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Stability analysis of advanced breeding clones of lemongrass for citral content, herb and oil yield using AMMI, GGE biplot, and YREM

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Abstract

The study analysed lemongrass using additive main effects and multiplicative interaction (AMMI), genotype +genotype-by-environment (GGE), and genotype-environment interaction biplot analysis. Stable lemongrass genotypes were identified using AMMI stability value (ASV), stability index, and yield relative to environment maximum (YREM) computation. To investigate the current study's stability and adaption patterns, a set of six advanced breeding clones and two control varieties, Krishna and CIM-Shikhar, were tested in triplicate over four years using a randomized complete block design (RCBD). The genotype x environment linear (G x E) component and genotype variance analysis were significant for herbage, oil, and citral content, respectively. The G x E interaction was found to be 19.12% (citral), 31.92% (herb), and 4.34% (oil) in the AMMI analysis of variance. Trait variation was found to be a stable factor in the performance of many genotypes; no genotype demonstrated high levels of stability across multiple characteristics. Stable clones with optimal performance were identified as clones 8 and 3 for citral content, clone 5 for herb yield, and clone 1 for oil yield, as indicated by the biplot of the mean yield and AMMI stability value. Clone 8 was found to be a stable clone for citral content, with a unity YREM based on estimations. Nevertheless, clones 7 and 6 were steady performers for both oil content and herb production, respectively. No clones demonstrated unity YREM for either characteristic.

Keywords: Clones, GGE bi-plot, AMMI, stability value, stability index

Introduction

Lemongrass, well known for its essential oil is cultivated in tropical and subtropical regions including Guatemala, China, India, Malaysia, and Sri Lanka (Vimala *et al*. 2022). Because of its high citral content, India is the largest producer of lemongrass oil. The oil is utilized in soaps, perfumes, and cosmetics, as well as the manufacture of vitamin A and β-ionones. It is grown in several Indian states, including Odisha, Kerala, Karnataka, Maharashtra, Telangana, and Andhra Pradesh (Kumar *et al*. 2022; Vimala *et al*. 2022).

An intrinsic challenge for plant breeding programs is figuring out how different genotypes interact with varied environmental conditions to produce varieties with better performance (Eberhart and Russell 1966; Gupta *et al*. 2015). In the final stages of a breeding program that culminate in cultivar release when there is a large amount of material available for each advanced genotype, it is possible to characterize each genotype in terms of its stability in adjusting to various environmental conditions (Acosta-Pech *et al*. 2017; Zoric *et al*. 2017). Genotype-by-environment interaction (GEI) has a substantial impact on crop development through plant breeding, primarily because it makes identifying breeding objectives more complex and confuses genotype comparisons with the test environment. To overcome these limitations, it is suggested that a deeper comprehension of the changes in plant adaptation linked to performance variances, particularly the GEI, is necessary (Xu 2016; Hassani *et al*. 2018). Genotype selection for higher performance is made more difficult by GEI. To evaluate different aspects of the GEI dilemma, plant breeders often use multi-environment Trails (MET) (Wrike, 1962; Eberhart and Russell 1966; Shukla 1972).

Multiple methods have been employed to differentiate stable from unstable genotypes in various crops and to characterise their surroundings. The most widely used techniques for evaluating yield stability in cultivar release programmes are undoubtedly regression-based and multivariate statistical studies. Multiple genotype responses to environmental factors can be included using multivariate statistical studies. The genotype by environment interaction (GE) and genotype main effect (G) method, or GGE, was created by Yan (2002) for MET graphical analysis. Plant breeders typically use METs to evaluate how well a genotype performs in relation to other genotypes in particular conditions.

The AMMI interaction model is used for analyzing crop yield trials, specifically focusing on genotype-environment interactions (GEI). It addresses the shortcomings of existing methods like Analysis of Variance (ANOVA) and Principal Component Analysis (PCA) by giving a more comprehensive study of GEI. The AMMI model is used for three key purposes: variance analysis, principal component analysis, and yield estimation accuracy. It aids in the selection of stable genotypes for agriculture under varying environmental conditions (Usha Rani *et al*. 2017; Mousavi and Nagy, 2020). The links between environments, genotypes, and their interactions are established with the help of the model. This understanding is critical for improving crop performance and stability (Purchase *et al*. 2000). Biplots, which include AMMI and GGE biplots, are graphical representations that show the relationships between genotypes (G), environments (E), and interactions (GEI). These plots are instrumental in revealing patterns and interactions in complex data sets involving genotype and environmental factors (Oladosu *et al*. 2017; Usha Rani *et al*. 2017). PCA is a commonly used technique to create these biplots. They help in identifying genotypes suitable for specific environments and those exhibiting stability across diverse conditions. Data on GEI and Singular Value Decomposition (SVD) are used to create biplots (Yan *et al*., 2000). The biplots are created by displaying these SVD-based components graphically (Gauch 1992).

The AMMI Stability Value (ASV), which gauges the stability of genotypes, is a metric that comes from the AMMI model. As per Temesgen *et al*. (2015), genotypes exhibiting lower ASV values are deemed more stable, indicating uniform performance in a variety of conditions. Another metric for evaluating the combination of high-yield performance and stability in genotypes is the Yield Stability Index (YSI). It considers ASV scores and yield rankings to determine genotypes that exhibit both high yield and stability (Ill´es *et al*. 2020). Another approach to assess the stability is an estimation of yield relative to the environmental maximum (YREM). It is used to assess how well a genotype is adapted to a specific environment and how close it comes to reaching the maximum yield achievable in that environment (Purchase *et al*. 2000; Ashwini *et al*. 2021; Kirankumar *et al*. 2023; Shivakumar *et al*. 2024).

