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Multi-model approach for optimizing cold-wave resilient maize selection: unveiling genotype-by-environment interaction and predicting yield stability

Chitra Bahadur Kunwar¹, Bikas Basnet^{2*} , Samjhana Sunuwar³, Deep Narayan Mahato⁴, Ramdas Chaudhari⁵, Jharna Upadhy¹ and Pragma Pokhrel¹

Abstract

Cold waves both significantly reduce yield & damage crops as well; unforeseeable nature of cold waves makes it challenging for farmers to manage risk. Thus, we aim to select maize hybrids that thrive under cold stress (both escaping early cold-waves and tolerating cold snaps); pinpoint stable, high-yielding hybrids ideal for regions prone to cold stress. In this investigation 2 years winter trial in Nepal was appraised on diverse maize hybrids for cold wave tolerant, stable across four stations with a Randomized Complete Block Design & 3 replications at each station. Likewise, this research employed 4 statistical methods both fixed effect and linear mixed model: genotype-environment interactions (AMMI), visually analyzes genotype performance and stability across environments (GGE) breeding values of genotypes for selection (BLUP), multiple traits for selection (MTSI). This scholarship revealed significant ($P < 0.001$) impacts of genotype, environment, and their interaction (GEI) on yield. This GEI, accounting for 100% of yield variance, was mainly captured by 3 principal components, with the first explaining 49%. Notably, mixed-effects models and biplots identified superior hybrids exhibiting both high average yields and consistent performance. GGE biplot analysis unveiled high-yielding and adaptable: GK3157, NK6607, RMH1899 Super, GK3254, RMH666, Shan 111, DKC9149, and Sweety-1. Further, BLUP and WAASBY analyses delineated the superior performers and stabilized hybrids for yield, with DKC9141, Uttam 121, NK6607, MM2929, RMH-666, GK 3254, and GK3157, and RMH-1899 super candidates for both high yield and stability. In Nepalgunj, Delta 3333, MM2122, and Shaan 111 excelled in both yield and stability, while Rampur favored Rampur Hybrid 6 and MM2424 for stability. Parwanipur and Tarahara shared similar winners for stability and yield, including MM2122, Shaan 111, and Delta 3333 in Parwanipur, and NK7884, MM2424, and Delta 2222 in Tarahara. Based on Multi genotype ideotype distance (MGDI), 9 hybrids were selected for yield and stability, including MM 2033, NK 6607, Sweety 1 so on; exhibited escape to cold waves whilst GK3254, TMMH-846, and MM-9442, were chosen for cold waves adapted hybrids. Moreover, by identifying cold-tolerant maize hybrids, this study has potential to mitigate risks for farmers (economic burden, crop failure) and bolster food security.

Keywords Mixed-effects models, Yield stability, Genotype-environment interaction, Cold wave, Hybrid maize, Nepal

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Introduction

Scientists have discovered the historical background of maize and have ascertained that its predecessors possessed a total of 10 chromosomes (Wei et al. 2007). After conducting a thorough analysis of duplicated genes, it has been revealed that the origin of maize is not a result of self-duplication but rather than a consequence of hybridization with approximate size of the maize genome is 2.4 Gb (Haberer et al. 2005). Around 9000 years ago, maize underwent the process of domestication through a singular event in southern Mexico, specifically derived from Balsas river teosinte (*Zea mays ssp. parviglumis*) (Yang et al. 2019). Teosinte seed is covered by a remarkably tough fruit-case that makes it arduous to eat. What's more conundrum is that, the presence or absence of the fruit-case is primarily determined by a single gene known as the fruit-case gene, which is classified as a unique type of regulatory gene-teosinte glume architecture or *tga1*, and therefore, the impact of a single gene can be remarkably transformative (Yang et al. 2019; Wang et al. 2005). In the discourse surrounding the cultivation of teosinte, an intriguing question persists: what impetus for ancient agriculturalists to cultivate a plant seemingly inferior as a crop? Anthropologist George Hill posits a compelling hypothesis: the appeal of teosinte may have rested in its popcorn-like popping trait. This distinctive characteristic likely facilitated easier digestion and enhanced their nutritional accessibility, potentially incentivizing early farmers to incorporate it into their agricultural practices as a food staple (Biointeractive 2015).

