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# Grain yield performance of spring maize under different agroecological zones

Sandesh Thapa<sup>1\*</sup>  and Sara Rawal<sup>1</sup>

## Abstract

Maize is an important cereal crop which ranks second in production after rice in Nepal with an increasing demand for livestock and poultry ration but declining in production. This experiment was conducted to screen top- and stable yielding maize hybrids in various agroecological zones of Nepal during the spring season. In this study, nine maize genotypes were evaluated across six environments in randomized complete block design with three replications. The additive main effect and multiplicative interaction (AMMI) ANOVA revealed that environment, genotype, and their interaction had a substantial effect on the grain yield and all five principal components (PCs) were significantly different ( $P < 0.0001$ ). AMMI stability value revealed that genotypes Rampur composite and Kanchan 101 were the most stable genotypes in all environments. The specific adaptation of genotype as explained by Which-won-where model suggest that Godavari in environment E2, Rajkumar in E4 and E5, and Bisco gold 941 in E1, E3 and E6 were the winning genotypes. Furthermore, the mean-versus-stability model revealed that genotype Kanchan 101 had above average yield with greater stability. In addition, biplot analysis revealed that 78.32% of variation is explained by PC1 and 11.41% by PC2 of the interaction effect. The genotype ranking based on revealed that genotype Kanchan 101 was close to the ideal line and Sano ghogha was at the greatest distance. Conclusively, AMMI and genotype and genotype by environment interaction (GGE) model explicates that genotype Kanchan 101 has both the high yield and stability across all agroecological zones. In future research on multi-year trial with emphasis other agronomic traits to assess the stability and priorities for the development of package of practices for maximizing the grain yield is recommended.

**Keywords** Yield stability analysis, AMMI model, GGE biplot, Maize, Genotype selection index

## Introduction

Globally maize is the highest produced crop followed by wheat and rice (FAO 2022) and has multiple food value both to humans and livestock and is of the energy efficient crop (whole plant after harvest still has food/feed value). With rising demand, maize is a significant cereal crop that helps to ensure the security of both food and feed. Maize is a day neutral C4 plant which can be grown anywhere in the world, from tropical regions that are

hot and humid to cool temperate regions (Shrestha and Koirala 2019). Maize is grown in spring season (Tripathi et al. 2022) globally but winter and summer growing has also been reported based on local agro-climatic conditions (Bahadur Kunwar and Shrestha 2014; Kandel and Shrestha 2020; Tripathi et al. 2022). Furthermore, researchers reported that, variation in season (Tripathi et al. 2022) and locations (Shrestha 2013) have considerable effect on performance of genotypes which shows direction for selection of genotypes based on agro-eco zones.

Maize is a staple food for consumption in mid-hills of Nepal where short supply/low production leading to food insecurity is a major concern. After rice maize is the second dominant crop in Nepal both in terms of cultivation

\*Correspondence:

Sandesh Thapa  
sand.thapa.2056@gmail.com

<sup>1</sup> Gokuleshwor Agriculture and Animal Science College, Baitadi, Nepal



and production but the productivity is still low as compared to other nations. The major reason behind low productivity is land fragmentation, use of open pollinated varieties, and traditional way of farming. Since maize cultivation is a major crop in mid-hills of Nepal after onset of rain in April/May, the lowlands receive more rain-water compared to upland due to which comparatively high yield is reported. However, the studies involving the effect of altitude from sea level as environment to evaluate the performance of hybrid maize and identifying the stable genotype/environment is not well studied which is important in context of agro-ecosystem with steep hills where altitude increases sharply within a region and has varied climatic conditions.

The overarching goal of any breeding program is to breed elite genotypes in relation to a particular environment, but the genotype-by-environment interaction (GEI) makes selection more complicated (Gauch 2013). The variation in performance of genotypes across the environment and years has been reported by several researchers which demonstrates that a genotype may not perform equally in all environments (Hongyu et al. 2014). Thus, it is of utmost importance to identify the stable and high yielding genotypes suitable for various environments. To select the genotypes when their performance across the environment is significant it is always essential to use robust statistical methods for evaluating the GEI interaction effects in crops. There are various statistical models for evaluating the performance of genotypes across environments viz. two-way ANOVA, regression models (general and Linear), Shukla's stability of variance, additive main and multiplicative interactions analysis of variance (AMMI ANOVA), and GGE biplots (Dang et al. 2024). The detailed study for yield stability analysis in Multi environment trial (MET) and the ease of reproducibility of GGE biplot has also been explained by (Pour-Aboughadareh et al. 2022). The ability of variance analysis and regression analysis is limited when any breeding program demands GEI evaluations. However, the AMMI ANOVA and GGE biplots can provide the robust evaluation of genotypes when GEI is significant and are easy to read/interpret graphical tools (Yan and Kang 2002). In addition to the GGE biplots, some indices like AMMI stability value (ASV) and Genotype selection index (GSI) aids in identifying the stable and high yielding genotypes across all the environments respectively approach (Pour-Aboughadareh et al. 2022).

