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STUDY ON VARIABILITY AND STABILITY ANALYSES FOR ESSENTIAL OIL YIELD AND RELATED TRAITS OF CITRONELLA (*CYMBOPOGON WINTERIANUS* **JOWITT.) GENOTYPES GROWN UNDER MEGHALAYA CLIMATIC CONDITIONS**

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Citronella is renowned for its aromatic and industrial significance worldwide and is gaining preference as an economical crop across Northeast India. However, the availability of varieties specific to essential oil purposes is limited. To increase production, it is essential to study the variability and stability of genotypes that can be used for essential oil purposes across different environments. An assessment of genetic variability among seven citronella genotypes for twelve quantitative traits revealed that fresh biomass yield and leaf area index are essential traits for increasing essential oil yield in citronella. These traits are controlled by additive gene action and can be improved through selection breeding methods. The environment plays a predominant role in determining the stable performance of genotypes. Significant variation due to genotypeenvironment interaction among citronella genotypes for oil yield was observed. Additive main effects and **ABSTRACT** multiplicative interactions (AMMI) model and genotype main effect plus G \times E interaction (GGE) biplot analysis provided a clear idea of genotype and environment interaction. Principal component analysis (PCA) studied using the AMMI model showed PC1 of 69.79% for plant height, 76.31% for the number of leaves per clump, 73.61% for fresh biomass yield per plot and 61.74% for essential oil yield of GEI sum of squares percentage. The results of the present study showed the influence of environments on the evaluated genotypes across seasons. The "which-won-where" biplot revealed that the *kharif* 2019 season was better for higher essential oil yield. Both biplots indicated that the genotypes JC-5, Mandakini and Bio-13 had higher fresh biomass and oil yield on average and they were also stable across the four evaluated seasons. These genotypes show potential for exploitation in future breeding programs.

Key words : AMMI model, Essential oil, Stability analysis, Fresh biomass yield, GGE biplot.

Introduction

Cymbopogon winterianus Jowitt, commonly known as Citronella ($2n = 20$), is widely recognized for its industrial importance due to its essential oil. It is broadly distributed in several tropical and subtropical regions and is extensively grown for its high-quality essential oil and bioactive chemical constituents. These constituents are majorly used in the production of cosmetics, soaps, perfumery and flavouring products worldwide (De Silva *et al*., 2020). Citronella has been known for its insect repellent, antifungal, antibacterial and insecticidal activities for a long period (Wany *et al*., 2014). In folk medicine, it is used to treat anti-inflammatory, antimicrobial, antitumor, respiratory, neurobehavioral, gastrointestinal disorders and other beneficial biological activities (Boukhatem *et al*., 2014; Madi *et al*., 2019).

The essential oils of *Cymbopogon* species mainly consist of monoterpene fractions such as citral (a mixture of geranial and neral), geraniol, citronellol, citronellal, linalool, elemol, 1,8-cineole, limonene, β -caryophyllene,

methyl heptenone, geranyl acetate and geranylformate, exhibiting marked variations across different species (Kumar, 2020). Additionally, essential oil components are significantly influenced by genetic and environmental factors like precipitation, temperature, humidity, postharvest factors and geographical conditions (Adhikari *et al*., 2015; Baruah *et al*., 2017). However, the composition of citronella essential oil is dynamic, undergoing changes influenced by various factors, including environmental conditions and genetic variability among different plant genotypes. Among these factors, seasonal variations play a significant role in shaping the chemical profile of citronella essential oil. Fluctuations in temperature, precipitation and humidity throughout the year can profoundly impact the biosynthesis and accumulation of essential oil constituents in Citronella plants (Sarma, 2002). The essential oils in *Cymbopogon* species are biosynthesized in rapidly growing leaves and stored in specific oil cells in parenchymal tissues (Dutta *et al*., 2018 and Munda *et al*., 2019).

