GRAIN YIELD STABILITY OF MAIZE (Zea mays L.) HYBRIDS USING AMMI MODEL AND GGE BIPLOT ANALYSIS

J. Shrestha*, S. Subedi, R. Acharya, S. Sharma and M. Subedi
National Plant Breeding and Genetics Research Centre
Nepal Agricultural Research Council, Khumaltar, Lalitpur, Nepal

ABSTRACT
Six maize hybrids; Khumal Hybrid-2, KML-5(A) × KYM-33, KML-8(A) × KYM-33, KWM-91 × KWM-93, KWM-92 × KWM-93, Super-951 were evaluated in a randomized complete block design (RCBD) with three replications at Bhotectaur (Sindhupalchok), Mandan Deupur (Kavrepalchowk), and Khumaltar (Lalitpur), Nepal to identify stable and superior hybrids. Hybrids were found significant (p<0.01) for grain yield. The effects of environment and genotype × environment (G × E) interactions on grain yield were found to be significant (p<0.01). The combined analysis showed that KWM-91 × KWM-93 produced the highest grain yield (8.89 t ha⁻¹) across all locations, followed by KWM-92 × KWM-93 (8.60 t ha⁻¹), which was at par with each other. The hybrids; KWM-92 × KWM-93 (bi=0.84, CV=18.54%, SD=1.59) and KWM-91 × KWM-93 (bi=1.16, CV=22.37%, SD=1.99) were found to be more stable, with regression coefficient (bi) nearly equal to unity (1) and grain yields above the grand mean yield. The GGE biplot revealed that KWM-91 × KWM-93 was the most responsive hybrid for Mandandeupur and Bhotectaur environments; whereas, KWM-92 × KWM-93 was for the Khumaltar environment. Hybrid KWM-91 × KWM-93 was the most stably yielding hybrid among all hybrids. This study suggests that KWM-91 × KWM-93 can be promoted for cultivation in mid hills of Nepal.

Keywords: Multi-environments, Maize hybrids, Stability, Yield

INTRODUCTION
With an area of 9,40,886 ha and a yield of 26,53,243 t, maize (Zea mays L.) is Nepal's second most important cereal crop (MoALD, 2020). It makes up 3.15% of national GDP and 9.5% of agricultural GDP. It contributes 24.97% of overall cereal production and covers 27.39% of total food crop land (MoALD, 2020). The yield

* Corresponding Author: jibshrestha@gmail.com

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potential of enhanced cultivars differs significantly from the national average yield. To increase yield potential even more, maize breeding efforts are focusing on producing high-yielding hybrid maize types. A mix of genetic and environmental factors determines the grain output. The Nepal Agricultural Research Council (NARC) has been instrumental in increasing maize productivity in Nepal. The current production capacity is insufficient to meet the demands of the country's rising population and assure food security.

One of the most essential responsibilities in the maize breeding program is to evaluate maize hybrids in various environments in order to generate high-yielding cultivars. To find and select the most stable and adaptable genotypes across a wide range of habitats, the genotypes should be screened in multiple environments spanning distinct ecological domains. Maize is a crop of great diversity that may be cultivated in many different agroecological zones (Ferdu et al., 2002). In all areas where they were adapted, the enhanced cultivars produced high and reliable yields (CIMMYT, 1991). Grain yield is influenced by genetics, environment, and management approaches, as well as their interplay (Messina et al., 2009). The interaction of these two explanatory variables provides insight into genotypes that are appropriate for specific situations. The impact of the environment is usually a significant factor in overall variation (Blanche et al., 2009).

Maize hybrid grain yields were affected by soil and climate conditions (Huang et al., 2017). The genotype (G) of the cultivar, the environment (E) in which it is cultivated, and the interaction between G and E (G × E) determine the level of performance of any feature. The occurrence of a strong genotype × environment (GxE) interaction poses a substantial difficulty in terms of genetic basis for phenotypic manifestation and makes selection challenging. GGE biplot analysis provides a framework for determining target testing areas and distinguishing high yielding and stable genotypes.