Recent years have witnessed the widespread adoption of the AMMI model and its associated parameters (ASV and GSI) in breeding programs, particularly for identifying elite genotypes based on yield and yield-governing traits in MET (Adjebeng-Danquah et al. 2017; Gauch 2006, 2013; Osiru et al. 2009; Shim et al. 2015; Zaid et al.

2022). Unlike the variance analysis, AMMI ANOVA integrates principal component analysis (PCA) with variance analysis to clarify the interaction effects between genotype and environment. By isolating the sum of multiple product terms from the interaction terms in the additive model, it enhances estimation accuracy (Chen et al. 2003, Dang et al. 2024). The AMMI model is effective in analyzing the primary effects of genotypes and the environment, along with GEI effects. GGE biplots also known as Genotype and Genotype by environment interaction is a graphical visualization of genotypes and environments based on principal components (first two principal components with large variation). These GGE biplots aids in visualizing the winning genotypes in corresponding environments, ranking of genotypes and environment, discriminativeness vs representatives of environment (Bos and Caligari 1995; Fan et al. 2007; Dia et al. 2016; Pour-Aboughadareh et al. 2022; Shim et al. 2015; Zaid et al. 2022). In addition, GSI is calculated as the sum of the rankings based on ASV scores and yield or performance rankings. Lower GSI values indicate genotypes that possess both high yield and stability (Adjebng-Danquah et al. 2017; Singamsetti et al. 2021).

The aim of this study is to examine the yield and yield stability of maize hybrids in different agro-eco zones in mid-hills of Nepal using AMMI and GGE biplot method such that high yielding and stable genotypes could be selected for each environment.

## Materials and methods

### Experimental site

The study was conducted in two Agro-ecological regions namely Terai and Mid-hill region of Nepal under six environmental conditions where Terai region consisted of one and remaining five environments consisted of mid-hill regions (Table 1). The differences in planting date across the environment is attributed to the onset of rain for planting in rainfed conditions and environment E6 is planted way earlier as the maize growing season on that region is around March–April which has proper irrigation facilities.

### Plant materials

The planting materials (genotypes) consisted of four commercial hybrid maize, three Open pollinated varieties. Two landraces (G8 and G9) were selected for comparison with commercial variety (Table 2).

### Design of experiment and agronomic practices

The experiment was conducted in a randomized complete block design (RCBD) which consisted of nine genotypes and three replicates in all six test environments. The experimental field was laid out plot size of 3 m × 4 m,

**Table 1** Agro-climatic conditions and planting dates with respect to different environments

Environment code	Study area	Agro-eco region	Latitude	Longitude	Moisture availability	Altitude (masl)	Temp. (Min)	Temp (Max)	Rainfall (mm)	Planting date
E1	Baitadi	Upland-Midhill	29.658057	80.548969	Rainfed condition	900	18.6	31.3	268.4	4th May 2022
E2	Baitadi	Lowland-Midhill	29.660513	80.545559	Rainfed condition	650	18.5	34.5	292.34	1st May 2022
E3	Baitadi	Lowland-Midhill	29.660513	80.545559	Irrigated condition	650	18.5	34.5	292.34	1st May 2022
E4	Darchula	High hill	29.74548	80.4902	Rainfed condition	1500	13.3	29.6	263.45	12th May 2022
E5	Baitadi	High hill	29.653822	80.547382	Rainfed condition	1549	16.45	30.3	291.43	12th May 2022
E6	Sunsari	Terai	26.656067	87.185807	Irrigated condition	61	21.4	36.78	553.54	15th March 2022

**Table 2** List of genotype used in experiment across all environments

Genotype Code	Genotype	Source	Grain color
G1	Bisco gold 941	Bisco Bio-science Pvt. Ltd., Hyderabad, India	Yellow
G2	Deuti	NMRP, Chitwan	White
G3	Godavari	Godavari Ganga Seeds Private Limited	Yellow
G4	Kanchan	Kanchan Ganga Seeds Private Limited	Yellow
G5	Manakamana-3	NMRP, Chitwan	White
G6	Rajkumar	Bio seed Research India, Pvt	Yellow
G7	Rampur composite	NMRP, Chitwan	Yellow
G8	Sano ghogha	Farmers variety	White
G9	Thulo ghogha	Farmers variety	White

space between blocks and plots were 0.6 m and 0.25 m, respectively. Each genotype was sown in four consecutive rows. The distance between plots was 0.5 m and blocks were at 1 m.

Land preparation was done by tilling the soil two times at a depth of 6-inch and leveling the field. Prior to sowing, moisture was ensured for germination in irrigated condition and in rainfed condition, and sowing was done after rainfall in respective study area. Farmyard manure (15 t ha<sup>-1</sup>) was applied during field preparation and inorganic fertilizers were applied respectively in the form of Urea (N), Diammonium phosphate (N and P) and Muriate of potash (K) at 180:60:40 kg ha<sup>-1</sup> as suggested by Tripathi et al. (2016). Urea was applied half of the requirement while sowing (50%) and remaining was applied in split dose at 1st and 2nd weeding at 20 and 45DAS, respectively.

