Cluster and stability analysis for high oil yield performance in lemongrass (*Cymbopogon flexuosus* Steud).

Abstract: The collected thirty-three germplasm of lemongrass including checks from Chhattisgarh and from different state of India. Experiment was conducted on Research cum instructional farm of IGKV, Raipur in RCBD design in three replications from 2021, 2022, 2023 and 2024. The plant geometry was maintained as 30X30cm. The observation was recorded in randomly selected five plants. The data was analysed for the genetic diversity and were studied using cluster analysis and stability analysis for oil content (%). D^2 Statistical analysis bared that thirty-three genotypes were divided into six different cluster in which cluster I consist of maximum genotypes whereas cluster IV, V and VI bear one genotype in each. The distance between cluster III and cluster V has maximum inter cluster distance (1005.88), greater the distance between two cluster the greater the divergence and *vice-versa*. Maximum inter cluster distance also implies that the hybridization made including parents from these cluster will produce enhance segregates for crop improvement and develop better cultivar. Cluster I and cluster IV inter cluster distance were less, it suggests that these clusters consist are more similar genotypes. In completion of diversity study lemongrass accessions C.G. CF-4 and C.G. CF-2 have potential to became highest oil yielding variety. Stability analysis was conducted for consecutive data from 2021-2024 in which for oil content (%) among all accessions in which C.G. CF-4 reported with highest oil content (%) among all check with 2.18% oil percentage and found stable genotypes across the environment.

Keywords: Diversity, stability, high oil content, herbage yield, lemongrass.

Introduction: Native to South East Asia, lemongrass (*Cymbopogon flexuosus* Steud) is a perennial tropical plant in the poaceae family. It is a tall herbaceous plant with massive stripped leaves and an uneven edge. (Akhila *et al*. 2010 and Anand *et al*.2010). Oil glands in the leaves, which contains 0.5–2% oil in them, are easily extracted by steam distillation. Because of high concentration of citral and gerenayl acetate, the oil is used to flavour soft drinks, aromatic detergents and soaps, add aroma to perfumes, cosmetics, and cover offensive odours in a variety of industrial items. The synthesis of ionones, which are utilised in fragrance, also involves citral. Insect repellent and carminative properties have been attributed to lemon grass, a medicinal herb (Sorru *et al*. 2016). Lemon grass is a perennial plant that is often grown in the subtropics and tropics. There are two kinds of lemon grass: *Cymbopogon citratus*, which is a West Indian species, and *Cymbopogon flexuosus*, an East Indian species. Native to South East Asia, and Australia have a number of species of lemon grass. Because of this, it is often referred to as tropical East Indian lemon grass. *Cymbopogon flexuosus* often known as the Cochin or Malabar grass, is an indigenous plant found in Sri Lanka, India, Thailand, and Burma. It is related to the West Indian lemon grass (*Cymbopogon citratus*). Beyond its culinary applications, lemongrass has also garnered attention for its medicinal properties, playing a role in traditional herbal remedies in many cultures it acts as source for the production of Vitamin A and β-carotene *etc*. [Bonzi *et al*.2013]. The oil of lemongrass used as folk medicine against the treatment of gastro intestinal disorder, neurological disorder, anti-inflammatory, anti-pyritic, anti-fungal, anti- cancerous and insecticidal and rich source of anti-oxidant. [Hanna *et al*. 2016; Baurah *et al*. 2017; Jeffers 1967]. Active constituents in lemongrass essential oil are myrcene, followed by limonene and citral along with geraniol, citronellol, geranyl acetate, neral, and nerol, which are beneficial to human health. A large part of lemongrass' expansion is driven by the plant's huge industrial potential in the food, cosmetics, and medicinal sectors.lemongrass has prioritized number one under aromatic crops in India due to its wide application on culinary, cosmetic, pharmaceutical and confectioneries. (Krishnendu *et al*, 2024) [ICAR DAMPR AICRP-MAP&B Report 2022-23]. Chhattisgarh ranked 8th state under development centre for medicinal and aromatic crops in India. Horticulture sector in Chhattisgarh has prioritised lemongrass crop number one in aromatic plant sector for research and development. As a versatile plant, it supports a range of ecosystems by providing food, shelter, and medicinal resources to both humans and wildlife. Its contributions to the development of natural products, such as essential oils with antimicrobial and anti-inflammatory properties, also expand the scientific knowledge base, offering potential solutions for health and environmental challenges. Furthermore, lemongrass's adaptability to various climates and its cultivation practices fosters a deeper understanding of agricultural diversity, resilience, and sustainability in the face of changing environmental conditions.