Understanding the seasonal variation in essential oil composition is crucial for optimizing citronella cultivation and extraction processes. It provides valuable insights into the ideal timing for harvesting, as well as the selection of cultivars with desirable chemical profiles for specific industrial applications. Moreover, exploring the seasonal dynamics of essential oil composition can contribute to the development of breeding programs aimed at enhancing the yield and quality of citronella essential oil. The unique environmental factors prevailing in Meghalaya may exert distinct effects on the chemical constituents of citronella essential oil, thereby necessitating region-specific insights for effective cultivation and utilization of this valuable plant resource.

Generally, a genotype's performance across various environments and seasons, either within a single location or across different locations, determines its stability, which is a prerequisite and must be studied. Several methods have been proposed to study and quantify $G \times E$ interaction. The most commonly used stability analysis methods include those proposed by Eberhart and Russell (1966), Finlay and Wilkinson (1963). Nowadays, additive main effects and multiplicative interactions (AMMI) analysis is predominantly used because it graphically represents interactions through biplots. The GGE biplot also provides a clear understanding of the GEI among genotypes (Yan *et al*., 2000 and Yan, 2001). In GGE, the genotype (G) is considered a main factor and the genotypeenvironment interaction (GE) is a major source of variation. These analyses help identify genotypes that perform well across different agronomic zones, aiding in regional recommendations and selection of test sites (Gauch *et al.*, 2011; Gauch, 2013; Magudeeswari *et al*., 2019). Using this information, AMMI and GEI analyses were performed on citronella genotypes to examine seasonal variation and identify genotypes with stable performance across various traits under different environmental conditions. The present study aimed to identify associations among yield-contributing traits and to determine stable citronella genotypes suitable for the climatic conditions of Meghalaya.

Materials and Methods

Plant Material and Experimental Design

A total of seven citronella genotypes namely Jalapallavi (G1), Bio-13 (G2) and Mandakini (G3) from the Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow, U.P. and JC-1 (G4), JC-2 (G5), JC-4 (G6) and JC-5 (G7) from the North East Institute of Science and Technology (NEIST), Jorhat, Assam were evaluated across four different seasons/cuttings. The first cutting was performed six months after planting in February 2019, followed by subsequent cuttings at fourmonth intervals: June 2019, October 2019 and February 2020 in the experimental farm of the College of Post Graduate Studies in Agricultural Sciences (CPGSAS), Umiam, Meghalaya (latitude 25⁰ 40' 51" N and longitude 91° 54' 39'' E, 950 m above mean sea level). The total rainfall, maximum, and minimum temperature data recorded during the crop period are presented in Fig. 1. The experiment was conducted using a Randomized Block Design (RBD) with four replications. Each genotype was planted in five-row plots with a spacing of 50×35 cm in plots measuring 2.0×2.0 m². Standard agricultural practices were implemented to ensure a healthy crop stand.

Sampling of essential oil

In each cropping season, 1000 g of fresh herbage from each Citronella genotype was shade-dried for 24- 48 hours and subsequently used for the extraction of essential oil. The herbage was trimmed into 2-3 inch pieces, and hydro-distillation was conducted for two to three hours in a Clevenger apparatus following standard protocols (Clevenger, 1928). The oil yield was recorded per kilogram of shade-dry weight. The extracted oil was then preserved in amber bottles under room conditions.

Data recorded

The observations were recorded on randomly selected five plants in each genotype for the following traits *i.e.*, plant height (cm), leaf length (cm), leaf width (cm), leaf area index, number of tillers per clump, number

Fig. 1 : Weather data (temperature, relative humidity and rainfall) recorded during the crop season *Rabi* 2018-19 (S1), Pre-*kharif* 2019 (S2), *Kharif* 2019 (S3) and *Rabi* 2019-20 (S4).

of leaves per clump, petiole length, fresh herbage weight per clump (g), shade dry weight per clump (g), fresh biomass yield per plot (kg), dry biomass yield per plot (kg) and essential oil yield (ml kg⁻¹) were observed on plot basis in all the four cropping seasons *i.*e., *Rabi* 2018- 19 (S1), Pre-*kharif* 2019 (S2), *Kharif* 2019 (S3) and *Rabi* 2019-20 (S4).