#### Data collection

Data was collected for grain yield across all the environments. All the cobs were harvested when plants in entire plot achieved physiological maturity and weighed in the field and grain moisture content was taken to convert the plot weight into tons per hectare at 12.5% moisture content (Eq. 1). Similar methodology has also been adopted by several researchers (Gurung et al. 2018; Tripathi et al. 2016; Kandel et al. 2020; Koirala et al. 2017, 2020).

$$\text{Grain yield} = \frac{\text{Field weight (kg)} \times 0.8 \times (100 - \text{Moisture content})}{\text{Plot size (m}^2) \times 87.5} \times 10 \quad (1)$$

#### Statistical analysis

##### AMMI analysis of variance

Data were subjected to analysis of variance using additive main effect and multiplicative interaction (AMMI) (Equation 2) to study the effects of genotype (G), environment (E) and genotype by environment interaction

(GEI) (Biswas et al. 2021; Farshadfar et al. 2011; Gauch 2006, 2013; Gauch et al. 2008).

$$Y_{ijr} = \mu + \alpha_i + \beta_j + \sum_{n=0}^N \lambda_n \delta_{in} y_{jn} + \theta_{ij} + \varepsilon_{ijr} \quad (2)$$

where:  $Y_{ij}$  = the average yield of genotype  $i$  in environment  $j$  for replicate  $r$ ,

$\mu$  = the grand mean;

$\alpha_i$  = main effect of  $i$ th genotype;

$\beta_j$  = main effect of  $j$ th environments;

$\lambda_n$  = IPCA for unique values;

$n, N$  = IPCA axis retained in the model;

$y_{jn}$  = Environmental eigen vector values for  $n$ th IPCA axis;

$\delta_{in}$  = Genotypic eigen vector values for  $n$ th IPCA axis;

$\theta_{ij}$  = the AMMI error and  $\varepsilon_{ijr}$  = the error term.

##### AMMI stability value (ASV)

AMMI stability value (Equation 3) was computed to rank the genotypes based on ASV, lower the ASV more stable is the genotype (Pour-Aboughadareh et al. 2022). Several researchers (Fan et al. 2007; Farshadfar et al. 2011; Gumede et al. 2022; Shojaei et al. 2021; Singamsetti et al. 2021; Zaid et al. 2022) have followed similar method for ranking stability of genotypes based on ASV in multilo-cation trials. ASV was computed using formula:

$$\text{ASV} = \left[ \left\{ \frac{\text{PCA1}_{SS}}{\text{PCA2}_{SS}} + \text{PCA1}_{score} \right\}^2 + (\text{PCA2}_{score})^2 \right]^{1/2} \quad (3)$$

where  $PCA1_{ss}$  and  $PCA2_{ss}$  are the sum of squares of PC1 and PC2 respectively;  $PCA1_{score}$  and  $PCA2_{score}$  are the score of genotypes of PC1 and PC2 axes in AMMI's model, respectively. The greater the absolute value of the PCA, the greater the adaptability of a given genotype to a particular environment. Conversely, lower ASV indicates that a genotype is more stable across different environments.

#### Genotype selection index (GSI)

The genotype selection index (GSI) was estimated through the formula (Equation 4) (Farshadfar et al. 2011) and was later ranked where genotype with lower value of GSI was found to be high yielding and more stable (Farshadfar et al. 2011; Shojaei et al. 2021; Singamsetti et al. 2021):

$$GSI = rY_i + rASV_i \quad (4)$$

where,  $rY_i$  and  $rASV_i$  is the  $i$ th rank of genotype based on average yield and AMMI stability of the  $i$ th genotype respectively.

#### GGE biplot analysis

Genotype by environment (GxE) interaction is crucial for identifying stable and high-performing genotypes across diverse environments. GGE biplot analysis offers a graphical representation of genotype and environment effects, aiding in the visualization of complex data. Stability indices like ASV complements GGE biplots, providing quantitative measures of genotype stability. Studies by Pour-Aboughadareh et al. (2022), Dia et al. (2016), Gauch et al. (2008), Shim et al. (2015), and Yang et al. (2009) demonstrate the effectiveness of

GGE biplot analysis to select stable and high yielding genotypes across various environment.

## Result and discussion

### AMMI analysis of variance

The AMMI analysis of variance revealed that, grain yield had significant effect on environment ( $P \leq 0.00001$ ), genotype ( $P \leq 0.00001$ ), and the interaction between genotype and environment ( $P \leq 0.00001$ ), accounting for 5.7%, 70.48%, and 23.77% of the variation, respectively (refer to Table 3). The significant Genotype by Environment (G×E) interaction underscores the varied responses of maize genotypes to environmental fluctuations, highlighting the need for testing genotypes across various locations. While a substantial portion of the variation is attributed to genotype, the significant G×E interaction complicates genotype predictability for specific environments, necessitating the use of additional stability statistics such as stability value and GGE biplot analysis (Dia et al. 2016). The low variability (2.63%) and high significance ( $P \leq 0.00001$ ) of replications reveals that the effect is strong and reliable with minimum random error. Similar findings with low but significant variability in replications has also been reported in multilocation trials by Al-Naggar et al. (2020) in maize and Saeidnia et al. (2023) in wheat.