Material and Methods: Thirty-three germplasm collected from different part of Chhattisgarh and Haryana state of India (*Figure* 1), including seven checks Pragati, Praman, CIM Shikhar, Neema, CKP-25, Krishna and C.G. LG-1, and during year 2021-2024 an experiment was carried out at the Research Cum Instructional Farm, Department of Genetics and Plant Breeding, College of Agriculture, IGKV Raipur (C.G.). Raipur is the capital of Chhattisgarh and is situated at latitude of 21˚13'16˝ NL, a longitude of 81˚1'43˝, and an elevation of 298.5 metres above mean sea level, The experiment was conducted in RCBD design in three replication, with a crop geometry of 30X30 cm. In order to fetch a good harvest, all recommended packages of practices was followed. Five plants were chosen at random from each accessions and observation were recorded for following traits viz. 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), citral content (%) and oil content (%) and some qualitative traits *viz*, culm colour, lead blade colour and leaf sheath colour. Oil was extracted from leaves at three-month interval by steam distillation method in clevenger apparatus. Through stability analysis for oil content (%) were studied and analysed by using Eberhart and Russell Model.

Statistical Analysis: Clustering of genotypes was done using wards hierarchal algorithm based on squared Euclidean distance (D^2 Mahanolobis method). To determine the trend of morphological variation, the observation recorded on traits were statistically analysed by using softwares Grapes Version 1.0.0. (Gopinath *et al*. 2020) and Past version 4.03. for constructing of dendogram DARwin version 6.0.21. Stability of the accessions were analysed by using Eberhart and Russell model.

Result and Discussion: The D^2 statistics, it was a multivariate analysis based on Euclidean cluster analysis. It was proposed by Mahalanobis (1936), measured divergence at two levels: Besides aiding in the selection of divergent parents for hybridization, D^2 statistics measured the degree of diversification and determined the relative proportion of each component character to the total divergence.

Thirty-three accessions further divided into six clusters. Cluster I contain the 21 accessions which is highest among all accessions, *viz*; C.G. CF 1, C.G. CF 10, C.G. CF 11, C.G. CF 12, C.G. CF 13, C.G. CF 14, C.G. CF 21, C.G. CF 16, C.G. CF 19, C.G. CF 17, C.G. CF 26, C.G. CF 3, C.G. CF 23, C.G. CF 5, C.G. CF 6, C.G. CF 7, C.G. CF 8, C.G. LG-1, Krishna (C), Nima (C), and Praman (C). These genotypes present in single cluster suggested that they are genetically similar and closely related than those belonging to other cluster. Cluster II consist seven genotypes *viz*. C.G. CF 2, C.G. CF 18, C.G. CF 20, C.G. CF 4, C.G. CF 25, CIM-Shikhar (C), and Pragati (C). The genotypes in this cluster were less next to Cluster I. Cluster III comprised of only two genotypes viz.C.G. CF 15 and CKP-25 (C). The small size of this cluster suggested that these genotypes were quite distinct from others cluster genotypes, indicating unique genetic traits. Clusters IV, V and VI consisted of a single genotype in each cluster was: C.G. CF 9, C.G. CF 22, and C.G. CF 24, respectively. These single accessions in clusters indicated that these genotypes had unique characteristics compared to other accessions.