The GGE biplot is created by plotting the first two principal components (PC1 and PC2) produced from the environment-centered data’s singular value decomposition. The effective evaluation of a stable genotype, which might be employed for cultivation, is aided by knowledge of GxE. The testing of genotypes for yield stability under changing environmental circumstances has become a necessary aspect of any breeding program. The AMMI model combines ANOVA for the main effects of the genotypes and the environment together with principal components analysis (ACP) of the genotype-environment interaction (Zobel et al., 1988; Gauch and Zobel, 1996). The AMMI stability value (ASV) can be determined using the AMMI model (IPCA1 and IPCA2) (Purchase et al., 2000). This ASV is comparable to Shukla, Eberhart, and Russell's genotype stability techniques (Purchase et al., 2000). The yield-stability statistic (YSI) could also be used to recommend varieties (Kang, 1993; Pazdernik et al., 1997). Kang (1993) proposed a superior stability index (I) that is free of all of the aforementioned flaws (Rao and Prabhakaran, 2005). For yield
stability, a new approach known as genotype selection index (GSI) is also used, that took into account the AMMI stability value and mean yield (Farshadfar, 2008). AMMI has been used to analyze GEI in maize (Badu-Apraku et al., 2012). The objective of these experiments was to identify high-yielding, stable hybrid maize.

**MATERIALS AND METHODS**

**Plant materials and experimental sites**

Khumal Hybrid-2, KML-5(A) × KYM-33, KML-8(A) × KYM-33, KWM-91 × KWM-93, KWM-92 × KWM-93, and Super-951 are maize hybrids obtained at the National Plant Breeding and Genetics Research Centre, Khumaltar, Lalitpur. These studies were conducted in Bhotechaur (Sindhupalchowk), Mandan Deupur (Kavrepalanchowk), and Khumaltar (Lalitpur), Nepal in 2020 and 2021 from March to September. Mandan Deupur is located at 85.66°E longitude, 27.67°N latitude. It has arid and poor quality soil. Average annual rainfall at Mandan Deupur (Kavrepalananchok) is 2595 mm. Bhotechaur is located at 85°30’53.4”E longitude, 27°47’40.2”N latitude and 1532 m altitude. It has loam soil. Average annual rainfall at Bhotechaur (Sindhupalchowk) is 2500 mm. Khumaltar is located at 85°02’E longitude, 27°4’ N latitude and 1350 m altitude. It has clayey loam soil. The average annual temperature is 17.8°C and average annual rainfall is 1150 mm. The climate is semi-temperate (Sherchand, 1998).

**Experimental design and cultural practices**

With three replications, the experiment was set up in a randomized complete block design. Plots were divided into four rows of five meters each, with a 60 cm × 25 cm gap between them (row to row × plant to plant). One week before sowing, 6 t ha⁻¹ of well-decomposed farmyard manure was integrated into the soil, and 180:60:40 kg N:P₂O₅:K₂O [(nitrogen (N), phosphorus (P), and potassium (K)] ha⁻¹ was administered via Urea, Diammonium Phosphate, and Muriate of Potash (MOP). During final land preparation, a half-dose of N, a full dose of P₂O₅, and a full dose of K₂O were used as a base dose.

The remaining half dose of N was split in halves and given 45 and 90 days after seeding. During the maize season, two hand weeding and hoeing sessions were performed. The first weeding was performed 18 days after sowing, and the second 36 days after that. At three key growth stages, the crop was irrigated: knee-high, tasseling, and milking.

**Data Collection and Observations**

Data were collected for grain yield. The following formula (Eq.1) was used to compute grain yield (kg ha⁻¹) at 12% moisture content using fresh ear weight.

\[
\text{Grain yield (kg ha}^{-1}\text{)} = \frac{\text{F.W.} \times \text{kg plot} \times (100 - \text{HMP}) \times 10000}{(100 - \text{DMF}) \times \text{NPA}} \tag{1}
\]
Where,
F.W. = Fresh weight of ear in kg per plot at harvest
HMP = Grain moisture percentage at harvest
DMP = Desired moisture percentage, i.e. 12%
NPA = Net harvest plot area, m²
S = Shelling coefficient, i.e. 0.8
This grain yield (kg ha⁻¹) was then converted back to grain yield (t ha⁻¹).