Moreover, all five principal components (PCs) were highly significant ( $P \leq 0.01$ ), consistent with findings reported by Gumede et al. (2022) regarding multi-environment trials. These extracted PCs offer insights into interaction effects, with the first two PCs carrying more information than the others, emphasizing the importance in GGE biplot analysis (Baraki et al. 2014). Both AMMI and GGE biplot methods offer extensive information in multi-location trials, and it is recommended

**Table 3** AMMI analysis of variance

Source	Df	SumSq	MeanSq	F-value	Pr(> F)	Percent of variation
Environment (E)	5	40.33	8.07	5.21	0.00001	5.70%
Replications	12	18.57	1.55	1.05	0.00001	2.63%
Genotype (G)	8	498.56	62.32	42.31	0.00001	70.48%
G × E	40	168.15	4.2	2.85	0.00001	23.77%
PC1	12	78.14	6.51	4.42	0.00001	46.5
PC2	10	57.36	5.74	3.89	0.00001	34.1
PC3	8	21.89	2.74	1.86	0.001	13
PC4	6	6.5	1.08	0.74	0.01	3.9
PC5	4	4.27	1.07	0.72	0.0158	2.5
Residuals	96	141.42	1.47	NA	NA	NA
Total	201	1035.18	5.15	NA	NA	NA

by researchers (Dia et al. 2016; Bishwas et al. 2021; Pour-Aboughadareh et al. 2022) to employ the AMMI model followed by GGE biplot analysis for selecting stable, resilient, and high-performing genotypes across diverse environments, thereby facilitating the identification of underperforming genotypes.

#### Performance evaluation of genotype across environment

The Genotype G1 (Bisco gold 941) reported the highest grain yield in three environments namely E1, E3, and E6 (Table 4). This was followed by G3 (Godavari) in E4, and G6 (Rajkumar) excelled in E4, E5 and E6. The average grain yield across all environments ranged from 4.19 t/ha to 9.37 t/ha, with G8 (Sano ghogha) being the lowest yielding genotype and G1 (Bisco gold 941) the highest. Among the genotypes, G7 (Rampur composite) exhibited the lowest ASV, demonstrating the greatest stability across various environments, followed by G4 (Kanchan 101). The genotypes with low GSI values indicate the most desirable genotypes for both the stability and high yielding (Grada and Ciulca 2013). Based on GSI rankings, G4 (Kanchan 101) and G7 (Rampur composite) were preferred for selection due to their lower indices, indicating their suitability. A similar methodology was also employed by Singamsetti et al. (2021) to identify the top-performing and stable genotypes in diverse environmental conditions.

#### Which won where model for identifying winning genotypes in each environmental condition.

The approach known as “Which Won Where” (WWW), utilizing a polygon view of GGE biplot datasets, stands out as a promising, highly effective, and widely accepted method for summarizing genotype and genotype-environment interactions (Pour-Aboughadareh et al. 2022).

In the WWW approach, a line originating from the biplot’s center and intersecting a polygon side perpendicularly represents the set of hypothetical environments where the two cultivars defining that polygon side perform equally (Yan et al. 2007). When environments are categorized into distinct sectors, it suggests that different genotypes excel in each sector, indicating genotype-environment interaction (Dia et al. 2016).

The genotypes positioned at the vertices of a polygon without nearby environmental indicators perform poorly, while those at vertices with environmental indicators are the best performers in those specific environments (Zaid et al. 2022). In our analysis, genotypes G8 (Sano ghogha) and G9 (Thulo ghogha) performed poorly across all environments. Genotypes less responsive to any testing environment are located within the polygon. Genotypes situated on the polygon’s edges but not enclosed by environmental indicators signify poor performance and are not recommended for cultivation in any environment (Bishwas et al. 2021).

Environmental indicators formed three sectors for grain yield, with genotype G3 (Godavari) was the winning genotype in environment E2, genotype G6 (Rajkumar) winning in E4 and E5, and genotype G1 (Bisco gold 941) winning in E1, E3, and E6 (Fig. 1). Our findings align with several researchers (Dia et al. 2016; Fan et al. 2007; Farshadfar et al. 2011; Shim et al. 2015; Zaid et al. 2022) utilized the Which Won Where model to identify high-yielding genotypes in specific environments.

