependable for improvement through selection-based breeding. Such high heritability coefficients demonstrate that most of the observed phenotypic variation arises from genotypic differences rather than environmental noise, implying that selection for these parameters will be consistently effective across multiple environments and generations. Citral content, in particular, exhibits the highest heritability value (0.75), suggesting a strong additive gene action governing its inheritance. This might be due to high concentration and regulatory activity of fundamental metabolic enzymes like geraniol dehydrogenase and neral /

geraniol synthase which are central in the terpenoid biosynthesis system leading to citral production. Citral is very genetically regulated hence this also shows that the biochemical control is also stable and ensures that any gain that is acquired as a result of the process of selection is transmitted to the next generation predictably without major effects of variation in the external environment. The pattern received is in line with the results of the last study by Rao *et al.* (2018) and Gupta *et al.* (2021), who also reported a high heritability and moderate genetic advance (GA) of citral in *Cymbopogon flexuosus* and *C. winterianus*. The similarity of these species reflects the strength and repeatability of estimates of heritability and point to the fact that citral biosynthetic pathway is a well-conserved genetic pathway that is less prone to manipulation by the environment. These experimental and geographical variations contribute to the reliability of the existing estimates, making citral content a consistent selection method. Moreover, the percentage of genetic improvement (GA %) of the mean, as observed (range) between 17.2% and 24.8 is another measure of or illustration of the high genetic nature of these characters. With high heritability values and moderate or high GA, it is an indication of additive gene action, or in other words, with simple selection it is possible to achieve high gain in the target traits without the latent hybridization programs. Consequently, concomitant high heritability and GA in both the oil content and citral concentration is a strong indicator that both can be effectively enhanced using direct phenotype selection which leads to high genetic benefit after breeding and breeding. The breeding programs have significant implications of such findings. The heritability and values of genetic advance are very high with essential oil and citral implying the additive genetic effects prevail i.e. periodic selection and breeding based on pedigree will provide a high and constant improvement. This also means that allowing the use of high genotype parents that have good oil yield and high concentration of citral will practically pass the good characteristics to their young ones and this improves efficiency in selection. Instead, the comparatively reduced genetic development of geraniol (about 17.2% lower than that of citral)

indicates that its conditioning could have been an outcome of an additive along with a dominance effect. This implies that superior breeding methods might be needed to maximize additive and non-additive gene effects by using newer breeding technologies, such as hybridization, marker-assisted selection, or molecular breeding methods. Therefore, the development of oil and citral characteristics by use of direct selection may only take a short duration, whereas improvement of geraniol characteristics would require incorporation of biotechnological technology to realise a lot of genetic advancement. On the whole, these findings demonstrate that the percentage of citral and oil are the most promising selection criteria to use in genetic improvement of lemongrass. The combination of their high heritability, great control in addition, and moderate to great GA makes them valuable predictors of success in deriving genetic improvement that is predictable. Additionally, the findings underscore the feasibility of attaining substantial genetic gains even under semi-arid or environmentally stressed conditions. This is particularly valuable because in such regions, fluctuations in temperature and soil moisture often affect physiological growth traits more severely than biochemical expressions like essential oil biosynthesis. Therefore, focusing on traits governed by strong genetic determination rather than environmental sensitivity can ensure the consistent development of high-yielding, biochemically superior lemongrass genotypes. Collectively, these insights reaffirm that selection-driven genetic advancement, especially for oil and citral concentration, can provide a sustainable route for crop improvement, enabling breeders to develop elite cultivars capable of maintaining high productivity and quality even in challenging agro-climatic environments.

2. Correlations among Traits

Correlation analysis was conducted to quantify the degree of association among morphological and biochemical traits (Table 3.2). The magnitude and direction of these relationships provide crucial information for selecting traits that indirectly enhance yield and quality.