The effects of GEI in lemongrass has not been thoroughly examined using advanced multivariate statistical approaches, according to a review of recent literature (Bhan *et al*. 2005; Lal 2012; Kumar *et al*. 2022). The purpose of this study was to examine the interactions between genotype and environment (year) in lemongrass using combination analysis, stability measure, biplot, and YREM and also to identify stable genotype(s) across environments.

Materials and methods

Origin of plant material

Open-pollinated Krishna seeds were used as the starting population for the breeding program. The open-pollinated seeds that were gathered from the cycle 4 population were used to create the current study materials. From this population, oil content of 1450 unique plants was examined which ranged from 2.00 to 2.80% (measured with the Clevenger apparatus) and six clones were chosen for the study. These six clones together with two control varieties (Krishna and CIM-Shikhar) were tested for three years. The control varieties *viz.,* Krishna and CIM-Shikhar used in the present experiment are popular varieties of lemongrass and nearly > 90 % of lemongrass cultivation area covered by these varieties in India.

Location and layout of the experiment

The study was conducted at the CSIR-Central Institute of Medicinal and Aromatic Plants, Research Centre Bengaluru, India, from June 2018 to June 2021. The experimental site was located at 11° 7′ N latitude, 77° 59′ E longitude, and 426 meters above mean sea level (MSL). The experiment was conducted in a randomized block design (RCBD) with three replications. Plants of each genotype were planted in four rows, 45 cm apart from one another and from row to row. Throughout the crop season, all prescribed package of practices were implemented, including fertilizer application (150:60:60 NPK per ha per year) and pest and disease management.

Data recording and essential oil extraction

Herbage yield (grams per plant) and oil content (%) were determined on fresh weight (grams per plant) basis. Data were collected from five plants from each genotype in each replication. Clevenger apparatus was used to extract essential oils from plant samples (Clevenger 1928). The extracted oil was stored at 4 degrees Celsius for subsequent analysis. Citral components of essential oils were separated and quantified using gas chromatography (GC) as explained in Lal *et al*. (2012).

Statistical analysis

In order to estimate the presence of variances among the genotypes, genotype by season (genotype by environment), the quantitative traits were subjected to analysis of variance (ANOVA) using Spar-2. The environments were regarded as random variables, but the genotypes were viewed as fixed variables.

AMMI analysis of variance: The data recorded on three traits across four environments were analyzed using AMMI analysis model as per Zobel *et al*. (1988) to estimate genotypic, environmental and interaction effects. The estimate of the response variable for the ith genotype in the jth environment is evaluated as per the equation below

$$Y_{ij} = \mu + \alpha_i + \beta_j + \sum k \, \lambda_k \, \gamma ik \, \delta jk + \varepsilon_{ij}$$

where, y_{ij} is the trait mean of the ith genotype in the jth environment, μ is the overall mean of the lemongrass genotypes, α_i and β_j are the genotype and environment deflections from the overall mean, respectively. While, singular value for the kth interaction principal component axis is represented as λ_k. Likewise, γ_{ik} and δ_{jk} are the genotype and environment principal components scores for axis k, ε_{ij} is the residual error.

To ascertain the degree of stability among the 8 clones (including checks) across environments, further statistical analysis was conducted to see if there was a significant interaction between the environment and the genotype. Multi-environment assessment (which-won-where pattern), genotype evaluation (mean versus stability), and tested environment raking (discriminative versus representative) form the basis of the resulting graph. The genotypes were ranked according to each stability parameter in ascending order. Standard deviation-standardized (scaling=0), environment-centered (centering=2), singular-value partitioning=2, and transformed (transform=0) served as the foundation for the biplots.

AMMI stability value (ASV): The ASV as described by Purchase (2000) was calculated as follows:

$$ASV = \sqrt{[\,(SS_{IPCA1} / SS_{IPCA2} * IPCA1_{score})^2 + IPCA2_{score}{}^2\,]}$$

Where, SS_{IPCA1} and SS_{IPCA2} are the sums of squares for the first and second IPCA axes, respectively. $IPCA1_{score}$ and $IPCA2_{score}$ **are the genotype scores for the first and second IPCA axes.**

Yield stability index (YSI): The new approach YSI was calculated by the following formulas:

$$YSI = RASV + RY$$

Where, RASV is the rank of AMMI stability value and RY is the rank of mean trait value of genotypes (RY) across environments. YSI incorporates both mean trait value and stability in a single criterion.

All the analyses were implemented using GEA-R (Genotype x Environment Analysis with R for Windows). Version 4.1 (2017-08-3).

Estimation of Yield Relative to Environment Maximum (YREM)

The identification and quantification of crossover Genotype-Environment Interaction (GEI) using a metric called YREM (Yield Relative to the Environmental Maximum). Crossover GEI occurs when the ranking of genotypes changes across different environments. In other words, some genotypes may perform well in one environment but poorly in another, leading to crossover effects. YREM is a metric used to quantify the potential decline in yield of test genotypes due to crossover GEI. A higher YREM value indicates that a genotype's yield potential is reduced to a lesser extent even when crossover GEI is present (Yan 1999). The formula for calculating YREM is given as:

$$YREM_{ij} = X_{ij} / MAX_{ij}$$

$YREM_{ij}$: represents the YREM value for the ith genotype in the jth environment. X_{ij}: This is the mean yield of the ith genotype in the jth environment. MAX_{ij} represents the yield of the highest-performing genotype in the jth environment. A higher YREM value indicates that the genotype is relatively closer to the best-performing genotype in terms of yield in that specific environment. The analysis, including the calculation of YREM values, was carried out using Microsoft Excel.