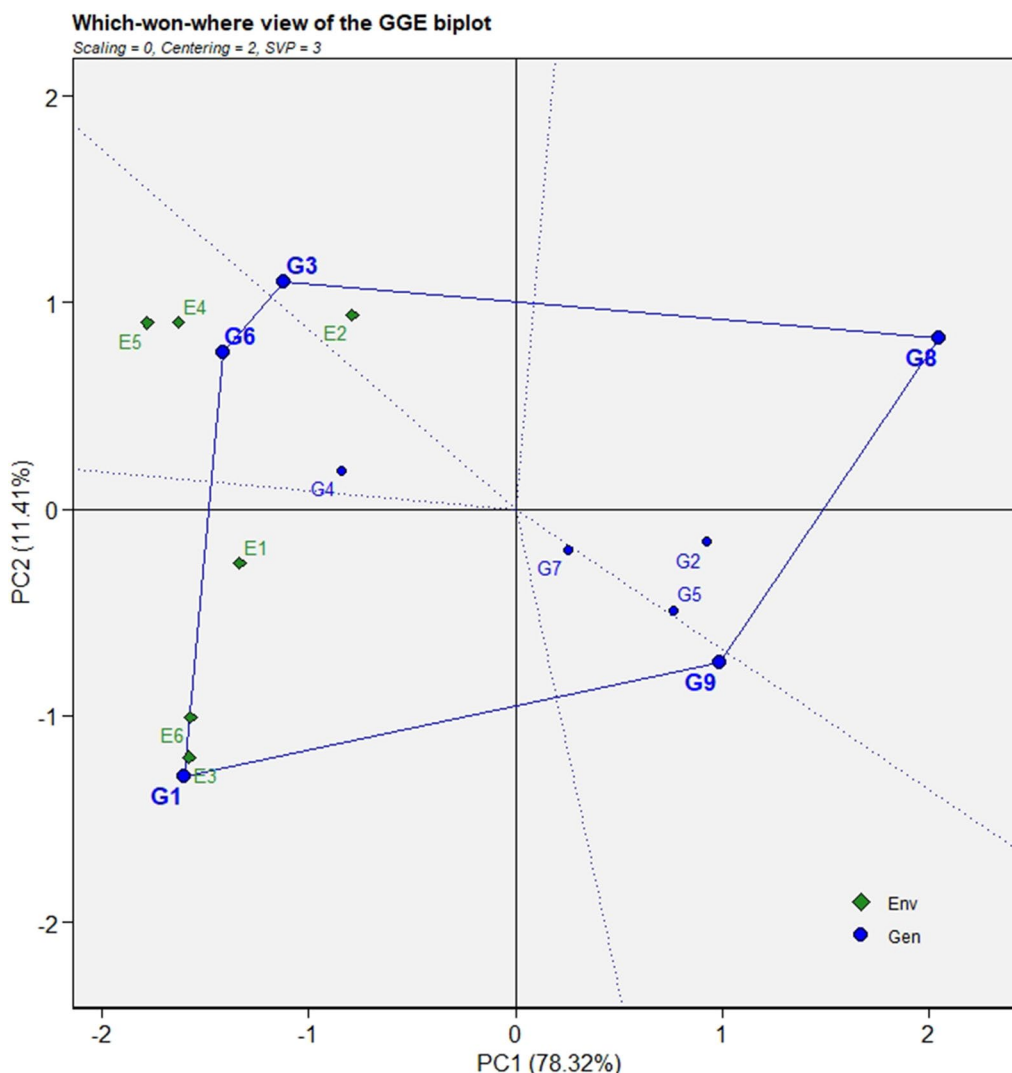
#### Mean vs stability of maize genotypes in six environments.

The assessment of stability patterns across diverse locations was conducted utilizing the mean versus stability analysis of the GGE biplot, as discussed by Fan et al. (2007), Osiru et al. (2009), Dia et al. (2016), and

**Table 4** Mean performance of genotype across environments, ranking of stability by AMMI stability value (ASV) and genotype selection index (GSI)

Genotype	E1	E2	E3	E4	E5	E6	Mean across env	Rank (A)	ASV	Rank (B)	GSI (A + B)
G1	9.52	6.99	11.70	8.20	8.11	11.70	9.37	1.00	1.95	9.00	10.00
G2	5.85	6.06	6.38	5.11	4.64	6.58	5.77	7.00	0.47	3.00	10.00
G3	8.26	8.86	8.10	9.58	9.41	8.52	8.79	3.00	1.30	6.00	9.00
G4	8.12	7.78	8.75	8.30	8.09	9.00	8.34	4.00	0.44	2.00	6.00
G5	6.16	5.88	7.04	5.08	4.63	7.17	5.99	6.00	0.61	4.00	10.00
G6	8.74	8.77	8.97	9.76	9.64	9.33	9.20	2.00	1.75	7.00	9.00
G7	6.76	6.55	7.49	6.17	5.80	7.67	6.74	5.00	0.35	1.00	6.00
G8	4.10	6.10	3.43	4.17	3.52	3.82	4.19	9.00	1.81	8.00	17.00
G9	5.93	5.47	6.99	4.49	4.01	7.07	5.66	8.00	1.06	5.00	13.00
Mean	7.05	6.94	7.65	6.76	6.43	7.87	7.12	–	–	–	–