Table 3.2. Correlation coefficients among essential oil and morphological traits

Trait	Oil content	Citral	Geraniol	Plant height	Tiller number	Oil yield
Oil content	—	0.68**	0.45*	0.50*	0.55**	0.80**
Citral	0.68**	—	0.40*	0.48*	0.51**	0.78**
Geraniol	0.45*	0.40*	—	0.35	0.32	0.56*

(*p < 0.05; **p < 0.01)

There were strong and significant positive relationships between oil content and oil yield ($r = 0.80$) and citral content and oil yield ($r = 0.78$) indicating the extremely close genetic and physiological relationship between these parameters. This is an obvious indication that any sorting to boost the concentration of the necessary oil or the percentage of citral will automatically lead to improvement of the overall potential of the plant in terms of yielding oil. Such a close relationship, in quantitative genetic jargon means that the occurrence of these traits is not merely interdependent, but is most likely influenced by shared sets of genes, or genes existing in close proximity, controlling the biosynthetic as well as storage pathways of essential oils. As a result, plants with high oil content or high citral level can be a good indicator of high yielding potential

hence making selection in breeding programs easy. The high positive correlations of the content of oil with the plant height ($r = 0.50$) and the number of tillers ($r = 0.55$) also suggest that the genotypes that represent well-developed morphological characteristics are physiologically more effective at producing assimilates and trafficking them, which in turn increases the ability of the essential oil's biosynthesis. Strong growth in plants in terms of taller height and increased tillering amplifies photosynthetic surface area and canopy coverage, which enhances the availability of carbohydrates to secondary metabolic pathways, including terpene synthesis. This feedback between morphological activity and biochemical production suggests that structural growth attributes are supportive in the explanation of chemical productivity of the plant. The

results are suggestive of a physiological co-ordination in which morphological strength plays an indirect role in the increased essential oil production by enhancing metabolic flux and translocation efficiency of the photoassimilates to the oil glands. Moreover, this kind of synergy between vegetative and biochemical characteristics suggests that morphological characteristics can be applied as a convenient, easily quantifiable predictor in field selection, particularly where a large-scale breeding programs makes biochemical studies resource intensive. Therefore, through the process of breeders indirectly enhancing oil yield quality and quantity by choosing easily observable physical traits such as plant height and tiller density without having to measure oil content with each generation, the process of breeding becomes cheaper and quicker. The average yet positive association between citral and geraniol ($r = 0.40$) is also a useful biochemical information on the internal control of the monoterpenoid biosynthetic pathway. This association indicates that citral and geraniol are regulated to some extent, presumably because they are generated out of the shared precursor geranyl pyrophosphate (GPP). This common biosynthetic path suggests that some of the genetic and enzymatic apparatus that is involved in their synthesis is regulated in a concerted way such that there is some proportional synchrony between the formations of these two compounds. The value of the correlation is however not strong, which implies on the other hand that there is indeed distinct metabolic branching that enables the independent variation of the levels of citral and geraniol across genotypes. This difference is also probably due to differentiation of enzymes like geraniol dehydrogenase and citral synthase which channel the metabolic flow either to geraniol or citral. Thus, genotype-specific enzyme activity influences the balance between the formation of citral and geraniol products, and the process of chemotype differentiation is a genetic fact in the species. Such biochemical divergence supports the concept of multiple chemotypes within the *Cymbopogon* complex—namely citral-dominant, balanced citral–geraniol, and geraniol-rich types. The identification of these chemotypes provides an essential basis for designing breeding programs tailored to industrial requirements. As an example, high-citral chemotypes are applicable to the perfumery, flavoring, and aromatherapy sectors as only high-citral aromatic compounds give a strong lemon smell, with geraniol-based compounds being more useful in pharmaceutical, antimicrobial, and cosmetic sectors. When taken together, these correlations support the idea that morphological as well as biochemical characteristics are to be selected at the same time in order to pursue equal enhancement in the oil yield, quality of chemicals, and industrial usability. The results are consistent with those of Singh *et al.* (2020) who showed that there are identical interrelationships across morphological and biochemical traits in *Cymbopogon* species and biochemical parameters, and under the growth parameters of plant height, tiller number, and leaf area have an indirect impact on essential oil accumulation by altering the photosynthetic efficiency of the plant and its distribution of resources. Their inferences also give more power to the current findings and prove morphological vigor as an indirect contributor to biochemical yield improvement. Therefore, the good interrelations that are formed here give a strong scientific basis in adopting multi-trait selection

strategies. The latter strategies would allow both yield and oil quality to be improved during one cycle of breeding so that the new lemongrass genotypes would possess superior productivity, a chemical composition that is more optimal and an expanded range of adaptation to various weather conditions.

