ARTICLE

Stable clone selection for oil and herb yield using GGE biplot model in climate-smart lemongrass

Crop Breeding and Applied Biotechnology
24(4): e501324419, 2024
Brazilian Society of Plant Breeding.
Printed in Brazil
http://dx.doi.org/10.1590/1984-70332024v24n4a56

K. V Ashwini[1], Gazala Parveen S[1] and Channayya Hiremath[1*]

Abstract: *Lemongrass, an industrially important aromatic grass known for its essential oil, exhibits phenotypic traits that are influenced by environmental factors. This study evaluated the stability and adaptability of 13 advanced lemongrass breeding clones over several years. Significant genotype and genotype × environment interaction (GEI) effects on herbage yield and essential oil yield highlighted the influence of genetic and environmental factors on these traits. The AMMI and GGE biplot analyses identified clone 5 as a high performing and stable genotype for herb yield. Meanwhile, clones 1 and 4 showed consistent performance across years and were desirable for essential oil yield due to their proximity to the abscissa of the biplot. These identified clones represent valuable genetic resources for future breeding programs aimed at developing superior lemongrass cultivars for commercial cultivation.*

Keywords: *Stability, AMMI, advanced breeding clones, multi-year, aromatic grasses*

INTRODUCTION

Lemongrass (*Cymbopogon flexuosus* Stapf) is a vital perennial aromatic grass, widely cultivated in tropical and subtropical regions for its essential oil. India is the leading global producer of lemongrass oil, followed by China and several Latin American countries. Known for its distinctive lemon scent, lemongrass oil finds applications in soaps, perfumery, and cosmetics. Indian lemongrass oil is especially valued for its high citral content, a key aromatic compound (Vimala et al. 2022) critical for synthesizing vitamin A and β-ionones (Kumar et al. 2022). In India, lemongrass is predominantly cultivated in Odisha, Kerala, Karnataka, Maharashtra, Telangana, and Andhra Pradesh (Kumar et al. 2022, Vimala et al. 2022). Lemongrass is believed to have originated in Malaysia or Sri Lanka, but it is now grown extensively in tropical regions such as the West Indies, Guatemala, Brazil, Congo, Tanzania, Thailand, Bangladesh, Madagascar, and China.

Genotypic stability is a critical consideration in plant breeding due to the significant influence of environmental conditions on plant growth and the temporal variations. To effectively assess the performance of genotypes under diverse conditions, it is essential to evaluate them across multiple environments and over several years (Gupta et al. 2015). This approach helps to understand how genotypes respond to varying growing conditions and ensures consistency in their performance over time. Plant breeding efforts primarily focus on evaluating breeding lines that serve as genetic resources for varietal

***Corresponding author:**
E-mail: channayyahiremath@cimap.res.in
ORCID: 0000-0001-8977-8254

Received: 25 July 2024
Accepted: 08 October 2024
Published: 05 November 2024

[1] CSIR - Central Institute of Medicinal and Aromatic Plants, Plant Breeding and Genetic Resource Conservation, Research Centre, Allalasandra, Yelahanka, Bengaluru, Karnataka 560065, India

development programs. The ultimate goal is to identify and select advanced breeding lines that possess desirable traits and demonstrate phenotypic stability across different environments and timeframes. Phenotypic stability is particularly important because breeders strive to develop high-yielding varieties that consistently perform well under a wide range of environmental conditions (Sunita et al. 2020).

The phenotype (P) of a genotype (G), which represents its observable characteristics is significantly influenced by its interaction with the environment (G×E). This interaction plays a critical role in determining the performance of a genotype under different environmental conditions. While, some genotypes may exhibit different traits when grown in different environments, others may exhibit consistently similar traits across a wide range of conditions (Fehr et al. 1991). Understanding this variability in genotype performance across environments is a key consideration in plant breeding. To evaluate and account for the variation resulting from the genotype-environment interaction (GEI), several biometric techniques have been developed to assess the stability and performance of genotypes. One widely used method is the additive main effects and multiplicative interaction (AMMI) biplot. This approach integrates additive main effects (genotypic effects) with multiplicative interaction effects (GEI), providing a robust statistical framework for stability testing. The AMMI biplot provides a reliable criterion for analyzing yield trials, especially in the presence of complex GEI.