The global maize yield has experienced an almost three-fold increase since 1961, rising from 2 tons per hectare to the current figure of 5.8 tons per hectare (Erenstein et al. 2022). It is anticipated that the United States will emerge as the foremost corn producer on a global scale in the year 2022–2023, boosting a substantial production volume of approximately 348.75 million metric tons. China-(2nd largest) and Brazil-(3rd largest) conclude the list of leading corn-producing nations (Shahbandeh 2023). Within Nepal during the timeframe of 2020–21, the area and production of maize are reported to amount to 979,776 hectares and 2997.733 metric tons (equivalent to 3.06 tons per hectare) (Promotion et al. 2012). In addition to selecting tolerant lines for optimal yields, biofortification offers a practical solution for addressing hidden hunger. Hybrids like ICTA HB-18, ICTA B-15, and BIO-MZN01 have been successfully enriched with essential nutrients like iron, zinc, and carotenoids. These advancements hold promise for enhancing the nutritional value of maize and improving food security for underprivileged communities (Basnet and Khanal 2022; Beswa et al. 2020).

Chilling stress harms maize by hindering growth, affecting reproduction, and lowering seed quantity. What's is more seed priming with Salicylic acid (20 mg/L) significantly boosted yield by 25% under this stress (Waqas et al. 2021). Chilling early in pollen development didn't hurt individual anther pollen yield, but Dent11's tassels chilled during earlier growth stages made 43–29% fewer pollen grains overall (Tranel et al. 2009). By the same token, extreme cold destroyed Nepal's winter maize crop, causing massive losses for farmers. A catastrophe history that lost almost all its yield, and over 12,000 farmers lost a total of Rs 107.38 million Madhesh Province of Nepal (Republica, 2018). Facing **hefty** economic losses, Nepal's National Maize Research Program (NMRP) investigated the performance and stability of hybrid maize hybrids from multinational companies. This study employed cutting-edge methods to assess how well these hybrids-maintained yield across diverse environments. To identify promising hybrids, the study utilized four different models, each chosen for its unique strengths and weaknesses. Although the AMMI stability model is a graphic-based tool for modeling GEI, it is unable to take into account a linear mixed-effect model within the structure (Olivoto and Lúcio 2020). AMMI (Additive main multiplicative Interaction) impotent to incorporate random effects and structured relationships between environments, which are essential features of LMMs (Linear Mixed Models). This can lead to biased estimates and limit its applicability in complex scenarios (Taleghani et al. 2023). Although Best Linear Unbiased Prediction (BLUP) is not a graphic-based tool to manage random GEI (Genotype Environment Interaction) structure, it can produce accurate response estimates. The WAASBY Weightage Average Absolute Structure by Yield), a mixed-model, incorporates all Interaction Principle Component Axes (IPCA) for stability analysis and treats hybrids as random variables (Munda et al. 2022). Emphasized on BLUP's WAASBY index is more resilient and insensitive to outliers because it is based on absolute deviations (Ndhlela et al. 2014; Nataraj et al. 2021). Over and above MTSI (Multi-Trait Stability Index) is a method used to select high-yielding and stable hybrids in multi-environment trials. Compared to AMMI, BLUP, and GGE biplot, MTSI has the advantage of considering both mean performance and stability simultaneously because it takes into account the correlation structure among variables (Olivoto et al. 2021). Hybrids with performance closer to the ideal, as determined by their position in the factor analysis results, receive higher MTSI scores (Benakanahalli et al. 2021).