Statistical analysis

Genotype means were compared $(p=0.05)$ using Microsoft Excel (Microsoft 365) for oil yield and its attributing traits. Individual and pooled season correlation analysis were performed using the metan package (Olivoto and Dal'ColLucio, 2019) in R version 4.2.3. Stable performing genotypes were identified using AMMI and GGE biplot analysis. The AMMI analysis, which differentiates the source of variation in to genotype, environment and $G \times E$ interaction was performed using GEA-R (Genotype by Environment Analysis with R) Ver. 4.1 software from CIMMYT, Mexico. The interaction effect was analyzed using principal components (IPCA1 and IPCA2). The GGE biplot providesa visual representation of biplots, helping to analyze G x E interaction, genotype ranking, and their performances (Yan and Kang, 2003).

Results and Discussion

Analysis of variability in citronella traits

Pooled analysis of variance showed that the genotypes and seasons were significantly different for all the traits (Table 1). The $G \times E$ interactions were significant for most of the traits studied, except plant height and leaf width, indicating a differential response of genotypes to variable seasonal stimuli. Variability studies showed that the highest genotypic coefficient of variation (GCV) was observed for fresh biomass yield, followed by dry biomass yield and leaf area index, while the lowest was observed for plant height, leaf width, and essential oil yield (Supplementary Fig. 1). The phenotypic coefficient of variation (PCV) was highest for fresh biomass yield, followed by dry biomass yield and shade dry weight per clump and lowest for leaf length and leaf width (Supplementary Fig. 1). Generally, GCV values are considered most reliable for use in breeding programs. Traits with high GCV to PCV ratios are mostly preferred, and in our study, fresh biomass yield and dry biomass yield recorded the highest GCV and PCV values, suggesting that selecting genotypes based on these traits could be advantageous.

The highest heritability was observed for all traits except petiole length and leaf length, which showed a moderate level of heritability (Supplementary Fig. 2). The genetic advance as a percentage of the mean was highest for fresh biomass yield, followed by dry biomass yield, and lowest for leaf width and the number of tillers per clump (Supplementary Fig. 2). Traits with high heritability and genetic advance are controlled by additive gene action. For such traits, selection methods in breeding would provide significant results. Similar findings were reported by Vashistha *et al*. (2013), Hanumanth Nayak *et al*. (2013), Ram Reddy and Jabeen (2016).

Correlation studies

Correlation analysis between 12 morphological traits revealed a positive significant association between essential oil yield and leaf area index, number of tillers per clump, number of leaves per clump, shade dry weight per clump (g), fresh biomass per plot (kg) and dry biomass per plot (kg) (Fig. 2). This shows that these traits play an important role in the production of high essential oil yield. Therefore, selecting genotypes based on these traits will facilitate the indirect selection of better genotypes in

Fig. 2 : Correlation coefficient studies on 12 quantitative traits of citronella genotypes.

mean are presented in Table 2. On a pooled basis, the genotypes JC-4 (46.47) and Mandakini (46.34) showed the highest mean value for leaf area index (LAI), whereas JC-1 (30.89) had the lowest LAI. Season S2 was the most favorable for producing higher fresh biomass yield per plot, as indicated by a higher seasonal mean (4.89 kg/plot), whereas S1 had the lowest seasonal mean (1.09 kg/plot) (Table 2). On a pooled basis, the genotypes Mandakini, JC-2 and JC-5 recorded the highest fresh biomass yield per plot. Seasonal effects showed that S1 (11.37 ml/kg) and S2 (10 ml/kg) had the highest pooled season mean, proving to be the best seasons with the most favorable weather for essential oil yield. The pooled mean for essential oil yield ranged between 12.21 ml/kg (JC-1) and 6.85 ml/kg (JC-4). Among the seven genotypes studied, four genotypes (JC-1, Bio-13, Jalapallavi and Mandakini) recorded a higher average mean essential oil yield over the pooled mean (Table 2). These outcomes indicate better growth and essential oil yield in seasons with higher rainfall and photoperiods, which are favorable for oil yield in many aromatic plants, such as *Cymbopogon*

Fig. 3 : AMMI biplot analysis of seven citronella genotypes evaluated under different seasons. [The seasons (S1, S2, S3) and genotypes (G1-G7) are same as mentioned in materials and methods section].

citronella. Similar results were reported in lemongrass by Singh *et al*. (2004). Fresh biomass yield per plot showed a significant positive correlation with plant height $(r =$ 0.70^{**}), number of tillers per clump ($r = 0.60$ ^{**}), number of leaves per clump ($r = 0.60$ ^{**}), fresh herbage weight per clump ($r = 0.85$ ^{**}) and shade dry weight per clump $(r = 0.85^{**})$ (Fig. 2). This indicates that all the above traits play an important role in increasing fresh biomass yield per plot.