Results and discussion

Analysis of Variance and AMMI Analysis

The single and combined ANOVA for herb yield per plant, oil content, and citral content revealed significant genotypic effects and GE interactions. Based on the AMMI analysis, all observable variables showed significant variation in the main effects of genotype (G), environment (E), and their interactions (GEI) with a significance level of $P < 0.001$(Table 1). This indicates that these factors significantly influence the observed variables. The genotype's main effect was found to have different levels of impact on the various observable variables. It ranged from 39.65% for herb yield, 70.86% for citral content, to 86.63% for oil content. These percentages represent the proportion of variation in each variable that can be attributed to the genotype alone. In the variation attributed to the genotype-environment interaction (GEI) for herb yield, the first and second interacting principal components (IPCs) accounted for 79.07% and 19.72% of the variation, respectively. This suggests that a substantial portion of the variability in herb yield can be attributed to the interaction between genotype and environment, with the first IPC having the most significant impact. Similarly, for the oil content of GEI SS, the first two IPCs accounted for 64.26% and 31.71% of the variation, respectively. Again, this indicates that genotype-environment interactions play a significant role in explaining the variability in oil content. Finally, for the GEI of oil content, the first and second principal components explained 73.09% and 25.94% of the variation, respectively.

One of the primary objectives of plant breeding is to identify and develop plant varieties or cultivars that can perform well under a wide range of environmental conditions. These cultivars should exhibit good and stable performance across various environments (Bishaw and Van Gastel 2009). Plant breeders aim to identify and develop cultivars with high genetic potential for productivity. This involves selecting plants with desirable traits that contribute to increased yield and other valuable characteristics. To assess the adaptability and performance of different cultivars, field evaluation trials are conducted over multiple years or in diverse environmental conditions. This helps in identifying how well a particular cultivar performs under various circumstances (Kang, 1993; Gauch and Zobel 1997; Tena *et al*. 2019). AMMI, GGE Biplot,

Table 1. AMMI ANOVA of eight advanced breeding clones of lemongrass herb yield, oil content, and citral content

Source of variation	Traits	Citral (%)		Herb yield (g/plant)		Oil content (%)	
	DF	1	2	1	2	1	2
ENV	3	13.33**	10.012	331740.10**	28.42	0.33**	9.03
GEN	7	40.43**	70.86	198338.70**	39.65	1.36**	86.63
ENV*GEN	21	3.64**	19.12	53217.97**	31.92	0.02**	4.34
PC1	9	6.20**	73.09	98186.45**	79.07	0.03**	64.26
PC2	7	2.83**	25.94	31481.06**	19.72	0.02**	31.71
PC3	5	0.15	0.97	2706.36	1.21	0.004	4.03
Residuals	64	0.49		6533.21		0.007	

*1. Mean sum of square and 2. Percent contribution of different sources of variation and YREM statistical models are employed to analyze genotype × environment interactions in plant breeding. They help breeders understand how different genotypes respond to different environments and how stable their performance is across these environments. These models can aid in the selection of well-adapted and stable varieties. By using these statistical models, plant breeders can identify "mega environments" or groups of similar environments. This categorization can help in tailoring cultivar recommendations to specific environmental conditions or regions (Kang 1993; Gauch and Zobel 1997). Ultimately, the goal of plant breeding is to recommend the best-performing and most stable genotypes for commercial cultivation by farmers in specific target environments. These recommended cultivars should provide reliable and high-yielding options for agricultural production (Anputhas *et al*. 2011).

The study employed classical ANOVA (Analysis of Variance) within a linear mixed model framework. Additionally, it utilized linear-bilinear models, including the additive main effect and multiplicative interaction (AMMI) model and genotype plus genotype-environment (GGE) models. These models are used to analyze the performance of different genotypes under varying environmental conditions. The study found a significant effect of the environment on agronomic traits, indicating that the field trials were conducted under diverse environmental conditions. This variation in environmental conditions led to variations in yield and other yield-related traits among the lemongrass clones. The significant variation in the GEI effect for the observed agronomic traits suggests that the main effects of genotype and environment alone cannot explain all the observed variation. This highlights the need to examine and understand GEI to assess the stability of the clones' performance across different environments. The present study emphasizes that the number of years for conducting experiments should be given more importance than conducting experiments in multiple locations. This implies that long-term data collection is crucial for assessing the stability of genotypes under varying environmental conditions (Singh *et al*. 2009; Singh *et al*. 2013; Kumar *et al*. 2023; Lal *et al*. 2023). The genotype × environment interactions must be considered when selecting superior genotypes. This suggests that breeders should account for how different genotypes perform under different environmental stimuli to make informed selections (Freeman, 1973). Previous research in lemongrass (Lal 2012; Kumar *et al*. 2023; Lal *et al*. 2023), vetiver (Lal *et al*. 2017 and 2022), citronella (Sunita *et al*. 2020) and Patchouli (Lal *et al*. 2023) also reported significant variation in the studied traits, indicating that GEI is a common and important aspect to consider in plant breeding.