Why do farmers harvest less than research stations, even in different countries? While some gaps are small, like in Zambia with 20% (Silva et al. 2023), others are

bigger, like China's 42%–58% range (Ren et al. 2020). Even Bhutan has a 37% difference (Wangmo et al. 2023)! What's holding farmers back? High genotype-environment interaction for grain yield complicates the search for superior Maize hybrids that perform consistently across diverse environments. Ameliorating Nepal's maize sector requires identifying stable, high-yielding, and tolerant as well as scape hybrids against the cold snaps. This innovative research fills a critical gap by utilizing stability analysis methods to select ideal hybrids for wider cultivation and recommendation as a empirical study of theoretical model. In Nepal only 17% opting for hybrid maize hybrids rests of OPV (Gairhe et al. 2021). Agrovet became a hub for cheap multinational hybrid seeds. Harrowing winter cold waves, known as "Sitlahar" results on leaving fields littered with barren cobs. In response, the government, determined to safeguard farmer livelihoods, implemented new regulations. Consequently, directives tasked the Nepal Agriculture Research Council (National Maize Research Program, Rampur, Chitwan, Nepal) with a critical mission: rigorously evaluate the stability and performance of commercially available maize hybrids under frigid cold waves across varied locations. This study evaluates optimal maize genotypes for resilience under cold waves stressful conditions because minimum temperatures for pollen abortion range from below 10 °C (Waqas et al. 2021; Shao et al. 2021). Nepal's Tarai region has a special climate called "sitlahar" in winter and "Lu" in summer. However, despite its pleasant climate, studies show a significant increase in cold days and extreme cold waves over the past 40 years. Usually, January, February, and December are the most-risky months for cold waves and extreme waves in the Terai region of Nepal. On average, the Tarai experiences 9.2–13.8 cold wave days and 1.4–3.8 extreme cold wave days each year (Shrestha et al. 2023). Regrettably, in Nepal, and many developing countries, cutting-edge methodologies and investigations involving maize hybrids cold tolerance and scape are remains under-researched and unexplored. Due to the facts that we propose a novel project based on farmers' field problems exploring cold-tolerant & high-yielding stable hybrid maize across 4 diverse research stations. We strive to mitigate cold stress impacts on maize production by leveraging state-of-the-art statistical methods; identify cold-escape & cold-tolerant hybrids for wider cultivation in term of stability, escape to cold snaps, and high yield that allies for boosting food security and farmer livelihoods. Thus, a multi-model approach combining AMMI, BLUP, GGE Biplot, and MTSI has not only identify genotypes with high yield under cold-wave stress but also predict their stability and adaptability across diverse environments, leading to more efficient selection of cold-wave resilient maize hybrids

compared to traditional single-model methods, assumed as an alternate hypothesis. This approach, utilizing mega-environment trials as a paradigm study, has the potential to significantly enhance food security. By optimizing selection, registration, and recommendations for various crops, it most likely to development of more resilient and adaptable hybrids, ultimately contributing to a more stable food supply.

Materials and methods

Plant materials, experimental site & design

In a two-year experiment conducted from 2020 to 2022, a total of 45 maize hybrids seeds- multinational companies and National Maize Research Program- were evaluated across diverse climates in Nepal. Four research stations were strategically selected to represent different climatic zones: the eastern humid lowlands (Tarahara), the central intermediate zone (Bara, Pawanipur), the maize research hub (Rampur-Chitwan), and the far-western drylands (Nepalgunj). A Randomized Block Design with three replications and factor levels was employed. Optimizing grain yield was the primary objective. To achieve this goal, a planting density of 66,666 plants per hectare was established using a spacing of 60 cm between plants and 25 cm between rows. The rows themselves were 5 m long. Sowing was conducted between November 13th and 23rd in both years, following a systematic progression from east to west. Location indicating the experimental site is given below in Fig. 1. Code associated for this study map is deposited on git hub Library of Bikas Basnet(<https://github.com/Bikasbasnettest/Loaction-map-in-R>).