The practical need to combine higher mean yield with superior stability has led to the development of the yield reliability concept (Eskridge 1990, Kang and Pham 1991). A reliable variety is characterized by consistently high essential yield across multiple years and locations (Annicchiarico 2002). By merging mean yield and yield stability into a single measure of genotype merit, the yield reliability index facilitates the selection and recommendation of superior varieties (Annicchiarico 2002). Previous studies on stability and GEI in lemongrass and other *Cymbopogon* species remain limited (Lal 2012, Kumar et al. 2022). Therefore, the aim of the present study was to evaluate the stability, reliability, and performance of 13 advanced breeding clones of lemongrass and provide recommendations for their potential commercial application.

MATERIAL AND METHODS

Materials

The present study utilized 11 advanced breeding clones and two check varieties of lemongrass (*Krishna* and *CIM-Shikhar*) (Supplementary Table 1).

Experimental site and design

The experiment was conducted at the CSIR-Central Institute of Medicinal and Aromatic Plants (CSIR-CIMAP) in Bengaluru, India, from June 2018 to April 2021. Meteorological data for the experimental period is provided in Supplementary Table 2. The study site is located at lat 11° 7’ N, long 77° 59’ E, alt 426 m asl. The soil at the site had a pH of 5.91 and an electrical conductivity of 0.07 dS/m. The experimental setup used a randomized block design (RBD) with two replications. The advanced breeding clones and check varieties were planted in two rows of 3.6 meters in length, with a spacing of 45 × 45 cm between plants. Recommended agronomic practices, including fertilizer application and pest and disease management, were consistently followed throughout the crop growth period.

Trait measurement

During the crop growth period, data were collected on herbage yield per plant (g) and essential oil content (%) from five randomly selected plants for each clone and check variety in each replication over four years. Essential oil was extracted using a Clevenger apparatus (Clevenger 1928) through hydro-distillation for three hours. Observations were recorded quarterly, and the trait means were calculated for statistical analysis.

Statistical analysis

Single-season analysis of variance (ANOVA) and combined ANOVA were performed by using the *Online tool for the analysis of a series of experiments in randomized block designs*. AMMI and biplot analyses were conducted by using GEA-R (*Genotype × Environment Analysis with R for Windows*), Version 4.1 (2017-08-3).

RESULTS AND DISCUSSION

The mean herb yield and essential oil content of the 13 advanced breeding clones of lemongrass are presented in Supplementary Tables 3 and 4, respectively. Advanced breeding clone 12 recorded the highest mean herb yield in two environments (2018 and 2019), while clone 3 excelled in the other two environments (2020 and 2021). Across all environments, clones 3 and 12 consistently outperformed the others, achieving the highest grand mean herb yield. Similarly, multiple advanced breeding clones produced higher essential oil content in three environments (2018, 2019, and 2020). Clones 3 and 6 stood out for their superior essential oil yield across all environments.

Single-season and combined ANOVA

The individual season analysis of variance (ANOVA) revealed significant ($p < 0.05$) differences among the evaluated lemongrass genotypes for both herb yield and essential oil content. The single-season and pooled ANOVA results are shown in Tables 1 and 2, respectively. The mean sum of squares for genotypes, environments, and GEI was highly significant ($p \leq 0.01$, $p \leq 0.05$) for both herb yield per plant and oil content. These significant differences among environments and genotypes highlight the influence of environmental conditions and genetic variability.

The observed significant variance due to GEI underscores the interaction between lemongrass genotypes and diverse environmental conditions. This interaction reveals that genotypes responded differently across seasons, underscoring the importance of evaluating genotypes under diverse environments. However, variance component analysis alone does not fully elucidate the details of GEI. Advanced statistical techniques, such as multivariate analyses including AMMI and biplot models, are more effective in dissecting and understanding these interactions (Khan et al. 2021). The environment's partitioning of the variance component revealed that both predictable (locations) and unpredictable (seasons) factors were key sources of variation. When GEI is analyzed in relation to predictable components, plant breeders can opt to select genotypes tailored to specific environments or develop genotypes that perform consistently across multiple environments (Dehghani et al. 2006).