**Statistical analysis**
To investigate differences among entries for the grain yield, data from each location
was treated to Analysis of Variance (ANOVA) individually, and then pooled across
locations to identify the G × E interaction. The significant G × E were used for
stability analysis of Eberhart and Russell model (1966). A stable genotype with unit
response was defined as one with a unit regression coefficient (bi=1) and a deviation
that did not deviate significantly from zero (S²d=0). As described by Eberhart and
Russell (1966), the behavior of the cultivars was assessed by the model (Eq. 2)

\[ Y_{ij} = m + b_i l_j + d_{ij} + e_{ij} \]  \hspace{1cm} (2)

where \( Y_{ij} \) = observation of the \textit{i}⁻\textsuperscript{th} (\textit{i} = 1, 2, ..., \textit{g}) cultivar in the \textit{j}⁻\textsuperscript{th} (\textit{j} = 1, 2, ...\textit{n})
environment, \( m \) = general mean, \( b_i \) = regression coefficient, \( l_j \) = environmental index
obtained by the difference among the mean of each environment and the general

\( \bar{E}_{ij} = \text{mean} (\sum_{j=1} l_j = 0) \),

\( \delta_{ij} \) = the regression deviation of the \textit{i}⁻\textsuperscript{th} cultivar in the \textit{j}⁻\textsuperscript{th}
environment and \( e_{ij} \) = residual error. \( d_{ij} = j \) - interaction of \textit{i}⁻\textsuperscript{th} genotype in the \textit{j}⁻\textsuperscript{th}
environment.

Tukey HSD test was used to evaluate the mean comparisons between genotypes at
5% levels of significance. RCBD was used to calculate variance components for the
ANOVA using the Statistical Tool for Agricultural Research (STAR 2.0.1) (IRRI, 2021). GEAR software version 4.1 was used to perform the stability investigation
(Pacheco et al., 2015).

**RESULTS AND DISCUSSION**

**Grain yield at various environments**
At Bhotchaun, Mandan Deupur, and Khumaltar, the maize hybrids had significantly
different grain yields (p<0.01) (Table 1). The genotypes differed significantly
(p<0.01) for grain yield over the locations. KWM-92 × KWM-93 had the highest
grain production of 6.80 t ha⁻¹ under the Bhotchaun conditions, followed by KWM-
91 × KWM-93 (6.67 t ha⁻¹). KWM-92 × KWM-93 (9.83 t ha⁻¹) yielded significantly
more grain than KWM-91 × KWM-93 (9.50 t ha⁻¹) under the Khumaltar conditions.
These findings were similar to those of Kafle et al. (2020), Shrestha (2016), Shrestha et al. (2019), Adhikari et al. (2018), Bastola et al. (2021), Dhakal et al. (2018), and Prasai et al. (2015), who observed significant differences in grain yield between maize genotypes. The genetic makeup of maize genotypes differed, resulting in differences in grain yield across the locations. Maize grain yield is the most important and intricate quantitative attribute, as it is controlled by several genes. Variations in maize production could be due to both environmental and genetic variables in different environments.

Table 1. Grain yield (t ha\(^{-1}\)) of maize hybrids across three locations (Bhotechaur, Mandan Deupur and Khumaltar)

| Hybrids               | Bhotechaur | Khumaltar | Mandan Deupur | Average |
|-----------------------|------------|-----------|---------------|---------|
| Khumal Hybrid-2       | 4.16c      | 5.71c     | 7.71b         | 5.86b   |
| KML-5(A) × KYM-33     | 4.50bc     | 6.83bc    | 8.50ab        | 6.61ab  |
| KML-8(A) × KYM-33     | 4.50bc     | 8.67ab    | 7.03b         | 6.73ab  |
| KWM-91 × KWM-93       | 6.67ab     | 9.50a     | 10.50a        | 8.89a   |
| KWM-92 × KWM-93       | 6.80a      | 9.83a     | 9.17ab        | 8.60a   |
| Super-951             | 4.50bc     | 5.50c     | 7.83b         | 5.95b   |
| Mean                  | 5.19       | 7.68      | 8.46          | 7.11    |
| CV (%)                | 15.04      | 10.06     | 9.9           | 11.27   |
| HSD (0.05)            | 2.2129     | 2.189     | 2.373         | 1.974   |
| P value, G            | 0.0043     | 0.0001    | 0.0059        | <0.001  |
| E                     | -          | -         | -             | <0.001  |
| G × E                 | -          | -         | -             | 0.004   |