AMMI model-based detection and characterization of GEI effects

The AMMI model combines two primary components, additive ANOVA (Analysis of Variance) and IPC (Interactions Principal Component) analysis. Additive ANOVA is used to identify the main effects of genotypes and environments. IPC analysis is utilized to explore the effects of genotype × environment interaction (GEI). The AMMI model is used because the observed performance of test genotypes in a specific environment may not accurately represent their true performance due to significant interactions between test genotypes and test environments (Inabangan-Asilo *et al*. 2019; Khan *et al*. 2021; Kumar *et al*. 2023; Lal *et al*. 2023). The model aims to separate GEI into two components: "signal" (repeatable and predictable patterns of interaction) and "noise" (non-repeatable and unpredictable interactions). The AMMI model employs multiple IPCs to effectively dissect GEI into "signal" and "noise" components. The initial IPCs capture most of the repeatable and predictable components of GEI, while later IPCs capture the non-repeatable and unpredictable components. The AMMI model is widely used in plant breeding studies due to its ability to analyze the impact of multiple environmental factors on genotypic performance. It helps understand genotype × environment interactions, which are crucial for making accurate predictions and selections in breeding programs (Crossa *et al*. 1990; Gauch, 1988; Gauch, 2013).

The present study AMMI analysis indicated that both environmental and genotypic effects were significant for herbage yield, essential oil yield, and citral content (Table 2). This suggests that these factors play a vital role in influencing the performance of tested clones and checks. Genotypic variance was found to be greater than environmental variance, indicating that genetic factors have a substantial impact on the traits, but environmental factors also play a significant role. While variance component methods may not be suitable for evaluating and understanding genotype performance over multiple years, the AMMI model is effective for representing genotype × environment (G × E) patterns.

This study found that the sum of squares (SS) attributable to the first two IPCs explained a significant percentage of the variation in GEI for citral content (99.03%), herb yield (98.79%), and oil yield (95.97%). This indicates that these IPCs captured a substantial portion of the predictable component of GEI. The significance of mean squares attributable to the first two IPCs indicated that the AMMI 2 model was the most suitable within the AMMI model family for capturing the predictable component of GEI. Selecting the best AMMI model is crucial for reliable estimates of genotypic performance and the selection of genotypes with highly predictable performance in future years (Bhan *et al*. 2005; Mukuze *et al*. 2020; Kirankumar *et al*. 2023).

From a grower's perspective, location is a constant, and yield consistency over years is a crucial aspect of genotypic performance. It suggests that growers rely on the stability of a cultivar's performance in their production environments over time (Annicchiarico *et al*. 2006; Bhagwat *et al*. 2018). The study points out the challenges of dealing with genotype × temporal environments (such as different years) because breeders cannot establish independent breeding programs for each year. The variability in genotype × year interactions, influenced by unpredictable climate conditions, makes it difficult to manage breeding programs for individual years (Spoorthi *et al*. 2021). Several researchers have identified substantial GEI for oil content, herb yield, and component features in citronella (Sunita *et al*. 2020), including Bhan *et al*. (2005), Lal, (2012), Kumar *et al*., (2022 and 2023) in lemongrass, and Lal *et al*., (2017) in vetiver.

Biplot pattern for elucidation of multivariate analysis.

Which-won-where' pattern

Figure 1 explains the use of the GGE biplot to analyze the performance of different genotypes across multiple environments for citral content, herb yield, and oil content. The polygon view of the GGE biplot is a graphical representation that helps identify winning genotypes across different environments. It involves drawing a polygon by connecting test genotypes that are farther from the biplot's origin. This polygon encompasses all the test genotypes. Equality lines, originating from the biplot origin, are drawn to divide the biplot into sectors. The vertex genotype in each sector of the biplot is considered the winning genotype for the environments whose markers fall into that sector. In this study, clone 8 consistently had the highest citral content across all four seasons and was placed in the sector that included all four environments. This makes it the winning genotype for citral content. Clones 4 and 5 were vertex genotypes in a different sector that contained no environments, and they did not produce the highest citral content in any year (Fig. 2a). The winning genotypes are supported by the actual data presented in Table 2, where clone 8 consistently ranked first in citral content. For herb yield, there was an equality line between clone 6 and clone 7, indicating that clone 7 performed better in E2 and E3, while clone 6 was better in E4. Clone 2 outperformed clone 7 in E1 (Fig. 2b). For oil content, clone 6 performed well in E1, E2, and E3, while clone 4 excelled in E4 (Fig. 2c). The equality lines divide the biplot into sectors, and the presence of different sectors suggests the existence of different mega-environments. In this study, for citral content, there was one sector, indicating the presence of one different mega-environment. Clone 8 was the consistent winner across all four environments. For herb yield and oil content, there were three and two sectors, respectively, suggesting the need to select different cultivars for different mega-environments.

The GGE biplot analysis is used to categorize genotypes and environments based on their performance. It provides a visual representation of the "which-won-where" pattern, showing which genotypes performed best in specific environments (Gauch and Zobel 1997; Fekadu *et al*. 2023; Yan *et al*. 2000). Vertex Genotypes, such as C-8, located far from the biplot origin create a polygon. Genotypes located at the vertices of a polygon are those with the highest yield in one or more environments within that sector. The results obtained from the GGE biplot analysis align with findings from previous research conducted by Fekadu *et al*. (2023), Kumar *et al*. (2022 and 2023) Lal *et al*. (2012, 2017, 2022 and 2023) indicating the consistency and usefulness of this approach in classifying genotypes and environments.