Climate data to study cold wave

In the context of examining the ramifications of cold waves during the pivotal Maize anthesis phase, which typically transpires between February 23rd and the initial week of March in Nepal, an analytical approach will be undertaken. To this end, a line graph will be employed for the visualization of the minimum and maximum temperature observations garnered from four distinct research stations. This data, pivotal for our investigation, was procured from the esteemed Department of Hydrology and Meteorology situated in Babarmahal, Kathmandu, Nepal Fig. 2. The mean relative humidity among the research stations exhibits notable disparities, with Tarahara recording 97.9%, Parwanipur reporting a significantly higher value of 99.3%, Chitwan at 98.2%, and Nepalgunj with 95.7%.

Statistical analysis

The data was entered into Excel and subsequently analyzed using R programming (R version 4.3.1 (2023-06-16 ucrt)). The metan package was employed for AMMI,

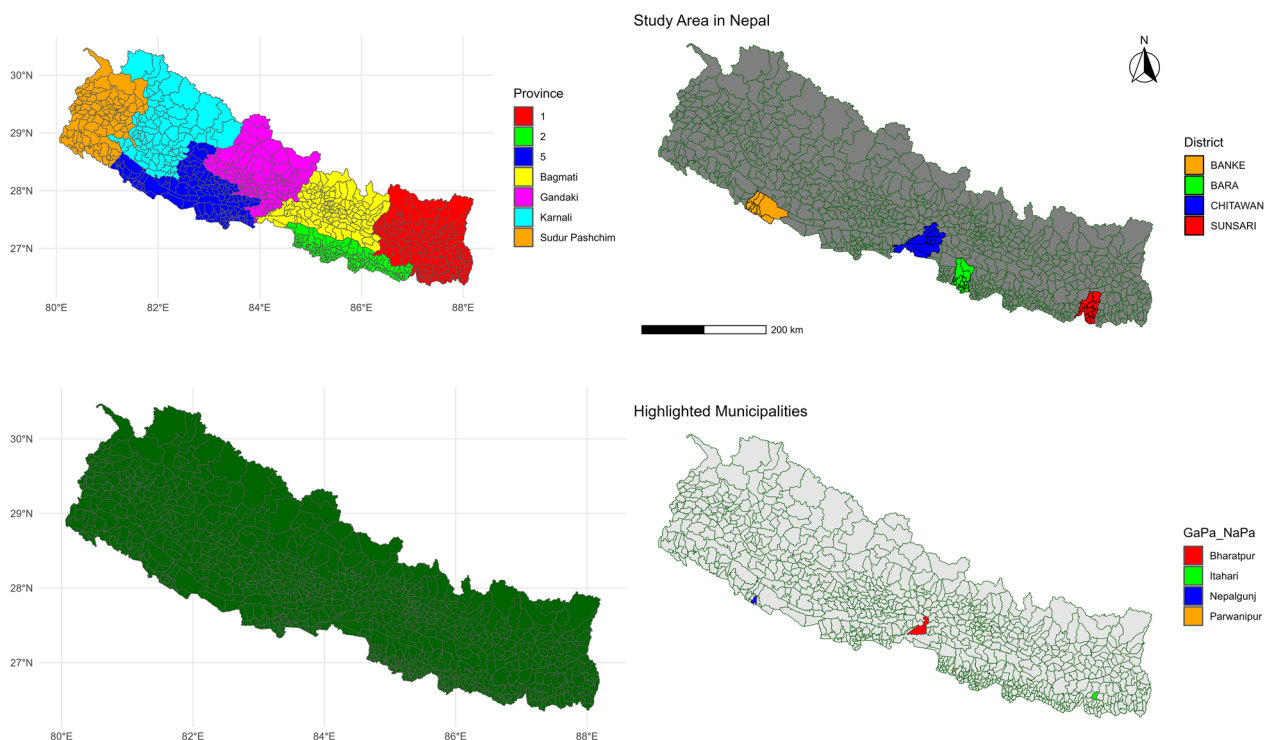


Fig. 1 Location of research stations in Nepal (2020–2022 winter season)