Table 1. Accessions included in different cluster based on D^2 analysis in lemongrass		
Cluster groups	**Total accessions**	**Name of a accessions**
Cluster I	21	C.G. CF 1, C.G. CF 10, C.G. CF 11, C.G. CF 12, C.G. CF 13, C.G. CF 14, C.G. CF 21, C.G. CF 16, C.G. CF 19, C.G. CF 17, C.G. CF 26, C.G. CF 3, C.G. CF 23, C.G. CF 5, C.G. CF 6, C.G. CF 7, C.G. CF 8, **C.G. LG-1(C), KRISHNA (C), NIMA (C), PRAMAN (C)**
Cluster II	7	C.G. CF 2, C.G. CF 18, C.G. CF 20, C.G. CF 4 , C.G. CF 25, **CIM- Shikhar (C)**, **PRAGATI (C)**
Cluster III	2	C.G. CF 15, **CKP-25 (C)**
Cluster IV	1	C.G. CF 9
Cluster V	1	C.G. CF 22
Cluster VI	1	C.G. CF 24

Inter and Intra-Cluster Distances: Highest inter cluster distance was reported between cluster IV and VI (1005.88) followed by cluster II and cluster IV (955.99) followed by cluster III and cluster VI (727.79). The distance between two cluster is the measure of degree of diversification. The greater the distance between two cluster the greater the divergence and *vice-versa*. Maximum inter cluster distance implies that hybridization made including parents from these cluster will produce enhance segregants for crop improvement and development-better cultivation. Lowest inter cluster distance determine less heterogeneity in the accessions which can be used for backcross programme (Singh *et al*. 2024).

Table 2: Inter and Intra cluster distance

Cluster Number	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6
Cluster 1	**128.39**	292.27	284.75	389.52	234.28	383.19
Cluster 2		**68.91**	460.50	955.99	632.18	248.09
Cluster 3			**174.31**	324.60	496.11	727.79
Cluster 4				**0.00**	358.48	1005.88
Cluster 5					**0.00**	756.17
Cluster 6						**0.00**