AMMI analysis

The AMMI-based analysis of variance revealed significant GEI for the traits under study, indicating that the performance of lemongrass genotypes varied across environments (Table 3). This underscores the importance of identifying stable lemongrass genotypes with consistent performance across diverse conditions. The % contribution to variation for herb

Table 1. Mean sum of squares of analysis of variance of lemongrass for herb yield and essential oil content in four seasons (S)

Source of variation	df	Mean squares							
		Herb yield (g)				**Essential oil yield** (%)			
		S1	**S2**	**S3**	**S4**	**S1**	**S2**	**S3**	**S4**
Replication	1	8,406.84	1,927.94	9,286.77	143.71	0.001	0.001	0.01	0.02
Treatments	12	216,235.56**	166,221.68**	156,436.19**	131,419.22**	0.11**	0.21**	0.17**	0.22**
Error	12	26,934.38	1,893.23	31.14	22,341.80	0.003	0.002	0.005	0.011

Table 2. Combined analysis of variance for herb yield and essential oil yield in different lemongrass clones

Source of variation	df	Mean squares	
		Herb yield (g)	**Essential oil yield** (%)
Environment	3	157,323.07**	4,542.30**
Replication	3	74.766	1.234
Genotype	12	1,476.06**	162.87**
Genotype × environment	36	1,216.22**	14.24**
Pooled error	48	1.010	1.000
CD (environments)		6.429	0.826
CD (genotypes)		1.347	1.340
CD (environment × genotype)		2.694	2.681

**: P < 0.05

Table 3. AMMI analysis of variance for herb yield and essential oil yield in lemongrass clones

Source of variation	df	Mean squares	
		Herb yield (g/p)	**Essential oil yield** (%)
Environment	3	258216.4**	0.13938**
Genotype	12	453841.7**	0.642**
GSI	36	63887.02**	0.02776**
PC1	14	122498.6**	0.0405**
PC2	12	34633.21**	0.02755**
PC3	10	16935.37**	0.01017*
Residuals	52	6907.92	0.00562

**: $P < 0.05$

yield was 63.91% from genotypes, 9.09% from environments (seasons), and 26.99% from GEI. For oil content, the contribution was 84.46% from genotypes, 10.95% from environments, and 4.58% from GEI. These results demonstrate that the largest source of variation for both traits was genotypic variance, followed by environmental factors and GEI. The GEI component was further subdivided into three principal components (PCs). The first principal component explained most of the variation observed for both herb and oil yield, followed by the second and third components. This aligns with findings from Lal et al. (2024), who conducted ANOVA by regression and reported significantly higher herbage yield, essential oil yield, citral content, and total essential oil yield in the check variety *Jor Lab L-8* compared to two other checks.

The relationship between the performance of lemongrass genotypes and the environment is effectively visualized using biplots, a highly useful tool for data interpretation. Based on the AMMI 1 biplot, advanced breeding clones 3, 4, and 5 exhibited positive interaction principal components analysis (IPCA) scores for herb yield (Figure 1a). Similarly, clones 1, 2, 3, and 6 demonstrated positive IPCA scores for essential oil content (Figure 1b). Notably, clone 5 was positioned closer to an IPCA score of zero for herb yield, and clone 1 for essential oil content, indicating their stability in performance across environments.

The clones 1 and 5 were identified as stable performers for herb yield (Figure 1a), as they were located near the origin in the biplot. For essential oil content, clones 3 and 6 were similarly identified as stable (Figure 1b).