The study suggests that the selection of superior genotypes should be tailored to each mega-environment to enhance productivity. For citral content, the study identifies only one

Table 2. Estimates of AMMI model-based parameters to assess the stability of eight advanced breeding clones of lemongrass herb yield, oil content, and citral content

Traits	Citral (%)						Herb yield (g)						Oil Content (%)					
Clones	Mean	Rank	ASV	RASV	SI	Av. YREM	Mean	Rank	ASV	RASV	SI	Av. YREM	Mean	Rank	ASV	RASV	SI	Av. YREM
1	78.32	7	0.79	4.00	4.79	0.94	539.23	7	1.47	7.00	8.47	0.60	2.04	3	0.64	1.00	1.64	0.93
2	79.28	2	0.82	5.00	5.82	0.95	750.27	2	0.63	4.00	4.63	0.86	2.08	2	0.74	3.00	3.74	0.95
3	78.25	8	0.20	1.00	1.20	0.94	583.95	5	0.53	3.00	3.53	0.67	1.94	5	0.99	5.00	5.99	0.89
4	77.81	5	1.02	6.00	7.02	0.93	613.74	4	0.32	2.00	2.32	0.72	1.99	4	1.52	7.00	8.52	0.91
5	78.79	6	1.76	8.00	9.76	0.94	544.30	6	0.27	1.00	1.27	0.61	1.92	6	2.03	8.00	10.03	0.88
6	78.82	4	1.08	7.00	8.08	0.94	738.39	3	1.00	5.00	6.00	0.77	2.16	1	1.31	6.00	7.31	0.99
7	79.06	3	0.60	3.00	3.60	0.95	817.65	1	4.01	8.00	12.01	0.92	1.31	8	0.87	4.00	4.87	0.60
8	83.63	1	0.37	2.00	2.37	1.00	482.20	8	1.22	6.00	7.22	0.56	1.32	7	0.68	2.00	2.68	0.60

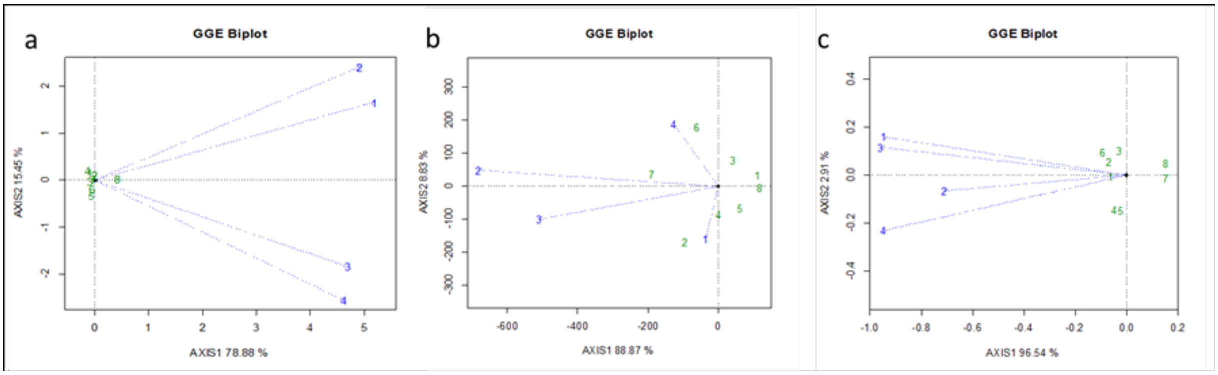

Fig. 1. Average environment coordination (AEC) view of GGE-biplot for identification of test genotypes relative to ideal genotypes for a. Citral (%), b. Herb yield plant^{-1} and c. Oil content (%)

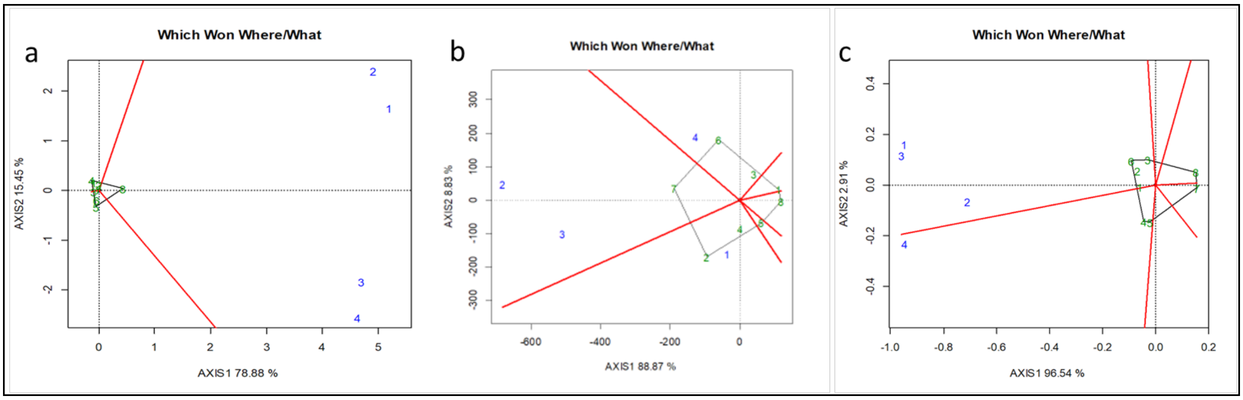

Fig. 2. Polygon view of GGE-biplot based on the symmetrical scaling for "which won-where" pattern of test genotypes and environments for a. Citral (%), b. Herb yield plant^{-1} and c. Oil content (%).