Different letters represent significant differences based on Tukey HSD test at P<0.05

The genotypic variation and genotypes and environment interaction were found to be significant in the pooled analysis of variance for grain yield (Table 1 and 2), indicating that differences in mean grain yield across locations could be due to differences in soil types, altitude, sowing date, sunshine hours and rainfall, humidity during the growing season. The maximum grain yield (8.89 t ha\(^{-1}\)) was achieved by KWM-91 × KWM-93, followed by KWM-92 × KWM-93 (8.60 t ha\(^{-1}\)) in a pooled analysis (Table 1). The presence of significant GE interaction in this research indicated that genotype yield performance varied between environments. In this investigation, the relative contributions of GE interaction effects for grain yield were similar to those found in previous studies (Saied, 2010; Tariku et al., 2013).

The grain yields of the hybrid genotypes differed significantly among the locations, according to the combined mean square analysis (Table 2). As a result of the
significant mean square analysis for location, it was discovered that the genotypes' genetic potentials were influenced by the environment as a result of the diversity in the environment.

Table 2. ANOVA results showing level of significance for the genotype × environment interaction for grain yield at three locations

| Sources of variation     | df | Sum Square | Mean Square | F Value |
|--------------------------|----|------------|-------------|---------|
| Replication              | 2  | 0.959      | 0.479       | 0.75    |
| Genotype                 | 5  | 78.168     | 15.633      | 24.36***|
| Environment              | 2  | 104.816    | 52.408      | 81.67***|
| Genotype × Environment   | 10 | 21.367     | 2.136       | 3.33**  |
| Error                    | 34 | 21.818     | 0.641       |         |

***Significant at P<0.001; **Significant at P<0.01

GGE Biplot analysis

Genotypes with better mean yield across test conditions and absolute performance stability are desirable for broad selection (Yan and Rajcan 2002; Yan and Kang 2003; Farshadfar et al., 2012). An ideal genotype produces the best yield in all of the environments studied and performs well. Yan and Kang (2003) and Akcura et al. (2011) define the ideal genotype as having the highest average value of all genotypes and being absolutely stable in that it does not exhibit any genotype by environment interaction, allowing for broad adaptation. The closest genotype to the optimum genotype was KWM-91 × KWM-93 (G1) (Figure 1). It's preferable to have a genotype that's closer to the “ideal.” Because they are close to ideal genotypes, the KWM-91 × KWM-93 (G1) was the most suitable genotype among the others (Figure 1).

As a result, this genotype should be recommended for distribution as a variety to help boost maize production in Nepal's mid-hill environment. The genotypes' mean yield performance, as well as their rank across settings, revealed that they have a lot of variance around the mean yield. This result is similar to result obtained by Sharifi et al (2017).

PC1 and PC2 accounted for 83.66% and 14.85% GGE sum of squares, respectively, and explained 98.51% of the overall variance in this study, according to GGE biplot analysis (Fig. 1, 2 and 3). A “which won where” graph depicts the distribution of prospective mega-environments. The vertex genotypes were KML-5(A) × KYM-33, Super-951, KWM-91 × KWM-93 (G1), KWM-92 × KWM-93 (G5), KML-8(A) × KYM-33 (G3), Khumal Hybrid-2 (G2) and Super-951, as shown in the biplot (Fig. 3). In accordance with Yan (2002)'s findings, the genotype(s) vertex in certain sectors may have a greater or highest yield than other parts in all environments.
The genotypes on the vertices are the furthest from the biplot origin and are thought to be the most responsive in one or all environments (Yan and Tinker, 2006). The mega-environments are separated by lines perpendicular to the polygon. For the Khumaltar, Bhotechaur, and Mandan Deupur sites, the hybrid genotype KWM-92 × KWM-32 was identified as a high yielding genotype. KWM-91 × KWM-93 was the most responsive hybrid for Mandandeupur and Bhotechaur environments; whereas, KWM-92 × KWM-93 was found most responsive in the Khumaltar environment. Badu-Apraku et al. (2008) and Badu-Apraku and Lum (2010) used the GGE biplot tool to identify early maturing maize cultivars suited for Striga-infested and Striga-free environments, as well as determine their stability performance across environments. Similarly, Oyekunle et al. (2017) employed the GGE biplot analysis to evaluate the performance of early-maturing maize hybrids and pinpoint ideal test sites in West Africa.