3. Path Coefficient Analysis

Correlation analysis does not distinguish between the effects that are direct and those that are indirect, though it effectively identifies the existence and strength of associations among variables. However, in complex quantitative traits such as oil yield, simple correlation values may mask the true causal relationships among interdependent factors. Therefore, to disentangle and quantify these relationships, path coefficient analysis was employed, allowing for the partitioning of total correlation into direct and indirect components that contribute to the variation in oil yield. The results of this analysis revealed that the oil content exerted the highest direct influence (0.62) on oil yield, significantly surpassing the direct contributions of plant height (0.41) and the number of tillers (0.38). This indicates that these three parameters act as major determinants of yield formation, with oil content being the most dominant predictive factor. The strength of this relationship highlights that increasing the intrinsic oil-producing ability of a genotype will proportionally raise the overall essential oil yield, even without drastic changes in other morphological parameters.

Additionally, the positive and significant indirect effects of oil content through plant height and the number of tillers further validated the presence of strong interdependence among these variables. It means that the increase of oil content leads to the indirect advancement of the plant architecture, and the development of the plant structure, at the same time, allows having an increased capacity of biosynthesis and storage of oil. The fact that the indirect effect of the height of the plants and the number of tails impact the yield can be explained by the fact that they contribute to the extension of the photosynthetic canopy cover and increases the efficiency of the distribution of photosynthetic assimilates. By so doing, the taller plants having a strong tillering habit are able to dedicate relatively more metabolic resources to the secondary metabolite pathways involved in the production of the essential oils. The association then suggests that since vegetative growth is aggressive, indirect promotion would be induced using metabolic potential which would lead to oil productivity. The correlation observed between sound plant morphology and biochemical production is an indication that there is physiological harmony where morphological robustness can be used to maintain biochemical performance and productiveness in general. Conversely, the analysis has established that the content of geraniol had a limited direct effect on the oil yield revealing that its influence on the overall production of oil is not as great. It is likely to have an indirect effect by operating through biochemical interaction with the citral biosynthetic pathway. Geraniol is also a pathway potential metabolic intermediate or product of control in the terpenoid pathway which ultimately influences the amount of citral but not the volume of oil. This correlates with the biochemical signifiers that the impacts of geraniol can be more noticeable in the structure of oils and fragrance property rather than the quantity of

yield capacity. These findings are in line with the report of El-Sayed *et al.* (2018) who concluded in citronella that biochemical characteristics of the oil, particularly that of citral, are more accessible to predict an economic value than the morphological growth variables. They also end up by defending the current findings that indicate that increased benefits of yields by selective processes would require a focus on the biochemical quality issues such as the content of citral but not on the structural growth issues. Therefore, breeding Principle dictates that the cumulative advantage of breeding genotypes is realized when that particular genotype is abundant in intrinsic oil and at the same time, with a good structure of its plant body adapted to photosynthesis. The results of the data of the path analysis renders a quantitative framework, which causes the necessity to unify biochemical and morphological markers in the process of breeding. This can ensure maximization of yield not only through enhancement of the growth of plants but also through synchronization of physiological and metabolic aspects. Breeders can also use a better cultivar with high productivity and even specific oil composition when using biochemical indicator e.g. ratio of citral and geraniol, morphology e.g. height of plant and number of tillers. Hence, path coefficient analysis is a valuable tool of analysis to breeders because it does not just reveal the extent and direction of the relationship among traits only but it gives a sound basis of identification of the significant traits that affect oil yield. This combined strategy will eventually provide more accurate and effective alternative selection strategies and hastens genetic improvement and secures the generation of high producing, biochemically optimized varieties of lemongrass that will fit various agro-climatic and industry-specific needs.