mega-environment encompassing E1, E2, E3, and E4. Within this mega-environment, the vertex genotype (clone 8) is highlighted as the best performer, suggesting that this genotype consistently yields the highest citral content across all tested environments. Three mega-environments are identified for herb yield. The first mega-environment includes E4 and E1, while the second consists of E2 and E3, E4. The third mega-environment is specific to E1. Within these mega-environments, the study indicates that genotypes (clone 2, clone 6, clone 7, and clone 8) perform best for herb yield. Two mega-environments were formed for oil content. The first one includes E4 and E1, while the second consists of E2 and E3, E4. Within these mega-environments, specific genotypes (clone 4, clone 5, clone 6, clone 7, and clone 8) were identified as the top performers in terms of oil content.

Yan and Tinker (2006), and Alidu *et al*. (2017) also stated that genotypes located on the vertices of the polygon perform either the best or the poorest in one or more environments. The identified mega-environments, which group together specific genotypes and environments based on performance, are not necessarily consistent from year to year. These groupings may vary depending on the climatic conditions and other factors in different years. To determine reliable and consistent mega-environments for lemongrass production, it is essential to conduct multiyear experiments (Fekadu *et al*. 2023). This approach helps account for the year-to-year variability and provides a more robust understanding of how genotypes perform across different growing seasons. The identification of mega-environments should be verified through multiyear experimental trials conducted in the specific target environments where crop is grown. This verification ensures that the identified groupings are applicable and reliable in real-world conditions (Fekadu *et al*. 2023; Gezahagn *et al*. 2023).

Discriminative ability and representativeness of test environments

The GGE biplot view demonstrates the discriminative capabilities and representativeness of the test environments. The length of the vectors reflects the test environments' discriminating capacity, and the angle between the vectors and the AEC abscissa (or AEA) indicates the test environments' representativeness. The average environment has the average coordinates of all test environments and is represented by the little circle at the end of the arrow on the AEA line that goes through the average environment and the biplot origin. In the present investigation, Figure. 3a, 3b, 3c illustrated the 'discriminativeness vs. representativeness' of the GGE biplot study for citral, herb yield, and oil content that were considered unique or ideal based on the lengths and angles of their environment vectors. For citral, E3 was identified as unique due to its short vector. For herb yield, E1 and E4 were noted as unique because of their short vectors. For oil content, E2 was singled out due to its short vector. Environments with long vectors are influential in discriminating among genotypes, indicating their capacity to distinguish differences. However, the ideal test environment for selecting superior genotypes is one with a long vector that forms a shorter angle with the AEC abscissa line. This indicates that it is both discriminative and representative. In this study, specific environments (E1 for citral, E2 and E3 for herb yield, and E3 for oil content) were identified as suitable for the selection of superior genotypes based on these criteria.

In this study, E1 (for citral), E2, E3 (for herb yield), and E3 (for oil content) are identified as having longer vectors. This suggests that these environments provide more information about the genotypes, making them better at discriminating between genotypes based on their performance. On the other hand, E3 (for citral), E1 and E4 (for herb yield), and E2 (for citral) have shorter vector lengths, coupled with smaller PC2 scores. These environments are considered to have lower discriminating

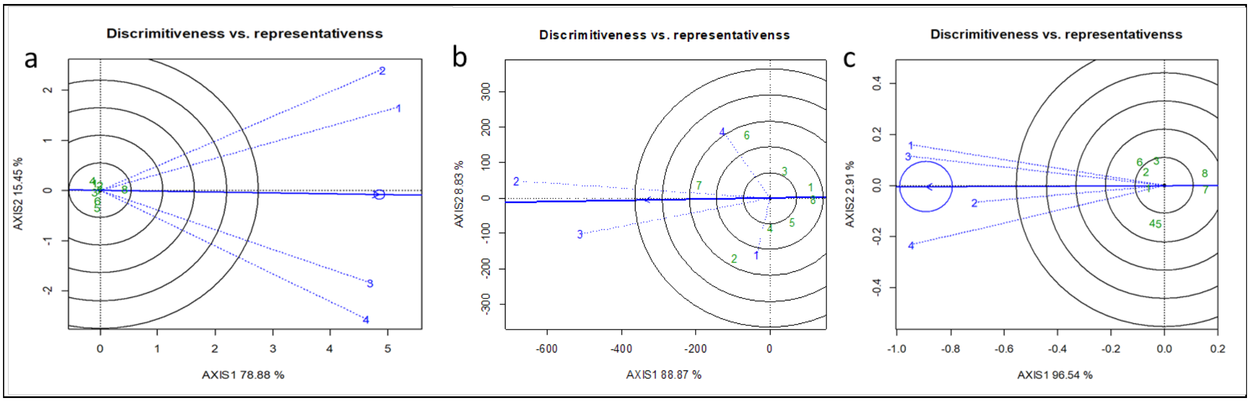

Fig. 3. Discriminative vs. representativeness view of GGE-biplot for a. Citral (%), b. Herb yield plant^{-1} and c. Oil content (%).

ability and offer less information about the genotypes (Alidu *et al*. 2017; Yan and Tinker, 2006; Yan *et al*. 2007, 2010). Environments with longer vectors and larger angles with the AEC (Average Environment Coordinate) abscissa are considered more suitable for identifying specifically adapted genotypes rather than for selecting high-yielding genotypes. These environments help in recognizing genotypes that excel under specific conditions (Yan and Tinker 2006; Yan *et al*., 2007; Khan *et al*. 2021). The most discriminating and representative environments are recommended as the best testing environments for the selection of high-yielding genotypes. These environments are likely to provide the most valuable information for breeding and cultivar selection (Khan *et al*. 2021).