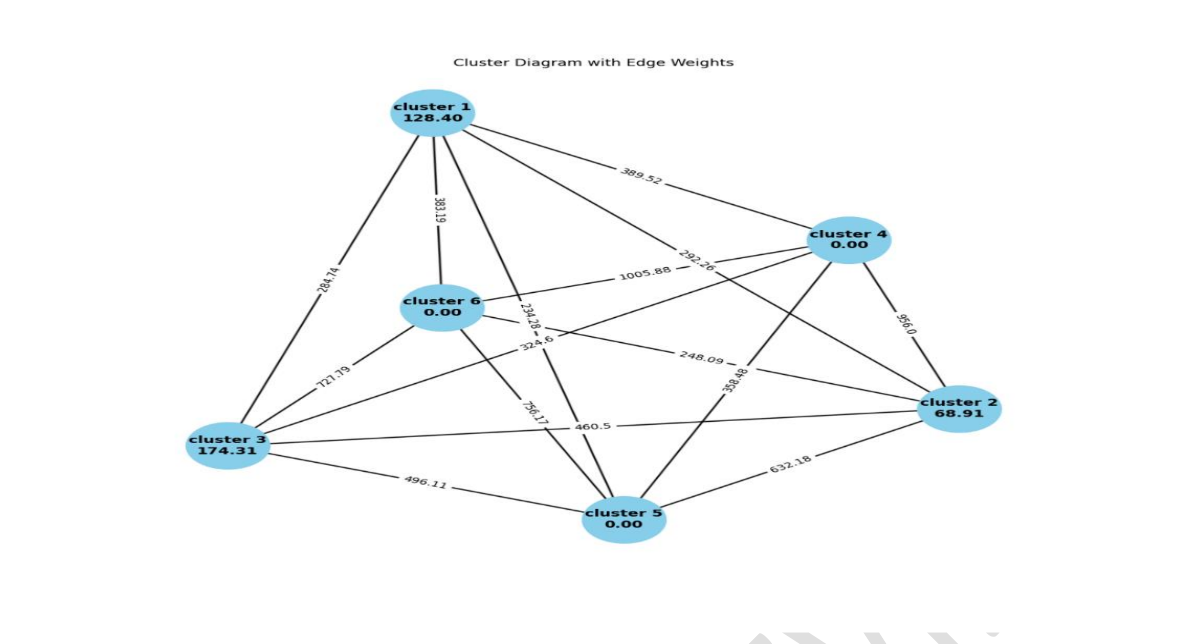

Figure **1: D^2 Euclidean inter and intra cluster diagram**

Cluster Group and Traits Contribution: Cluster I had a total herbage yield of 408.33 and exhibited a combination of moderate plant height (cm) (102.62 cm), culm length (cm) (13.77 cm), and culm diameter (cm) (1.13 cm). The genotypes in this cluster had an intermediate leaf blade length (cm) (73.93 cm) and leaf blade width (cm) (1.45 cm), resulting in a moderate leaf area index (1.03556). The total number of tillers per plant was 27.25, and the number of leaves per culm was 5.14. Notably, this cluster showed a moderate oil content (%)(1.67) and a high citral content (%) (84.23), making it valuable for breeding programs focused on improving oil yield and citral concentration.

Cluster II depicts the highest plant height (cm) (135.28 cm) and culm length (cm) (25.32 cm), indicating robust growth. It also had a higher culm diameter (cm) (1.20 cm) and leaf blade length (cm) (99.99 cm), with a leaf blade width (cm) of 1.70 cm and a higher leaf area index (1.40). This cluster also showed the highest number of tillers per plant (47.28) and a high number of leaves per culm (5.28). The oil content (%) was slightly higher than Cluster I (1.77), but the Citral content (%) was lower (74.48). The total herbage yield (312.619) was substantial, making this cluster suitable for breeding programs aiming at increasing biomass and leaf area index.

Cluster III were recorded for the shortest plant height (cm) (56.20 cm), culm length (cm) (6.16 cm), and culm diameter (cm) (0.72 cm). The leaf blade length (cm) (39.02 cm) and width (0.56 cm) were also the smallest, resulting in the lowest leaf area index (0.3). Despite the low herbage yield(g) (35g), this cluster had a moderate number of tillers per plant (28) and leaves per culm

(4.33). The oil content (%) (1.04) and citral content (%) (72.59) were relatively lower, making this cluster less favorable for high-yield breeding programs but potentially useful for specific traits such as a compact growth habit.

Cluster IV presented moderate values for plant height (cm) (85.63 cm), culm length (cm) (15.40 cm), and culm diameter (cm) (1.18 cm). The leaf blade length (cm) (63.74 cm) and width (2.48cm) contributed to the highest leaf area index (1.67). This cluster had a high number of tillers per plant (34.66) and leaves per culm (5.66), but lower oil content (%) (0.88). The citral content (%) was high (80.24), and the herbage yield was moderate (150). This cluster was suitable for breeding programs focusing on a high leaf area index and citral content (%).

Cluster V featured moderate plant height (cm) (88.96 cm), shorter culm length (cm) (8.03 cm), and smaller culm diameter (cm) (0.87 cm). It had a moderate leaf blade length (cm) (64.51 cm) and width (1.41 cm), resulting in a moderate leaf area index (1.02). The number of tillers per plant was the highest (51.66), but the number of leaves per culm was lower (4.33). The oil content (%) was low (0.70), and the citral content (%) was the lowest (55.0133), yet the herbage yield was high (335), indicating this cluster was useful for breeding programs aiming to increase the number of tillers and overall herbage yield despite lower oil and citral content (%).