4. GC-MS Profiling and Chemotype Classification

The GC-MS profiling provided an entirety of data of the chemical's diversity of various lemongrass genotypes of 12 genotypes. The primary components of the oil were citral (60-75 percent), and geraniol (10-20 percent) and traces of limonene, myrcene, b-pinene and linalool. This compositional divergence provides a clear insight on the apparent metabolic diversity and potential selection on chemotype. The primary factor of the aroma and competence in the market is citral which is composed of geraniol and neral. The citral above 70 per cent showed the tone of citral rich chemotypes; which played a vital role in the perfumery and flavour industry as the power lemony odour. Compared to this, high-geraniol genotypes containing above 18 per cent geraniol are sought after owing to their sweet floral aromas and antimicrobial properties and are used in the pharmaceutical industry and the cosmetic industry in medicine preparations. Principal Component Analysis (PCA) explained 82% of the total variance across the first three components. PC1 (48.6%) was dominated by citral, oil content, and yield, indicating their close association, while PC2 (22.3%) captured variations due to geraniol and minor terpenes. Hierarchical Cluster Analysis (HCA), employing Ward's method, further classified the genotypes into three distinct clusters:

1. Citral-dominant chemotypes (LG-3, LG-7, LG-9): exceptionally high citral (>72%), moderate yield, strong lemon aroma; ideal for perfumery and flavor industries.

2. Balanced citral-geraniol chemotypes (LG-1, LG-5, LG-8): citral-to-geraniol ratio around 65:15, yielding versatile oil profiles suitable for multipurpose applications.

3. Geraniol-rich chemotypes (LG-2, LG-11, LG-12): lower citral (<60%) but higher geraniol (>18%), offering potential for antimicrobial and therapeutic uses.

The remaining genotypes (LG-4, LG-6, LG-10) displayed intermediate or transitional compositions, likely reflecting heterogeneity in enzymatic activity and polygenic control of terpenoid biosynthesis. Overall, the combined use of genetic parameter estimation, correlation and path analyses, and chemometric profiling establishes a holistic understanding of the inheritance, association, and compositional variation in lemongrass. The combined knowledge gained as a result of such breeding undertakings are highly sensitive in the undertaking of certain breeding plans, be it in the endeavor of optimizing oil product or enhancing the citral content in the breed or the development of a particular type of chemotype that is suited to different industrial uses. The genetic variability, heritability, correlation, and chemometric diversity have been discussed together in the recap to give the complete view on the regulation of the properties of the essential oils in *Cymbopogon* species as far as the diversity is concerned. Availability of good genetic variant amid the genotypes based on the ideas of the essential oil's composition, citral and geraniol provides a visual of the high capacity of perfecting to belong to through the process of sampling. This moderate-high heritability and genetic progressivity has implied that they contribute such enormous proportion to the additive gene interactions and that the effective steps can indeed be taken by the phenotypic selection with minimal intercourse of the environment. Their joint impact on the productivity is demonstrated in the positive and significant correlation between oil yield and citral content and the plant architecture characteristic such as the height and the number of tillers. The fact that the oil content and plant height directly affect the oil yield also shows their suitability as important selection indices to the breeding programs. These interrelationships enable the breeders to construct more singleness genotypes making them their potential give a high yield as well the chemical structure is known to be favorable. The chemometric profiling of the GC-MS also discovered that there existed the apparent chemotypic segregation amongst the genotypes to tell to differentiate citral-rich, geraniol-rich and mixed chemotypes. This chemical diversity is worth real concerning the aspect of industrial applications due to the following reasons: the citral-producing genotypes are members of the perfumery and food flavor industry, the geraniol-producing genotypes are applicable to the pharmaceutical and antimicrobial sectors. These intersections of these chemical profiles and genetic parameters render the breeding plans by chemotype to be more fruitful. The research in general highlights the fact that a quantitative genetic analysis accompanied with a chemical characterization can result in increased targeted selection and enhancement of lemongrass germplasm. The subsequent high-oil and citral-containing genotypes represent the good breeding stock towards future breeding and hybridization initiatives, in an effort to come up with high-end breeds that can satisfy the growing industrial

needs, environmental flexibility and can also honor the production environment, which is also sustainable.