GGE Biplot

Polygon view of the GGE biplot

In a GGE biplot, the environment is represented as a vector and the genotype as a point within a coordinate system. The polygon view of the GGE biplot is constructed by connecting the points of extreme genotypes to form a polygon. “Sectors” are delineated by thick lines radiating from the origin, intersecting each side of the polygon, and separating the coordinate space into zones. These zones, separated by the thick lines, represent mega-environments. Genotypes located within the same sector are closely related in their performance. The genotypes at the vertices of the polygon are either the best or the worst performers in the environments that fall within their respective sectors. This biplot view provides a clear and practical framework for identifying and recommending genotypes best suited for specific environments. For the current study, the results revealed that all four seasons used to assess stability in lemongrass were located within the same sector of the polygon for both herb yield and essential oil content. The “which-won-where” biplot for herb yield (Figure 2a) indicated that seasons 1 and 2 fell within the same sector or mega-environment, where advanced breeding clone 12 emerged as the best performer. Conversely, seasons 3 and 4 were grouped in a separate sector, where clone 3 produced the highest herbage yield. For essential oil content (Figure 2b), clone 6 was identified as the top yielder in season 1, while clone 5 outperformed others in season 2.

Discriminativeness vs. representativeness of the environments

This analysis helps evaluate the discriminating ability and representativeness of the test environments (in this case, seasons). For herb yield, environments 1, 2, and 3 were more discriminating compared to environment 4 (Figure 3a). In contrast, for essential oil content, environments 2 and 4 showed greater discriminating ability (Figure 3b). The biplots also facilitated the identification of the most representative environment among the tested environments. This was

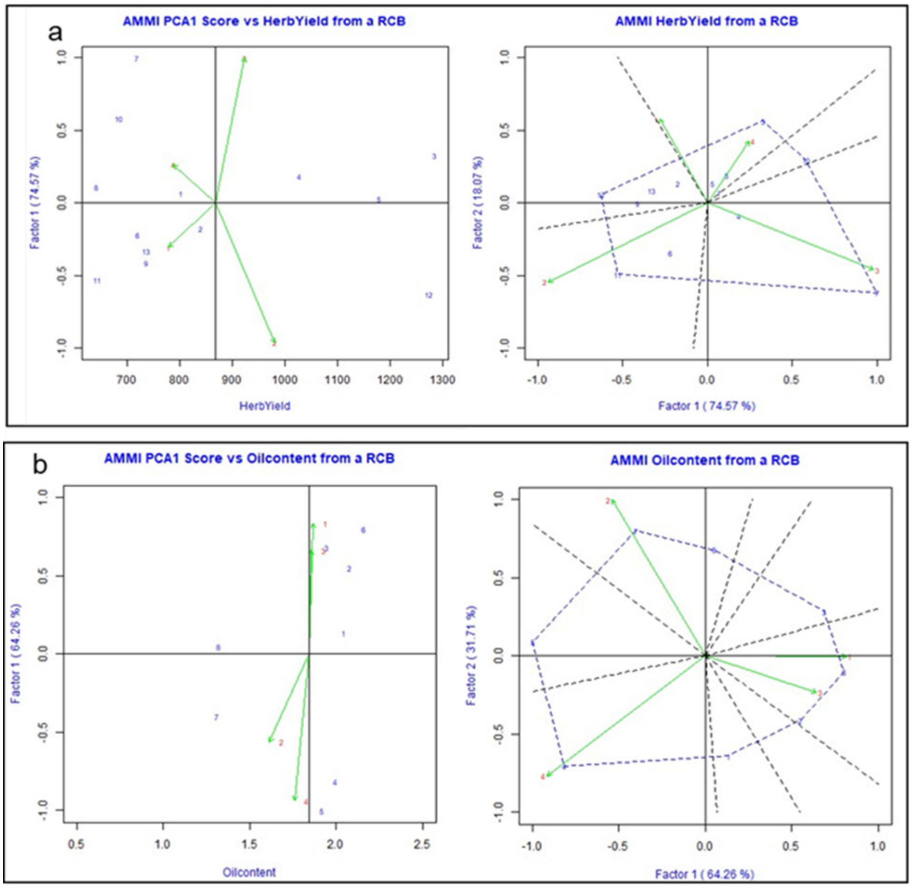

Figure 1. AMMI biplots for herb yield (a) and essential oil yield (b).