Cluster VI had the tallest plants (154.76 cm) and the longest leaf blade (114.54 cm). The culm length (cm) (20.50 cm) and diameter (1.15 cm) were also significant. The leaf blade width (cm) (1.72 cm) and leaf area index (1.45) were high. The total number of tillers per plant was (42), with a moderate number of leaves per culm (4.50). The oil content (%) (1.04) was comparable to Cluster III, and the citral content (%) (63.35) was moderate. The herbage yield (188.17) indicated good biomass production. This cluster was valuable for breeding programs targeting height, leaf blade length (cm), and overall biomass production. [Gupta *et al*. 2019; Sharma *et al*. 2018; Singh *et al*., 2021] revealed significant genetic variability among different lemongrass accessions, aiding in the identification of genetically distinct clusters that could be used in breeding programs to enhance specific traits by using cluster analysis, this present study evaluated the genetic variability and interrelationships among key agronomic traits in lemongrass. Similar results were taken by Ekka *et al*., 2024 for morphological assessment in lemongrass.

Table 3: Cluster group and trait contribution

Cluster group	Plant height (cm)	Culm length (cm)	Culm diameter (cm)	Leaf blade length (cm)	Leaf blade width (cm) s	Leaf area index	Total no. of tillers/ plant	No. of leaves /culm	Oil content (%)	Citral content (%)	Herbage yield (g)

Cluster 1	102.62	13.76	1.13	73.93	1.45	1.03	27.25	5.14	1.67	84.23	408.33
Cluster 2	135.28	25.32	1.20	99.99	1.70	1.40	47.28	5.28	1.77	74.48	312.61
Cluster 3	56.20	6.16	0.72	39.02	0.56	0.30	28.00	4.33	1.04	72.59	35.00
Cluster 4	85.63	15.40	1.18	63.74	2.48	1.67	34.67	5.66	0.87	80.24	150.00
Cluster 5	88.96	8.03	0.87	64.51	1.41	1.02	51.66	4.33	0.70	55.0	335.00
Cluster 6	154.76	20.50	1.15	114.54	1.72	1.45	42.00	4.50	1.04	63.35	188.17