Figure 1. Comparison of maize hybrids with the ideal genotype, G1: KWM-91 × KWM-93, G2: Khumal Hybrid-2, G3: KML-8(A) × KYM-33, G4: Super-951, G5: KWM-92 × KWM-93 and G6: KML-5(A) × KYM-33
Figure 2. GGE biplot showing ranking of maize hybrids for mean yield and stability. G1: KWM-91 × KWM-93, G2: Khumal Hybrid-2, G3: KML-8(A) × KYM-33, G4: Super-951, G5: KWM-92 × KWM-93 and G6: KML-5(A) × KYM-33

Figure 3. Polygon view of GGE biplot showing the identification of winning hybrid maize varieties with respect to the environments. G1: KWM-91 × KWM-93, G2: Khumal Hybrid-2, G3: KML-8(A) × KYM-33, G4: Super-951, G5: KWM-92 × KWM-93 and G6: KML-5(A) × KYM-33
Stability analysis using Eberhart and Russell model

Finlay and Wilkinson (1963) employed a variety of stability measurements in the past, including linear regression slopes as a measure of stability. In assessing genotype stability, Eberhart and Russell (1966) emphasized the importance of considering both linear and nonlinear components in Genotype × Environment Interaction. The name "stable variety" has been applied to a variety that performs consistently in all environments, according to this model. As a result, the stable variety has a high mean (Xi), unit regression (bi=1.0), and the smallest deviations from regression (S²di=0). The adaptiveness of the tested genotypes over the evaluated environments is explained by the coefficient of regression (bi). Varieties with a b-value close to unity and a greater mean grain yield have a better average stability.

A genotype with low mean, bi<1 and with non-significant S²di do not respond well to improved environmental conditions, and hence could be considered specifically suited to poor environments (Eberhart and Russell, 1966). In terms of stability, genotypes with a high mean, bi>1, and non-significant S²di are regarded below average. In favorable environments, such genotypes produce well, while in unfavorable conditions, they produce poorly. As a result, they are well-suited to a variety of environments (Eberhart and Russell, 1966).

Table 3. Mean grain yield (t ha⁻¹) and stability parameters for six maize hybrids across three locations

| GEN                | Mean | Sd   | CV (%) | bi   | S²di | R²  |
|--------------------|------|------|--------|------|------|-----|
| KWM-91 × KWM-93    | 8.89 | 1.99 | 22.37  | 1.16 | -0.21| 1.00|
| Khumal Hybrid-2    | 5.86 | 1.78 | 30.30  | 0.97 | 0.60 | 0.87|
| KML-8(A) × KYM-33  | 6.73 | 2.10 | 31.22  | 0.99 | 2.88 | 0.65|
| Super-951          | 5.95 | 1.71 | 28.76  | 0.87 | 1.23 | 0.75|
| KWM-92 × KWM-93    | 8.60 | 1.59 | 18.54  | 0.84 | 0.72 | 0.82|
| KML-5(A) × KYM-33  | 6.61 | 2.01 | 30.39  | 1.15 | 0.10 | 0.96|
| Mean               | 7.11 |      |        |      |      |     |

CV=Coefficient of variation, bi = regression coefficient, Sd = Standard deviation, S²di = the deviations from regression, R² = coefficient of determination (Eberhart and Russell 1966).