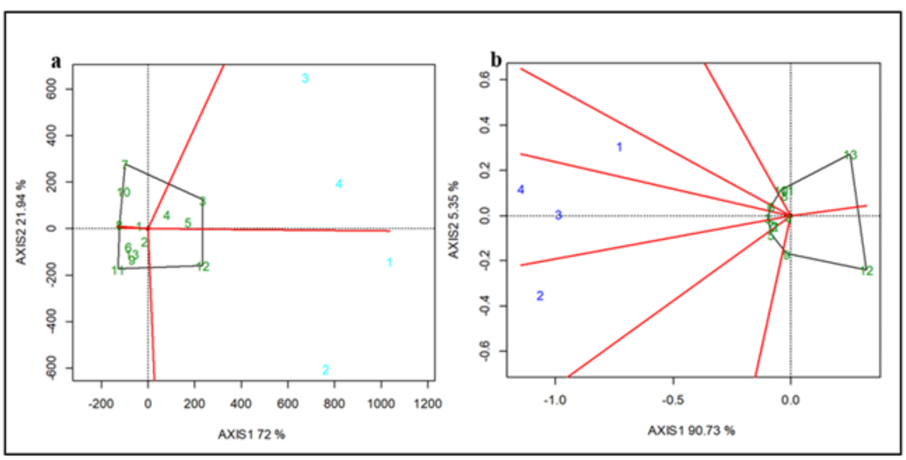

Figure 2. Polygon view of the GGE biplot based on symmetrical scaling, illustrating the "which-won-where" pattern for (a) herb yield and (b) essential oil yield.

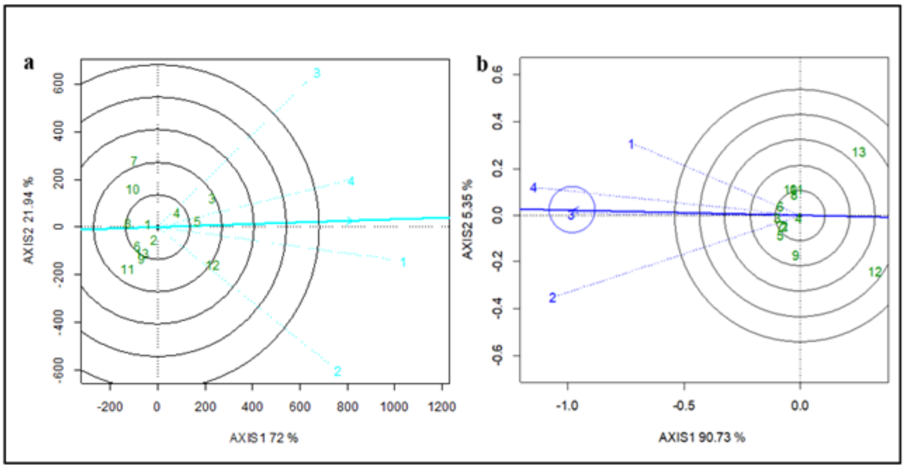

Figure 3. Discriminative and representativeness view of the GGE biplot for (a) herb yield and (b) essential oil yield.

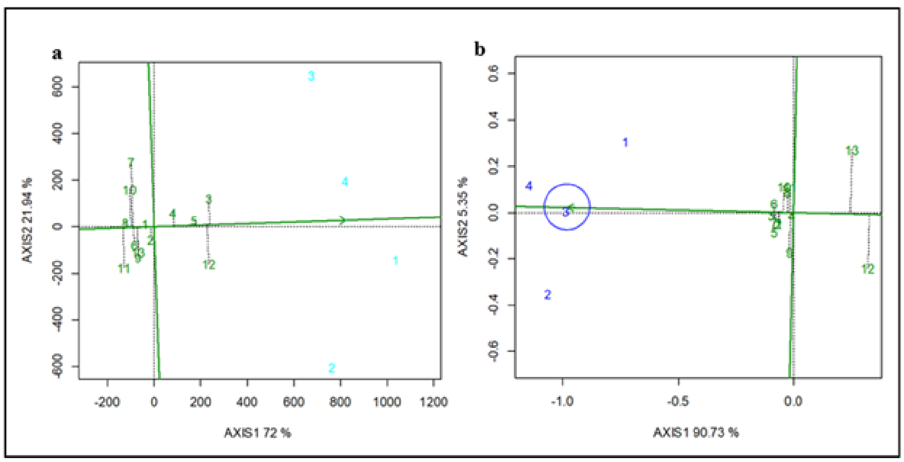

Figure 4. Average Environment Coordination (AEC) view of the GGE biplot based on environment-focused scaling, illustrating mean performance stability for (a) herb yield and (b) essential oil yield.