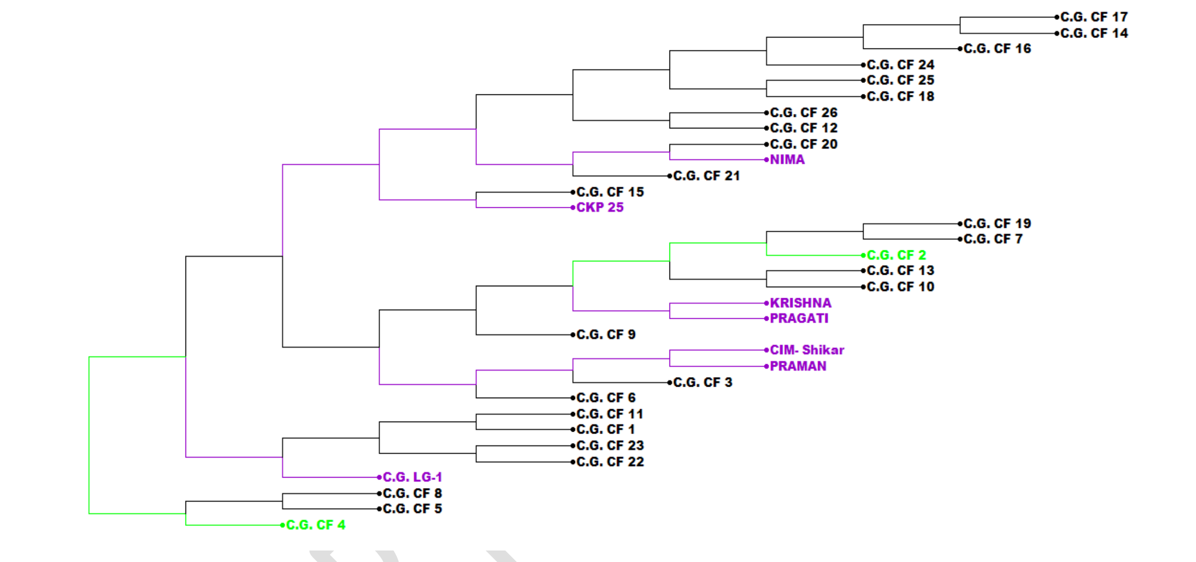

Figure **2: Dendrogram construction of thirty-three germplasm accessions**

Stability Analysis for oil yield (%): The oil content (%) among the genotypes showed a wide range of variation, with a population mean of 1.03%. The topmost accessions C.G. CF-4 (2.20 %), CIM shikhar (C) (1.91%), and C.G. CF-12 (1.94%), showed increase value over the population mean. On the lower side, accessions C.G. CF-25 (0.41%) and C.G. CF-26 (0.46%) had much lower oil content (%).

The regression coefficient (bi) and deviation from regression (s^2di) provide information about accessions under varying environmental conditions. Overall, C.G. CF-4, CIM shikhar (C), and C.G. CF-12 were identified as high oil yielding accessions in terms of oil content (%).

Similar outcomes found for mean performance and environmental indices, evaluated the stability of biomass and oil yield in lemongrass genotypes. Similar results obtained by Panwar *et al.*, 2023 in *ocimum* for essential oil yield.

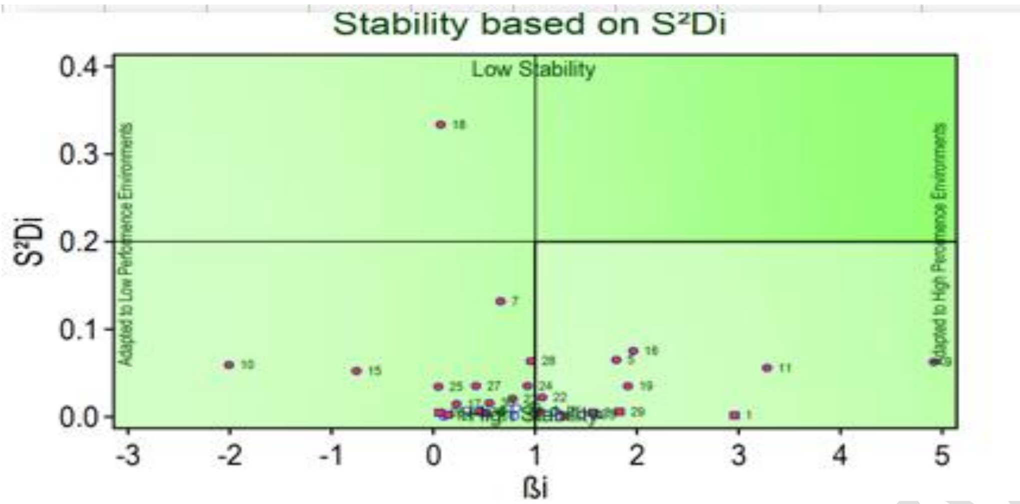

Figure 3: **Graphical representation of Regression coefficient for Oil content (%)**