Mean performance vs. Stability patterns

The AEC view of the GGE biplot is used to visualize the mean performance and stability of genotypes. The AEC arrow points in the direction of higher mean performance, indicating which genotypes perform better on average across the test environments. Genotypes located closer to the AEC arrow exhibit higher mean performance, while those located in the opposite direction have lower mean performance. The length of the projections of genotypes from the AEC provides information about their stability. Shorter projections indicate greater stability, while longer projections suggest lower stability.

The "Mean vs. Stability" view simplifies the assessment of genotypes based on their mean performance and stability across a range of environments (Fig. 4a, 4b, 4c). Biplot consists of two straight lines like the AEC abscissa (vertical) and the AEC ordinate (horizontal). In the present study, the "Mean vs. Stability" pattern of the GGE biplot revealed that a significant percentage of the variation (94.33% for citral content, 97.70% for herb yield, and 99.45% for oil content) in the studied traits was explained by genotype (G) and genotype-environment interaction (G × E). Clone 8 consistently produced higher citral content in all four environments, as indicated by its position relative to the AEC arrow. For herb yield, clone 7 followed by clone 2 and clone 6 were not only high-performing but also stable across the tested environments. In the case of oil content, clone 2 exhibited higher oil yield followed by clone 3 and clone 6 in all environments. Clone 2 was also highly stable.

The GGE biplot analysis revealed significant differences in citral content, herb yield, and oil content performance among testing environments. The genotypes on the left side of the ordinate were regarded adaptable or high yielding, whilst those on the right side of the ordinate were judged nonadaptable or low yielding. Genotypes positioned distant from the second PC ordinate in either direction, on the other hand, were unstable, and those closer to the ordinate in either direction were stable (Berhanu *et al*. 2023). The AEC ordinate line crossed through the origin of the GGE biplot, and genotypes with yields above the grand mean were situated on the left side of the AEC ordinate, while genotypes with low yields were located on the right side of the AEC ordinate. The genotype vector projections that are parallel to the AEC ordinate or perpendicular to the AEC abscissa in either direction represent the degree of genotypic stability across testing conditions (Yan 2001; Yan and Hunt 2002). Clones 8 for citral, 2 and 7 for herb yield, and 2 for oil content were considered stable genotypes among the high-yielding clones.

AMMI Model-based Stability Parameters

AMMI Stability Value (ASV) is a measure of stability that provides an objective way to assess how stable are the genotypes across different environments. It is calculated as the distance

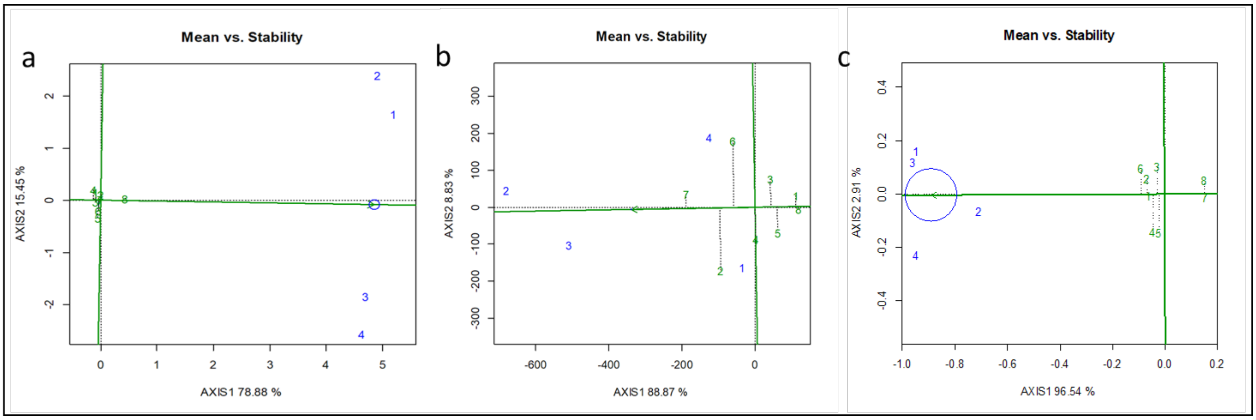

Fig. 4. Biplot based on environment-focused scaling for the mean performance vs. stability of test genotypes for a. Citral (%), b. Herb yield plant^{-1} and c. Oil content (%).

from the origin (zero) in a two-dimensional scatter plot of IPC 1 scores against IPC 2 scores. In this study, ASVs were estimated using both IPC 1 and IPC 2 because these components significantly contributed to the total genotype-environment interaction (GEI) variance for citral content, herb yield per plant, and oil content. For citral content, clone 3 and clone 8 were identified as stable genotypes with lower ASV estimates (0.20 and 0.37, respectively). For herb yield per plant, clones 5 (ASV = 0.27) and 6 (ASV = 0.32) were considered stable across the four environments. For oil content, clones 1 (ASV = 0.64) and clone 8 (ASV = 0.68) were identified as stable, although it's noted that clone 8 had lower oil yield compared to other clones. Lower ASV values indicate greater stability, suggesting that the genotypes' performance is less affected by varying environmental conditions. It's important to note that stability does not necessarily mean the highest performance. Clone 8, for example, was stable for oil content but had lower oil yield compared to other clones.