determined by the angle between the environmental vectors and the Average Environment Coordinate (AEC) axis, which passes through the biplot origin and represents the average of all test environments. For herb yield, environment 1 was the most representative of all test environments (Figure 4a), whereas for essential oil content, environment 3 emerged as the most representative (Figure 4b). These findings suggest that herb yield data collected from environment 1 and essential oil yield data from environment 3 are more reliable and can serve as benchmarks for evaluating genotypic performance.

Mean vs. stability of genotypes

The performance and stability of advanced breeding lines of lemongrass for herb yield and essential oil content were assessed using the Average Environment Coordinate (AEC) method (Yan 2001, Yan and Hunt 2002). In this method, an average environment is represented by a small circle in the biplot, based on the overall average PC1 and PC2 scores of the environments. A line is then drawn through this point and the biplot origin. Perpendicular lines extending from this axis represent genotypic stability. Greater distances from the origin along these lines indicate higher GEI and lower stability (Yan and Hunt 2002). The biplot analysis revealed that advanced breeding clones 8,

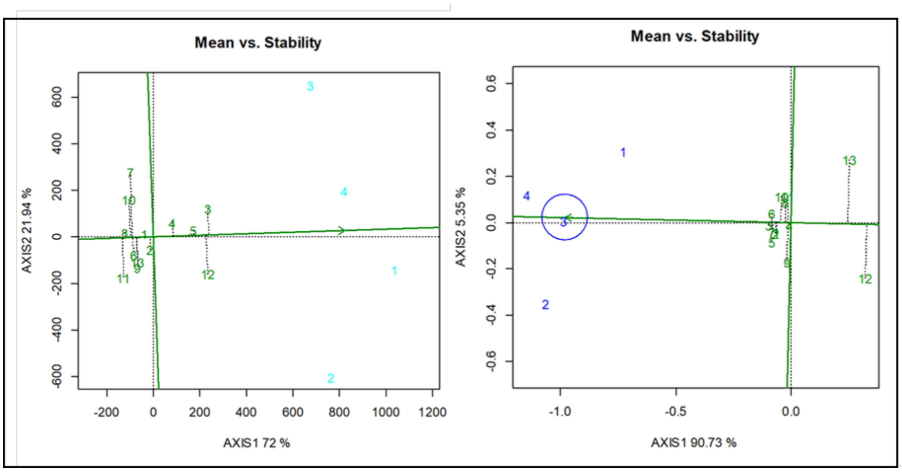

***Figure 5**.* Mean vs. stability view of the GGE biplot for (a) herb yield and (b) essential oil yield.

***Figure 6**.* (a) General field view of the advanced breeding clones of lemongrass. (b) Representative image of clone 5 (B2 6-4).

1, and 5 were highly stable for herb yield (Figure 5a). For essential oil yield, clones 4, 3, and 6 exhibited stability, as they were positioned closer to the AEC abscissa (Figure 5b). Using the same biplot, high-yielding genotypes were also identified. Advanced breeding clones 4, 5, 3, and 12 were recognized as high herb-yielding lines, as they were located on the right side of the biplot. The overall field view of the experimental site is shown in Figure 6a. However, for a genotype or variety to be considered desirable, it must exhibit both superior performance and consistent yield across environments (Yan and Tinker 2006). Based on this criterion, clones 5 (Figure 6b) and 4 were identified as high herb yielders with stable performance. Conversely, none of the clones demonstrated a relatively higher essential oil yield with good stability.

CONCLUSION

This study demonstrated significant variation among the 13 advanced breeding clones of lemongrass, the environments, and their interactions. The AMMI analysis of variance highlighted a significant GEI for all traits studied. Based on the AMMI1 biplot, advanced breeding clone 5 was identified as stable for herb yield, while clone 1 showed stability for essential oil content. The polygon view of the GGE biplot identified clone 12 as a superior herb yielder in environments 1 and 2, while clone 3 excelled in environments 3 and 4. For essential oil content, clone 6 performed best in environment 1, clone 5 in environment 2, and clone 3 in environments 3 and 4. Additionally, clone 5 for herb yield and clone 4 for essential oil content were considered ideal for their respective traits. These promising clones should undergo multi-location trials to validate their stability and performance before being recommended for commercial cultivation.