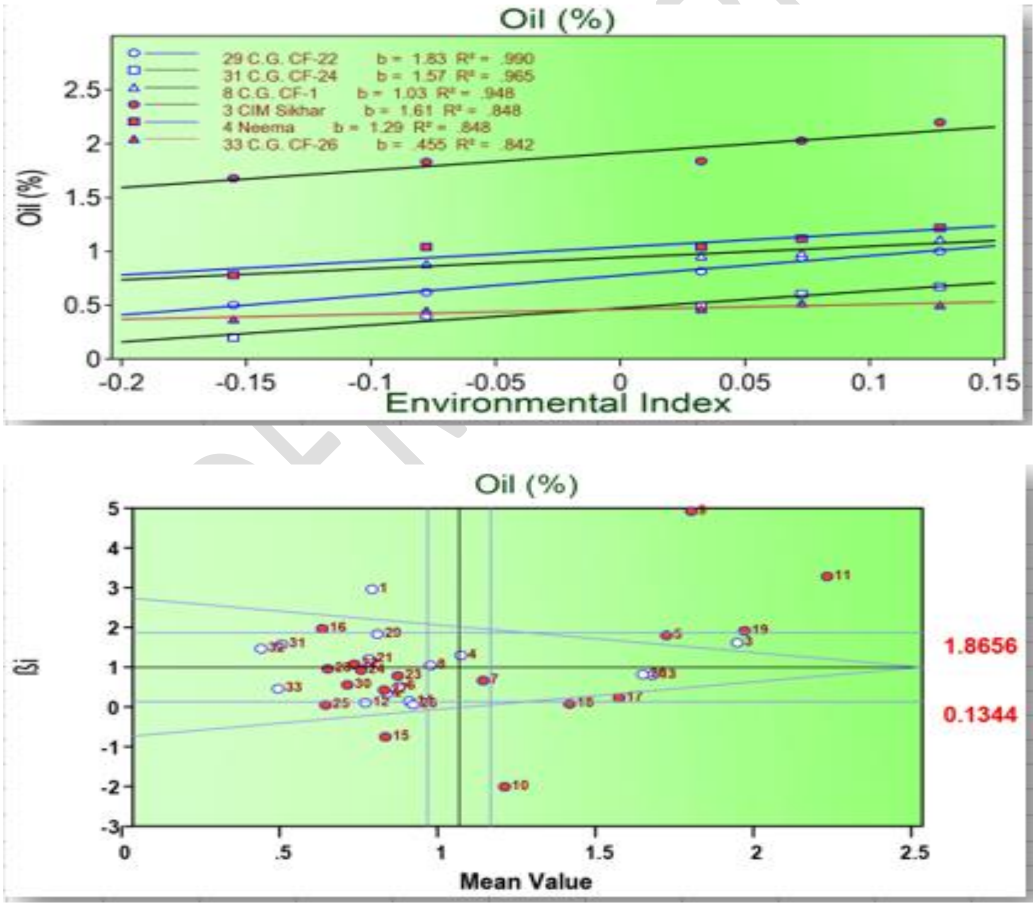

Figure **4**: Graphical representation of Regression coefficient for oil **content (%)**

Conclusion: Cluster analysis grouped 33 lemongrass genotypes into six distinct clusters based on their genetic similarity, aiding breeders in identifying suitable genotypes for breeding programs. Cluster I, with 21 genotypes, exhibited moderate genetic variability and traits such as intermediate plant height (cm) and high citral content (%), making it valuable for breeding programs targeting oil yield and citral concentration. Cluster II, comprising seven genotypes, showed robust growth and the highest biomass, making it suitable for breeding programs aiming to increase biomass and leaf area index. Cluster III, with two genotypes, had compact growth and lower yields, potentially useful for specific traits. Clusters IV, V, and VI each contained a single genotype, indicating unique characteristics. Cluster 4 had high leaf area index and citral content (%), Cluster V had the highest number of tillers and herbage yield despite lower oil and citral content (%), and Cluster VI featured tall plants and high biomass production. Highest inter cluster was recorded between cluster IV and VI (1005.88) hence the genotype found in these clusters are more diverse and hybridization if made from these two clusters will produce better segregates.

C.G. CF-4, CIM Shikhar (C), and C.G. CF-12 were identified as high oil yielding accessions in terms of oil content (%). The present study depicts these accessions with high stability and adaptability, and can be used as become parent for breeding programs for improving oil content (%). [Patra *et al*. 2017; Sharma *et al*. 2018; Kumar *et al*., 2019].

Conflict of interest: The author shows no conflict of interest.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

No Generative AI has been used during writing of manuscript.

Option 2:

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc. have been used during the writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology

Details of the AI usage are given below:

1.

2.

3.