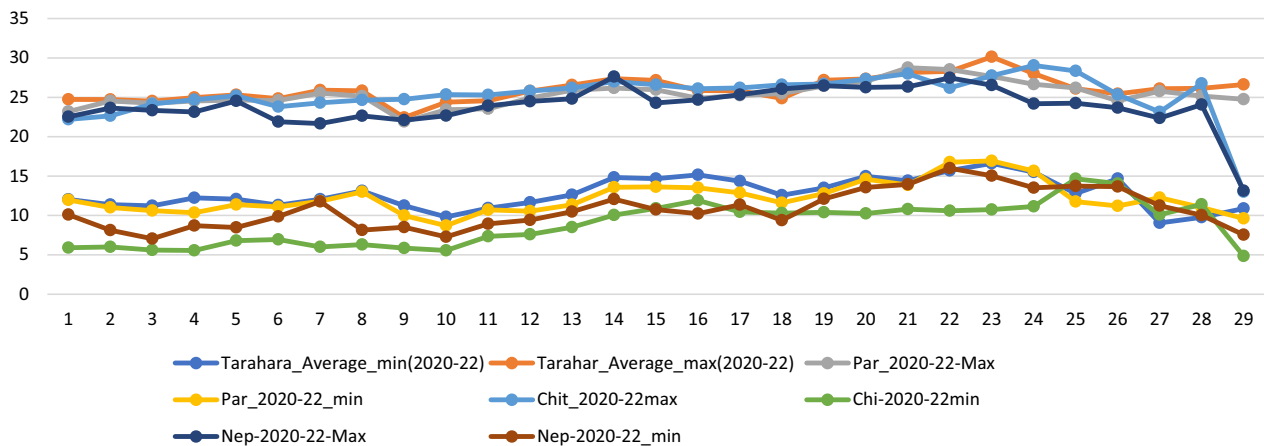


Fig. 2 Depicting the weather patterns from February 2020 to 2022 that coincide with the Days to Anthesis of hybrid maize Hybrids

GGE, BLUP, and MTSI model analysis. Joint analysis of variance (ANOVA) was used for pooled analysis. In this study, the year variable was considered as a crucial blocking effect to mitigate the impact of year-to-year variations on genetic differences among genotypes. To illustrate, adverse weather conditions in a single year can obscure true genetic disparities in yield performance. Therefore, only the average data from the four locations over a two-year period was used for stability analysis.

This approach ensures more reliable and discernible results by minimizing the influence of annual fluctuations. For the AMMI model, the performs_ammii function facilitated multiplicative interaction analysis, while plot_scores and ammi_indexes functions were utilized for AMMI biplot visualization and index value calculation, respectively. Index types 3, 4, 6, 8, and 10 were used for environment calculations (stability, representativeness, ranking, genotype ranking, and environmental

relationships, respectively). The 'gge' function provided insights into genotype performance and stability across environments, and plot_waasby generated WAASBY plots. BLUP analysis was achieved using the gamem_ met function. Finally, ggplot2's ggsave function enabled high-resolution figure export. Code used for this study is deposited on git-hub libraries (<https://github.com/Bikasbasnettest/PCA-cluster-biplot-AMMI-MTSI-WAASBY-ANALYISS-CODE->).

For linear mixed model

$$y = X\beta + Zu + e$$

where, β is the data vector of the fixed unknown effect (the average value of the block in each environment), u is the GEI+genotype effect, X and Z represent the matrix involving β , u , and Y , and ϵ is the random error vector (Olivoto and Lúcio 2020).

Additive main effects and multiplicative interaction

Compute the additive main effects and multiplicative interaction (AMMI) model. The estimate of the response variable for the i^{th} genotype in the j^{th} environment () using the AMMI model, is given as follows:

$$y_{ij} = \mu + \alpha_i + r_j + \sum_{k=1}^p \lambda_k \alpha_{ik} t_{jk} + p_{ij+\epsilon_{ij}}$$

where, λ_k is the singular value for the k^{th} interaction principal component axis (IPCA); α_{ik} is the i^{th} element of the k^{th} eigenvector; t_{jk} is the j^{th} element of the k^{th} eigenvector. A residual p_{ij} remains if not all p IPCA are used, where $p \leq \min(g-1;e-1)$ (Ndhlela et al. 2014).