Mean performance of citronella genotypes over different seasons for oil yield related traits

The mean essential oil yield-related traits of seven genotypes studied across four seasons and the pooled (Verma *et al*., 2019) and *Ocimum basilicum* (Kumar *et al*., 2011).

Genotype × Environment interaction analysis

Genotype and environment interaction (GEI) is an important source of variation for all crops. The stability of a genotype represents a stable response across environmental conditions. Based on this idea, genotypes with minimum variance for traits across different environments are considered stable (Banik *et al*., 2010). Therefore, analyzing $G \times E$ interaction is necessary for breeders to plan the distribution of new varieties and identify genotypes with specific and general adaptation across environments (Chandrasekhar *et al*., 2020; Patil

PL: petiole length (cm), FHW: fresh herbage weight (g), SDW: shade dry weight (g), FBY: fresh biomass yield (kg/ha), DBY: dry biomass yield (kg/ha), EOY: essential oil yield

et al., 2020). The analysis of $G \times E$ interaction using the AMMI model is extensively utilized due to its ability to predict multiple environmental impacts, reveal $G \times E$ interaction and provide accurate trait assessment (Nowosad *et al*., 2017; Choudhary *et al*., 2019; Munda *et al*., 2020).

Analysis of AMMI model

ANOVA of AMMI analysis revealed significant differences among environments for all traits. Significant differences among genotypes were observed for all traits as well (Table 3). The proportion of variation due to environments was highest, indicating significant differences among environments and their influence on genotypes. The $G \times E$ interaction was found to be significant for most characters except plant height. The mean sum of square value for $G \times E$ interaction was higher than genotype mean sum of square, indicating the differential response of genotypes to environments. AMMI analysis showed that over 60% of the variation was contributed by environments for all traits except petiole length, leaf area index and number of leaves per clump, which accounted for over 30% of the variation. The contribution of $G \times E$ component to the total variation was above 10% for petiole length, leaf area index, number of leaves per clump and essential oil yield. The genotypic contribution to total variation observed was above 10% for all traits. This environmental variation might be due to varied rainfall patterns, minimum and maximum temperatures, and relative humidity across the seasons (Fig. 1), which could have impacted essential oil yield and other traits. Furthermore, the significant genotype and environment interaction specify the differential expression of genotypes across the studied seasons, necessitating the study of genotype responses for different seasons. Thus, the genetic variation can be explained by different AMMI models. In the present study, the contribution of IPCA1 was higher for DBY, FBY, SDW, NT, NL, PL (more than 70%).

AMMI biplot for fresh biomass and essential oil yield is presented in Fig. 3. The x-axis represents mean values, and the y-axis represents the IPCA1 values. IPCA1 and IPCA2 of essential oil yield explain around 62% and 32% of the variations, respectively. The results revealed that Bio-13 and JC-1 were the highest essential oil yielding genotypes, among which Bio-13 was more stable. S2 and S3 did not classify the genotypes in a similar form, as they had close to 90p angles between them, whereas S1 and S2 classified genotypes better than S3 and S4, since they had the longest vector length. The genotypes Mandakini, JC-4, and Bio-13 were nearest to the origin, hence more stable for essential oil yield across all seasons (Fig. 3). The genotype Jalapallavi had a better essential oil yield than Mandakini, JC-4, and JC-5 in the second season (S2), while Jalapallavi, JC-2 and JC-5 were unstable across the seasons due to higher IPCA scores and dispersed

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Table 2 continued....

positions from the origin when plotted between IPCA1 and IPCA2.