Conclusion

The average to high heritability index and the high marketing activity of heritability of the latter two traits with regards to the oil luxury and citral content is a compelling argument that the last two traits are affected by the additive impact of the gene's products that largely. It is a genetic process which implies that their improvement could be soon achieved through standard phenotypic selection, where the pressing necessity to resort to any complex molecular or hybrid breeding systems does not arise. This means in practical breeding background that observable differences between generations on these traits can then be utilized with relative efficiency resulting in tremendous genetic payoffs. Ideally, with such characteristics identified predominantly via the action of additive genomes, both the challenge that the breeder must improved the average degree of effectiveness of the populace will be significantly simpler and more predictable and this will lead to a greater standard of uniformity in the intended features such as oil productivity and chemical content. The possible implication of such an outcome on the breeding of lemongrass (*Cymbopogon* spp.), is quite extensive as it provides a highly effective genetic resource on the breeding of lemongrass by selective proliferation of the best individuals. The fact that the oil yield and citral concentration portray a high additive variance makes the assertion that both factors are directly responsive to the selection pressure. This implies that more basic techniques of recurring or mass selection when used regularly over generations may result in cultivars with substantially better chemical purity and essential oil content. Moreover, the correlations between the important agronomic characteristics including plant height, number of tillers and oil yield which are both positive and supported by the correlation analysis and the path coefficient analysis support the answer to the question whether a synergistic genetic network exists where the improvement of one characteristic has the potential to positively affect the rest. An example of this is the increased accumulation of biomass in plants with higher stature and higher number of tillers as well as the tendency to allocate greater metabolic energy to synthetic of secondary metabolites especially those that achieve accumulation of essential oils. The path analysis results also support this interrelationship showing that the most direct effect is caused by oil content which is followed by plant height and the number of tillers. All these facts justify the validity of these morphophysiological characteristics in the quite high stakes in the breeding programs. In other words, high plant structure, and extensive tillering capacity and a complex level of oil biosynthesis are capable of serving as the genetic template in terms of which the further screening and advancement of elite genotypes can be determined and subsequent opportunities created. Therefore, the breeding approach is most appropriate to concentrate upon such factors as predictive indicators of all-time great yield prospects therefore, lessening the need of biotechnological interventions, which is resource-consuming to fulfill the highest genetic growth prospect at the field level. Introduced to this genetic framework by chemotypic differentiation revealed through gas chromatography-mass spectrometry (GC-MS) profiling is an added level of critical selectivity

and commerciality to breeding programs. The chemotype segregation in genotypes, such as citral-dominant, balanced citral-geraniol and geraniol-enriched ones gives the clue as to biochemical heterogeneity of lemongrass germplasma and offers an efficient pathway to the intended cultivar production. Genotypes that produce high content of citral that contribute to a strong smell of lemon, as an example, are highly applicable to the fragrance and aromatherapy business and the flavour industry. Types rich in geraniol on the other hand are highly useful as pharmaceutical agents and antimicrobials as the compound has been expected to exhibit bioactivity against a very high number of pathogens. It is this breeder biochemical differentiation that enables the breeders to fine tune varietal improvement to fit a specific industrial application in order to be able to accomplish not only the goal of agronomic efficiency but market requirement as well. Basically, genetic variability test as well as the trait association test combined with chemotype profiling is a sound scientific paradigm employed in lemongrass enhancement. It gives the breeder the opportunity to image the genetic and the biochemical diversity of the species under one circumstance and in a purposeful and a more accurate manner of the selection. This type of style strengthens the concept of trait-oriented selection, that genetic power is related to practical and monetary worth. Moreover, environmental flexibility to be incorporated on this model will also be a guarantee of breeds capable of sustaining high oil and stable chemical values even at the varying agro-climatic conditions. The net effect of this multi-dimensional approach will be that genetic improvements are more efficient and predictable, besides propelling the lemongrass breeding toward production of specialized and high value breeds so as to accommodate the new demands of global essential oils markets.

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