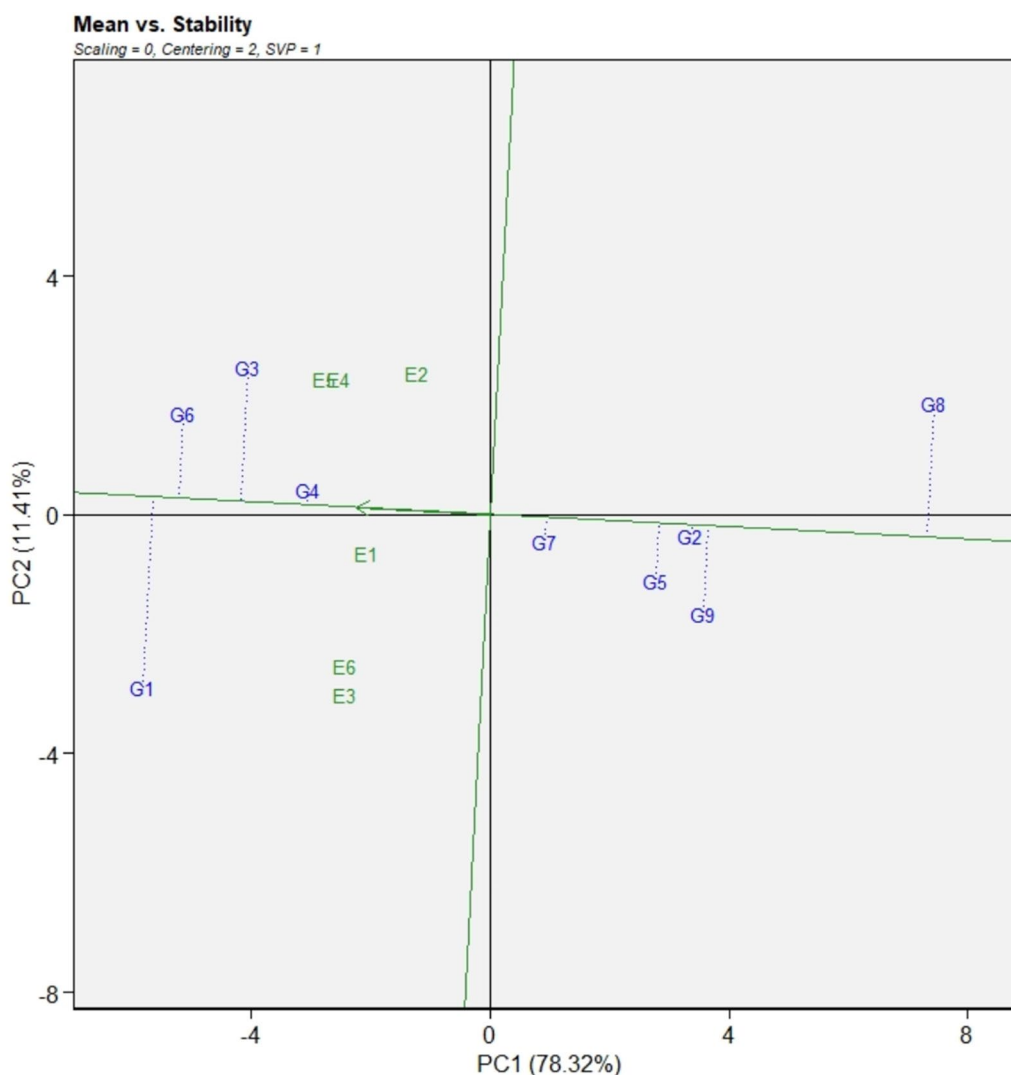
P.S. Refer to Table 2 for name of genotypes



**Fig. 1** GGE biplot analysis of nine genotypes showing which won where pattern across six environments. represents the genotype, represents the environment and dotted line emerging from the center divides the biplot into various sectors

Kaliyamoorthy et al. (2020). This method enables the evaluation of genotypes based on their average performance and stability across different environments. The biplot diagram features vertical and horizontal average environment coordination (AEC) lines, each indicating higher mean performance for the studied trait, which in this case is grain yield (Yan 2016). Our study’s mean versus stability analysis unveiled that 89.61% of the variation in grain yield could be elucidated. From Fig. 2, it was observed that the highest mean yield was recorded in G1 (Bisco gold 941), while the lowest was in G8 (Sano ghogha). Genotype G4 (Kanchan 101)

demonstrated above-average yields with greater stability, whereas genotypes G3 (Godavari) and G6 (Rajkumar) exhibited above-average yields but lower stability. Conversely, genotypes G7 (Rampur composite) and G2 (Deuti) displayed below-average yields but demonstrated higher stability. The ideal lines, representing the highest yield and absolute stability, are positioned at the arrowhead, with the distance of other lines indicating their desirability. Genotype G4 (Kanchan 101), situated closest to the AEC, emerged as the most stable line. Yue et al. (2020) utilized the mean versus stability approach to identify stable and desirable agronomic



**Fig. 2** GGE biplot showing mean vs stability analysis of genotype. represents the genotype, represents the environment

traits across various locations and high-yielding, stable genotypes. Similarly, researchers such as Shim et al. (2015), Dia et al. (2016), and Zaid et al. (2022) have employed mean versus stability analysis to pinpoint the most stable genotypes in multi-environment trials involving rice, wheat, and maize. In addition, Greveniotis et al. (2024) reported that GGE biplot is effective to identify the stable and high yielding genotypes across the environments (Figs. 3, 4).