ACKNOWLEDGEMENTS

The authors extend their sincere gratitude to the Director of CSIR-CIMAP, Lucknow, for the invaluable support and provision of laboratory and experimental field facilities essential for this study.

DATA AVAILABILITY

This paper contains all of the data that supports the study's conclusions. Supplementary files can be obtained by contacting the corresponding author.

REFERENCES

Annicchiarico P (2002) Genotype × environmental interactions; challenges and opportunities for plant breeding and cultivar recommendations. FAO, Rome (**FAO plant production and protection paper, 174**).

Clevenger JF (1928) Apparatus for the determination of volatile oil. **The Journal of the American Pharmaceutical Association 17**: 345-349

Dehghani H, Ebadi A and Yousefi A (2006) Biplot analysis of genotype by environment interaction for barley yield in Iran. **Agronomy Journal 98**: 388-393.

Eskridge KM (1990) Selection of stable cultivars using a safety-first rule. **Crop Science 30**: 369-374.

Fehr WR, Welke GA, Hammond EG, Duvick DN and Cianzio SR (1991) Inheritance of reduced palmitic acid content in seed oil of soybean. **Crop Science 31**: 88-89.

Gupta P, Dhawan SS and Lal RK (2015) Adaptability and stability-based differentiation and selection in aromatic grasses (*Cymbopogon species*) germplasm. **Industrial Crops and Products 78**: 1-8.

Kang MS and Pham HN (1991) Simultaneous selection for yielding of stable crop genotypes. **Agronomy Journal 83**: 161-165.

Khan MMH, Rafii MY, Ramlee SI, Jusoh M and Mamun MA (2021) AMMI and GGE biplot analysis for yield performance and stability assessment of selected Bambara groundnut (*Vigna subterranea* L. Verdc.) genotypes under the multi-environmental trials (METs). **Scientific Reports 11**: 22791.

Kumar A, Jnanesha AC, Kumar VM and Lal RK (2022) GGE biplot vs. AMMI analysis of genotype-by-environment data on essential oil yield in lemongrass [*Cymbopogon flexuosus* (nees ex. Steud) wats.] grown in semi-arid tropical regions of southern India under different agro-climatic conditions. **Biochemical Systematics and Ecology 103**: 104439.

Lal M, Gogoi A, Gupta T and Begum T (2024) Deciphering the stability indices using additive main effect and multiplicative interaction model for high yielding registered variety of Lemongrass (Jor Lab L-8). **Journal of Essential Oil Bearing Plants**: 1-2.

Lal RK (2012) Stability for oil yield and variety recommendations' using AMMI (additive main effects and multiplicative interactions) model in Lemongrass (*Cymbopogon species*). **Industrial Crop Products 40**: 296-301.

Sunita M, Neelav S and Mohan L (2020) GxE interaction of 72 accessions with three-year evaluation of *Cymbopogon winterianus* Jowitt. using regression coefficient and Additive Main effects and Multiplicative Interaction model (AMMI) Industrial. **Industrial Crops and Products 146**: 112169.

Vimala Y, Lavania UC, Singh M, Lavania S, Srivastava S and Basu S (2022) realization of lodging tolerance in the aromatic grass, *Cymbopogon khasianus* through ploidy intervention. **Frontiers in Plant Science 13**: 908659.

Yan W (2001) GGE biplot - a window application for graphical analysis of multi-environment trial data and other types of two-way data. **Agronomy Journal 93**: 1111-1118.

Yan W and Hunt LA (2002) Biplot analysis of multi-environment trial data. In Kang MS (ed) **Quantitative genetics, genomics and plant breeding**. CABI Publishing, Wallingford, p. 289-303.

Yan W and Tinker NA (2006) Biplot analysis of multi environment trial data: principles and application. **Canadian Journal of Plant Science 86**: 623-645.