Analysis of Genotype-Environment interaction through GGE Biplots

The biplot analysis of genotype and environment interaction provides the best way of visualizing the interaction pattern of genotypes and environments (Yan and Tinker, 2006; Yue *et al*., 2020) and also results in a possible existence of different environment groups in a region (Yan and Kang, 2003). The GGE biplots were constructed using principal components 1 and 2 (PC1 and PC2). The biplot analysis based on the performance of seven citronella genotypes for four seasons, with respect to fresh biomass yield and its attributing traits, indicated that 87.32% of the variation was explained by PC1 and 29.64% variation was explained by PC2 for fresh biomass yield (Fig. 4). Similarly, 60.41% of the variation was explained by PC1 and 22.64% variation was explained by PC2 for essential oil yield, respectively.

The grouping of different environments and their best-suited or yielding genotypes (Fig. 4) revealed that among the four seasons, S2 and S1 were the most discriminating seasons as their vector lengths were longer than other test seasons. For essential oil yield, S3 was found to be the most representative season than S2 and S4 seasons. Thus, the S3 season was useful for selecting specifically adapted genotypes. The genotype Jalapallavi was the winner in the S1 season, whereas Bio-13 was the winner for the S4 season and JC-1 was the winner for the S2 and S3 seasons (Fig. 4). The equality line between the genotypes Jalapallavi and JC-2 indicates that Jalapallavi was better than the genotype JC-2 in all seasons for essential oil yield. The genotypes Mandakini, JC-2, and JC-4 were not appropriate for growing in any seasons for essential oil yield, since none of the seasons fell in their sectors. In Fig. 5, the biplot explained 83.05% of the total variation. The genotype JC-1 had the highest mean essential oil yield, whereas JC-4 had the lowest essential oil yield across seasons, while the genotype Mandakini had a mean essential oil yield similar to the grand mean. The genotypes JC-1, Bio-13 and JC-4 were stable genotypes as their projection is closer to the AEC abscissa, while the genotype Jalapallavi was highly unstable because it had a lower than expected essential oil yield in the S1 and S4 seasons but higher than expected in the S2, whereas its essential oil yield in the S3 season was

Pre-kharif 2019 (S2), Kharif 2019 (S3) and Rabi 2019-20 (S4).

Pre-kharif 2019 (S2), Kharif 2019 (S3) and Rabi 2019-20 (S4)

Fig. 4 : GGE biplot analysis (Which–Won Where biplot) for fresh herbage and essential oil yield of seven genotypes evaluated under different seasons. [The seasons (S1, S2, S3) and genotypes (G1-G7) are same as mentioned in materials and methods section].

just as expected from its average essential oil yield.

Similarly, for fresh biomass yield, the genotypes JC-1 and JC-5 had almost low PCA1 scores (indicating low oil yielding) and also low PCA2 scores (indicating high stability) across environments, and they were found within the polygon. Hence, they were considered less responsive to the environments. Genotype ranking based on their performance under the environments, explained by a line drawn passing through the biplot origin and environment (Fig. 5), indicates that the genotype Jalapallavi is a low yielder (further away from the AEC x-axis), while genotypes Bio-13, JC-5, JC-4 and JC-4 were high fresh biomass yielders (closer to the AEC x-axis arrow). Even though JC-2, JC-4, and Mandakini had long projections from the AEC x-axis, they were unstable genotypes for fresh biomass yield. The genotypes JC-5, Jalapallavi and JC-1 had very few projections and were found to be

stable genotypes for fresh biomass yield. JC-5 is considered a desirable genotype with an average fresh biomass yield.

This study suggests that the traits of fresh biomass and essential oil yield in citronella are controlled by additive gene action and hence could be improved by selection methods in breeding. The above traits were positively influenced by leaf area index and dry biomass yield. Therefore, selection for earliness could favourably influence oil yield. The existence of a significant amount of variation due to genotype-environment interaction among the citronella genotypes for oil yield, as explained by GGE and AMMI biplots, was observed. Both biplots indicated that JC-5, Mandakini, and Bio-13 were found to have higher fresh biomass and oil yield on average, and they were also stable genotypes, which could be exploited in future breeding programs.

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