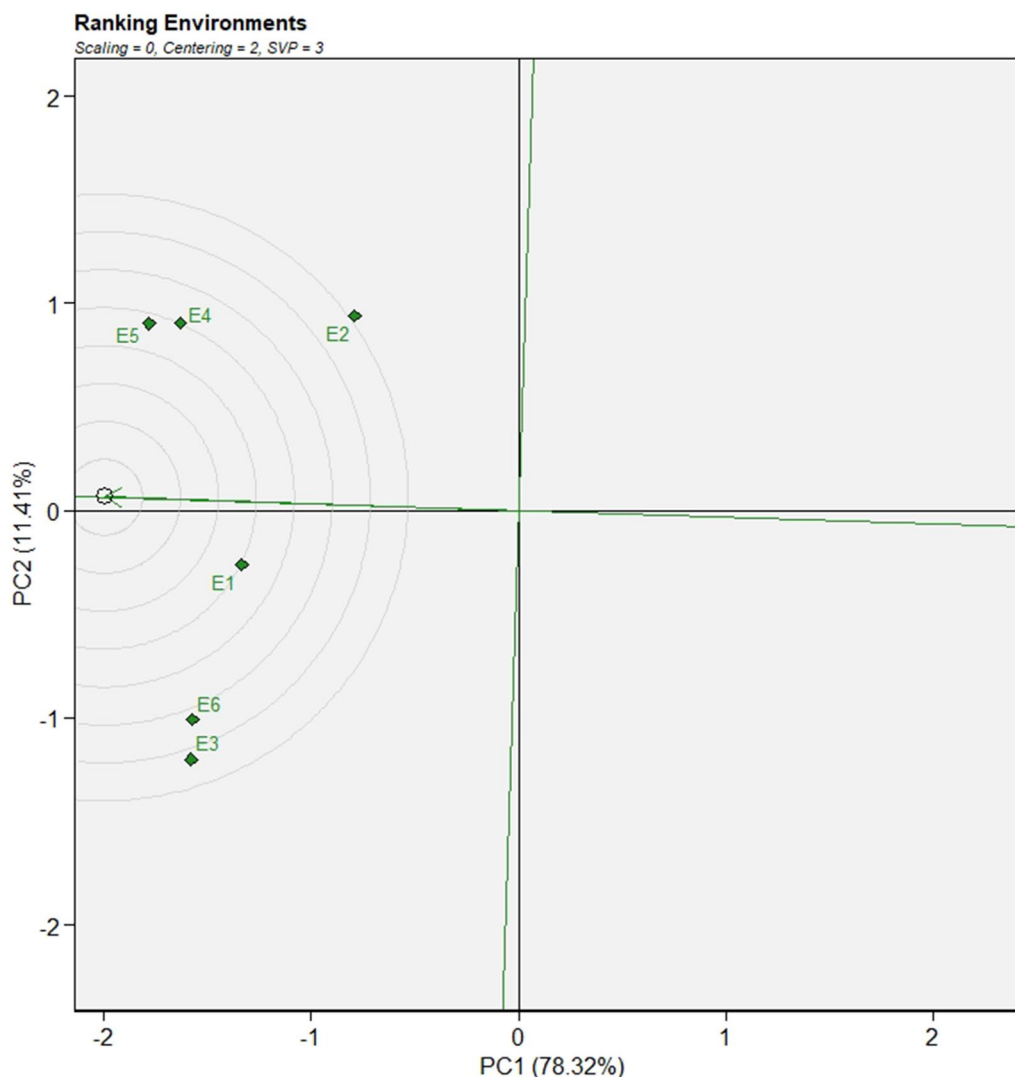
**Ranking genotype and environment**

The examination of GGE biplots facilitated the identification of optimal testing environments for all entries. Within the biplot, the green arrow indicates the ideal testing environment, situated at the center of the

innermost circle. Designating genotypes as random samples of entries, our study determined that environment E1 emerged as the ideal testing ground, with the ranking of environments following this order: E1 > E5 > E4 > E6 > E3 > E2 (Fig. 3).

In the GGE biplot illustrating the ranking of genotypes in relation to the ideal genotype, notable distinctions among the assessed genotypes were evident. The blue arrowhead signifies the ideal genotype, positioned at the center of the concentric circles, which performs optimally across all testing environments. Should no entry align precisely with the center, the nearest entry to the concentric circle is considered ideal. Environments are regarded as random samples of testing