The Stability Index (SI) is indeed a valuable tool for selecting genotypes that exhibit both high mean values for specific traits and stability across different environments. It simplifies the simultaneous selection process by considering both performance and consistency. In the present study, Clones 3 and 8 were identified as the best clones for citral content (Table 2). Their low SI values indicate that they not only have high mean citral content but are also stable across the different environments tested. Clones 4 and 5 were identified as the best and most stable clones for herb yield. Clone 1 was noted as being stable across the four environments for oil content.

The simultaneous selection of genotypes with the desired performance for mean yield and stability is made easier by SI, which considers both mean yield and stability in a single criterion. Low SI genotypes are thought to provide high yields and stability. Clones 3 and 8 for citral, clones 4 and 5 for herb yield, clones 1 for oil content with a lower magnitude of SI, and clones 3 and 8 for oil content and stability were considered the best genotypes in the current study. Numerous researchers have also discovered genotypes that are stable throughout time, including Vaijayanthi *et al.* (2017), Kavya and Rangaiah, (2019), Ashwini *et al.* (2021), Berhanu *et al.* (2023) and Kirankumar *et al.* (2023).

Yield Relative to Environment Maximum (YREM)

Considering that YREM is a simple statistic

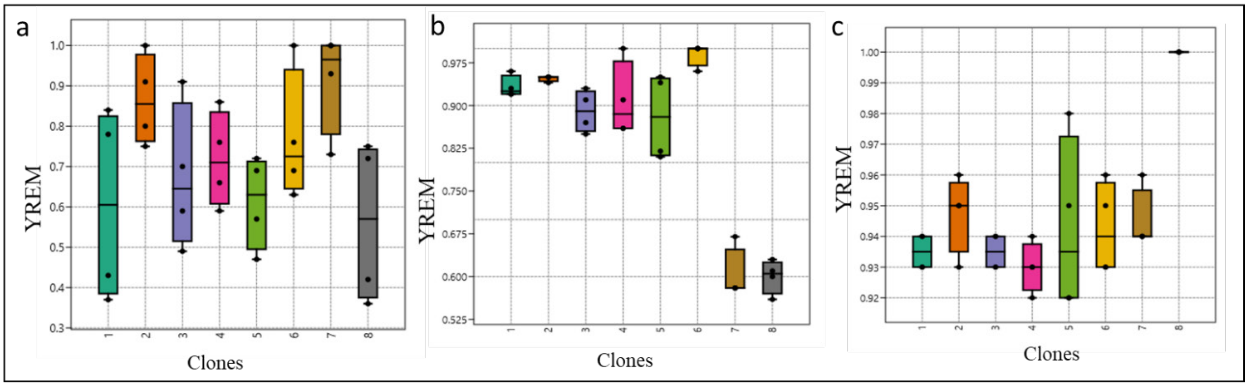

Fig. 5. Box-Whisker plots depicting YREM comparative performance of lemongrass clones differing in a. Citral (%), b. Herb yield plant^{-1} and c. Oil content (%)

that is independent of genotypes' attendance, it could be used as a predictor of genotypes' performance in future years. In the present study, unit YREM of clone 8 (Table 2 and Fig. 5) indicates that its interaction with the four test environments is of non-crossover type for citral content. Unit YREM of clone 8 also indicates that it remained the highest citral content in all the four environments and its citral yield potential as assessed in the present study is attainable in all the test temporal environments without any loss, even if there exists cross-over GEI. Thus, clone 8 has significantly higher citral yield potential and stability than all the clones. The average YREM of herb yield and oil content of lemongrass clones across seasons ranged from 0.56 to 0.92, and 0.60 to 0.99 which indicate 8–44%, 1–40%, loss in herb yield plant^{-1} and oil content respectively attributable to crossover GSI.

According to YREM, an intuitive and genotypes' attendance-independent assessment of the test genotype's performance (Yan 1999), clone 8 suffers a considerably lower loss in citral than other clones, indicating that its interaction with the four test environments is of the non-crossover type. Clone 8's unit YREM further suggests that it remained the highest yielder in all four environments, and its citral yield potential, as determined in this study, is attainable in all test temporal environments without loss, even if cross-over GEI exists. However, for herb yield and oil content, none of the clones shared one YREM, indicating a yield loss due to clone's crossover GSI. In the absence of crossover GSI, the average YREM of clones tested in various conditions must be 1.0. Any deviation in a genotype's YREM from 1.0 is attributed to a loss in achievable yield due to crossover GSI (Yan 1999). YREM has also been used by Ashwini *et al*. (2021), Spoorthi *et al*. (2021), Kirankumar *et al*. (2023) and Shivakumar *et al.* (2024) to detect crossover GEI and identify stable genotypes.

Conclusion

The study found that the genotype had the greatest influence on lemongrass herb yield, citral content, and oil content performance followed by the GEI and environmental effects. This implies that the genetic characteristics of different lemongrass genotypes significantly affect these traits. AMMI and GGE Biplot Models are helpful for visually analyzing data from multi-environment trials and estimating the interaction effects of genotypes in different environments. Based on AMMI, GGE biplot models, GSI, and YREM it was determined that clone 8 (CIM-Shikhar) check variety outperformed compared to all other clones and identified as stable for citral content. Clones 7 and 5 were stable for herb yield. While, clone 1 was found to be stable for oil content.

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