References:

- Akhila, A. 2010. Essential oil-bearing grasses: the genus *Cymbopogon*. CRC Press, Taylor and Francis Group, p.108.
- Anand, K.K., 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. [http://dx.doi.org/10.6165/tai.2010.55(3).197]. 14-25
- Bonzi, S., Somda, I., Sereme, P. and Adam, T.(2013). Efficacy of essential oils of *Cymbopogon citratus* (dc) stapf , Lipia multifolia moldenke and hot water in the control of seed borne fungi phoma sorghina and their effects on Sorghum bicolor(L.) moench seed germination and plants development in Burkina Faso. *Net J Agric Sci*. 1(4):111-115.
- Ekka, A., Tirkey, A., Kujur, V.K. and Verma, S.K. 2024. Utilizing ISSR and DAMD markers and agro morphological traits for genetic refinement of some elite lemongrass (Cymbopogon flexuosus Steud). *International Journal of Advanced Biochemistry Research*, Vol. 8(7): 1039-1049.
- Gopinath, P. P, Parsad, R, Joseph, B, Adarsh, V. S. (2020). GRAPES: General Rshiny Based Analysis Platform Empowered by Statistics. DOI: 10.5281/zenodo.4923220
- Gupta, M. L., *et al*. (2019). "Stability analysis of lemongrass genotypes for essential oil yield." Journal of Essential Oil Research, 31(5), 428-435.
- Hanaa ARM, Sallam YI, El-Leithy AS, Aly SE (2012) Lemongrass (*Cymbopogon citratus*) essential oil as affected by drying methods. Ann Agric Sci 57:113–116. https://doi.org/10.1016/j.aoas.2012.08.004
- J. Baruah, B. Gogoi, K. Das, N.M. Ahmed, D.K. Sarmah, M. Lal, B.S. Bhau. Genetic diversity study amongst Cymbopogon species from NE-India using RAPD and ISSR markers. *Industrial Crops and Products*. 2017.95:235-243. https://doi.org/10.1016/j.indcrop.2016.10.022.

- Jeffers JNR. Two case studies in the application of principal component analysis. *Appl. Stat.* 1967;16**:** 225-236.
- Krishnendu Adhikary, Pradipta Banerjee, Saurav Barman, Bidyut Bandyopadhyay and Debasis Bagchi, 2023. Nutritional Aspects, Chemistry Profile, Extraction Techniques of Lemongrass Essential Oil and It's Physiological Benefits, *Journal of the American Nutrition Association*, 1-19.
- Kumar, A., & Singh, B. (2019). Morphological and anatomical adaptations of lemongrass (*Cymbopogon citratus*) to water stress. *Plant Physiology and Biochemistry*, *135*, 220-228. https://doi.org/10.1016/j.plaphy.2018.10.015
- Panwar, N.S., Kumar, A., Mallik, S.S., Diwedi, V.K., Singh, P.B. and Kumar, G. 2023. Stability Analysis for Biomass and Essential Oil Yield in Basil (Ocimum basilicum) Germplasm. *Indian Journal of Plant Genetic Resources*, *25*(02), 180-182.
- Patra, N. K., 2017. "Genotypic stability in lemongrass." Agricultural Science Digest, 25(1), 37-39.
- Sarrou. E., Chatzopolou, P., Theodoros, K.V and Katiosis, S. 2016. Herbage yield and essential oil composition of sweet basil (*Ocimum basilicum* L.) under the influence of different mulching materials and fertilizers. *Journal of Medicinal Plants Studies* 4(1): 111-117.
- Sharma, A., Patel, R., & Singh, B. (2018). Association and path coefficient analysis for essential oil yield and its contributing traits in *Cymbopogon flexuosus*. *Journal of Applied Research on Medicinal and Aromatic Plants, 6*, 123-130.
- Sharma, S., Habib, S. Sahu, D and Gupta, J. 2021. Chemical properties and therapeutic potential of citral, a monoterpene isolated from lemongrass, *Med. Chem.* 17 (1):2–12.
- Singh R., Verma PK., Behl R. K., Arya RK., 2024. Studies on Identification of Stable Genotypes of Lemongrass for Semi-Arid Regions. *Ekin J.* 10(1):22-26.