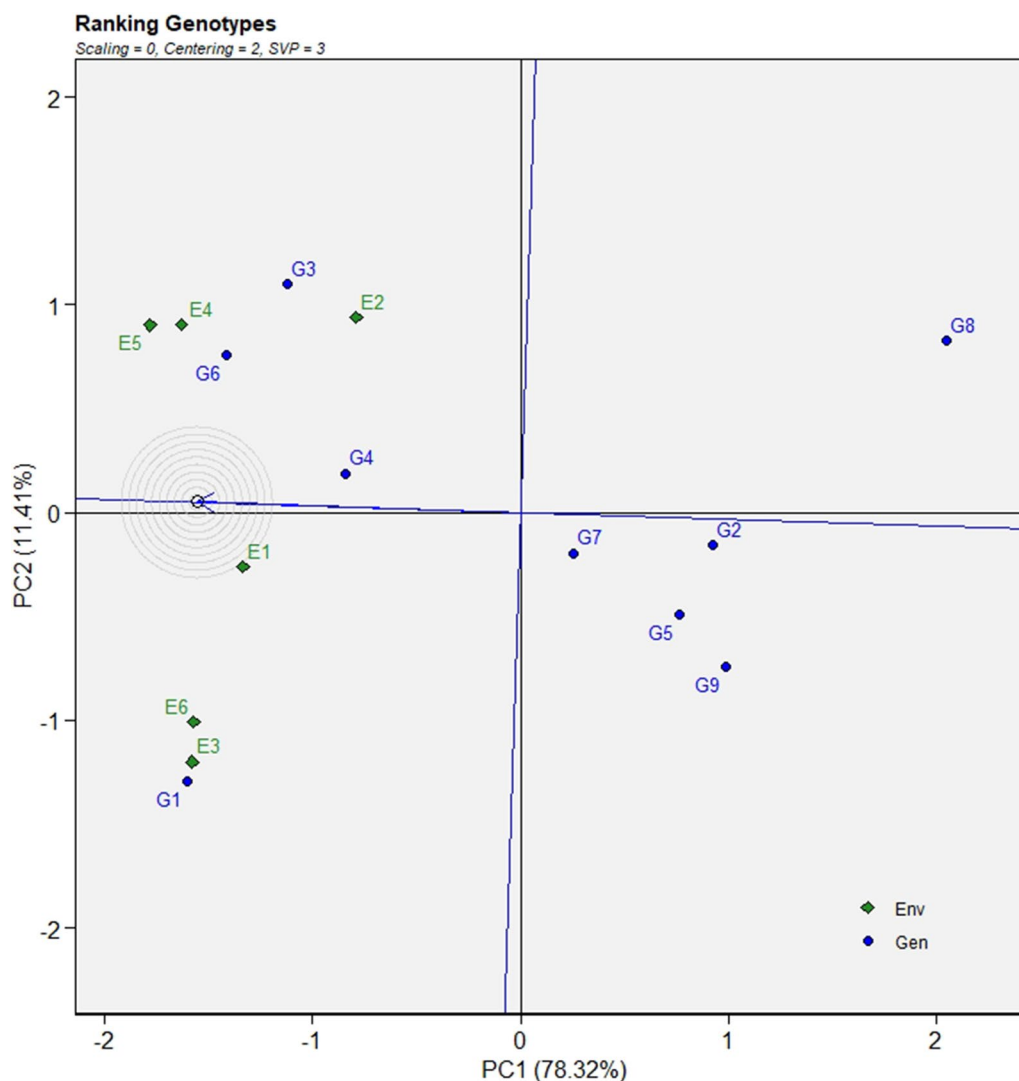
**Fig. 3** The ranking based biplot approach for identifying most ideal environment

environments, while genotypes serve as concentration points.

The ranking of genotypes (Fig. 4) is delineated as follows:  $G4 > G6 > G3 > G1 > G7 > G5 = G2 > G9 > G8$  (with genotypes G5 and G2 sharing identical rankings, indicated by an equal sign in descending order). The methodology employed for ranking genotypes and environments conforms to the framework outlined by Yan and Tinker (2006). The attractiveness of genotypes, ascertained through their closeness to the ideal genotype, is consistent with findings by Zaid et al. (2022) and supported by research conducted by Fan et al. (2007) and Yue et al. (2020).

## Conclusion

This study reported that genotype, environment, and genotype by interaction effect have significant effect on stability of genotype and as per AMMI model 78.32% variation was explained by PC1 and 11.41% by PC2. Stability of genotype using GSI rank and mean vs. stability plot concluded that genotype Kanchan 101 was most stable and above average yielding genotype, and which won where model reported that genotype Godavari was winning genotype in E2, Kanchan 101 in E4 and E5, and Rajkumar in E1, E3, and E6 respectively. Ranking of environment revealed that E1 as most ideal environment and ranking of genotype revealed that genotype Kanchan 101 as most ideal genotype followed



**Fig. 4** The ranking based biplot approach for identifying most ideal genotype

by Rajkumar and Godavari. Concludingly, for breeding programs genotype Kanchan 101 can be used which is high yielding and relatively stable line and for farmers high yielding genotype Kanchan 101 and Rajkumar is recommended for cultivation as they are stable across all environments and should further be evaluated along with other agronomic traits in multi-year trial such that genotypes could be used for commercial production of maize.

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**Author contributions**

Both authors S.T and S.R contributed equally to the project and manuscript. Both authors read and approved the final manuscript.

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**Availability of data and materials**

All data generated or analysed during this study are included in this published article.

**Declarations**

**Ethics of approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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