Thus, genotypes KWM-91 × KWM-93 and KML-5(A) × KYM-33 have regression coefficient (bi>1) which were relatively more responsive to improving environment, whereas genotypes KWM-92 × KWM-93, Super-951, KML-8(A) × KYM-33, and Khumal Hybrid-2 have regression coefficient (bi<1) which are relatively less responsive to improvement in environmental conditions (Table 5). Similar results were found by Seife, and Tena, (2020). The regression coefficient nearer to unity
indicating their stable performance over all the environments. The genotypes KWM-91 × KWM-93 (bi=1.16, CV=22.37%, R²=1.0, GM=8.89 t ha⁻¹) and KWM-92 × KWM-93 (bi=0.84, CV=18.54%, R²=0.82, GM=8.60 t ha⁻¹) were found to be more stable with grain yields above the grand mean yield (Table 3). According to Petersen (1988) and Finlay and Wilkinson (1963), genotypes with regression slope (b) significantly greater than unity are better adapted to high yielding environments (favorable environment), while genotypes with regression slope significantly lower than unity are better adapted to low yielding environments (unfavorable environment).

**AMMI Analysis**

The genotype accounted for the majority of the total variation (89.54%), whereas location accounted for only 51.29%. The first principle component axis (PCA 1) of the interaction captured 92.48% of the interaction sum of squares according to AMMI analysis (Table 4). The second principle component axis (PCA 2), in turn, explained 7.51% of the GEI sum of squares. PCA1 had a significant mean square at P=0.01, however PC2 had a non-significant mean square at 0.01 and contributed 100% of the entire GEI. Almohammed et al. (2019) reported similar findings.

**Table 4.** Partitioning of the sum of squares (SS) and mean of squares (MS) from the AMMI analysis of six maize hybrids yield performance evaluated across three environments.

| Parameters  | DF | SS   | MS   | F value | P value | PORCENT | PORCENAC |
|-------------|----|------|------|---------|---------|---------|-----------|
| ENV         | 2  | 104.81 | 52.40 | 82.82   | 0       | 51.29   | 51.29     |
| GEN         | 5  | 78.16 | 15.63 | 24.70   | 0       | 38.25   | 89.54     |
| ENV × GEN   | 10 | 21.36 | 2.13  | 3.37    | 0.00341 | 10.45   | 100       |
| PC1         | 6  | 19.761 | 3.29  | 5.13    | 0.00076 | 92.48   | 92.48     |
| PC2         | 4  | 1.60  | 0.401 | 0.625   | 0.647   | 7.51    | 100       |
| PC3         | 2  | 0     | 0     | 0       | 1       | 0       | 100       |
| Residuals   | 36 | 22.77 | 0.632 |         |         |         |           |
Figure 4. Biplot of the first interaction principal component axis (IPCA1) versus mean yields.

The AMMI biplot depicts the relationships between the first interaction principal component axis (IPCA1) and genotype and environment means (Fig. 4), with the biplot accounting for up to 100% of the total sum of squares. The sum of squares for genotype, environment, and PCA 1 was 38.25%, 51.29%, and 92.48% (Table 4).

Figure 5  Biplot of the first interaction principal component axis (IPCA1) versus the second interaction principal component axis (IPCA2) for maize hybrids
The IPCA 1 versus IPCA 2 biplot (i.e., AMMI 2 biplot) (Fig. 5) depicts the magnitude of genotype-environment interaction. The genotypes and environments that are the furthest away from the origin are the least stable. When genotypes and environments are in the same sector, they interact positively; when they are in opposite sectors, they interact negatively (Osiru et al., 2009).

The first two AMMI components’ genotypic and environmental scores are used to create a biplot (Vargas and Crossa, 2000). Furthermore, when IPCA1 was plotted against IPCA2, Purchase (1997) observed that the genotypes that scored closest to the center of the biplot (Figure 5) were the most stable. As a result, Figure 5 indicated that G1 (KWM-91 × KWM-93) was the most stable, and hence was closer to the biplot's center. Almohammedi et al. (2019) reported similar findings.

CONCLUSION

The genotype × environment interaction (GEI) and mean squares of environments (E) were both significant in the analysis of variance. Significant differences in grain yield amongst hybrid maize genotypes within environments indicated that there was a lot of variation. Result shows, maize genotypes KWM-91 × KWM-93 and KWM-92 × KWM-93 produced better grain yields and were more adaptable to favorable environmental conditions. Maize hybrids differed in terms of yield stability in different environments. GGE biplot and AMMI stability analysis revealed that KWM-91 × KWM-93 was the more stable and superior genotype. This study suggests that farmers can grow this genotype for higher production in Bhotechaur, Mandan Deupur, Khumaltar and other similar environments.

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