Weighted average of absolute scores

Compute the weighted average of absolute scores for quantifying the stability of g hybrids conducted in e environments using linear mixed-effect model (Olivoto et al. 2019). The weighted average of absolute scores is computed considering all Interaction Principal Component Axis (IPCA) from the Singular Value Decomposition (SVD) of the matrix of genotype-environment interaction (GEI) effects generated by a linear mixed-effect model, as follows:

$$WAASB_I = \sum_{K=1}^p |PCA_{jk} \times EP_K| \frac{1}{\sum_{K=1}^p EP_K}$$

where $WAASB_I$ is the weighted average of absolute scores of the i^{th} genotype; $IPCA_{jk}$ is the score of the i^{th} genotype in the k^{th} Interaction Principal Component Axis (IPCA); and is the explained variance of the k th IPCA

for $k=1,2,\dots,p$, considering $p \leq \min(g-1;e-1)$ (Olivoto et al. 2019).

Multi-trait genotype-ideotype distance index (MTGIDI)

$$MGIDI_i = \sqrt{\sum_{j=1}^f (F_{ij} - F_j)^2}$$

where $MGIDI_i$ represents the index of multi-trait genotype-ideotype distance for the i th genotype. F_{ij} represents the score assigned to a given genotype concerning a specific factor, denoted by "i" for the i^{th} genotype and "j" for the j^{th} factor. The variables g and f correspond to the total number of hybrids and factors included in this analysis, F_{ij} is the j^{th} score of the ideotype. The genotype exhibiting the lowest MGIDI is more proximate to the ideotype and, consequently, is expected to showcase desirable values for all scrutinized traits (Olivoto and Nardino 2020, nd).

The proportion of the MGIDI index for the i th row (representing genotype or treatment) explained by the j^{th} factor (x_{ij}) is utilized to assess the strengths and weaknesses of hybrids. This proportion is computed as

$$w_{ij} = \sqrt{\frac{D^2_{ij}}{\sum_{j=1}^f D^2}}$$

Results

Combined analysis of variance of AMMI and ANOVA model

The impact of environmental factors and the genetic makeup of maize plants on yield was found to be statistically significant, as determined by a comprehensive analysis of variance (ANOVA). The observed significance level ($p < 0.05$) underscores a robust influence of these factors on maize yield. The subsequent joint ANOVA analysis revealed several key statistical parameters. Additionally, the coefficient of variation (CV) was found to be 11.47%, indicating the relative variability of the yield. Further, the residual standard error, which measures the dispersion of data points around the fitted regression line, was determined to be 0.82. Furthermore, the analysis of interaction effects showed a mean sum of positive and negative interactions ($MSR+/MSR-$) of 3.43, representing the combined impact of environmental and genetic factors on yield variation. Moreover, further decomposition through principal component analysis (PCA) elucidated the total variance in yield. Specifically, three interaction principal components collectively accounted for 100% of the total variance. Notably, the first principal component explained 49% of the total variance,

emphasizing its significant contribution. Additionally, the second principal component contributed 28.4%, with the combined contribution of the first two components amounting to 77.4% of the total variance (Based on DMRT-(Duncan Multiple Rang Test), average yield of the tested genotypes is depicted in Fig. 3 with the highest yield of DKC-9149 (8.81 tons/ha).

Table 1). Based on DMRT-(Duncan Multiple Rang Test), average yield of the tested genotypes is depicted in Fig. 3 with the highest yield of DKC-9149 (8.81 tons/ha).

AMMI (1 and 2) biplot analysis for maize hybrid yield analysis

AMMI1 bi-plot helps visualize the genotype by environment interaction (GEI) effect by plotting the mean yields of environments and hybrids against their respective IPCA1 scores (Khan et al. 2021). The x-axis of the bi-plot represents the average yield, with points to the right indicating higher-yielding hybrids and environments, and points to the left indicating lower-yielding hybrids and unfavorable, low-yielding

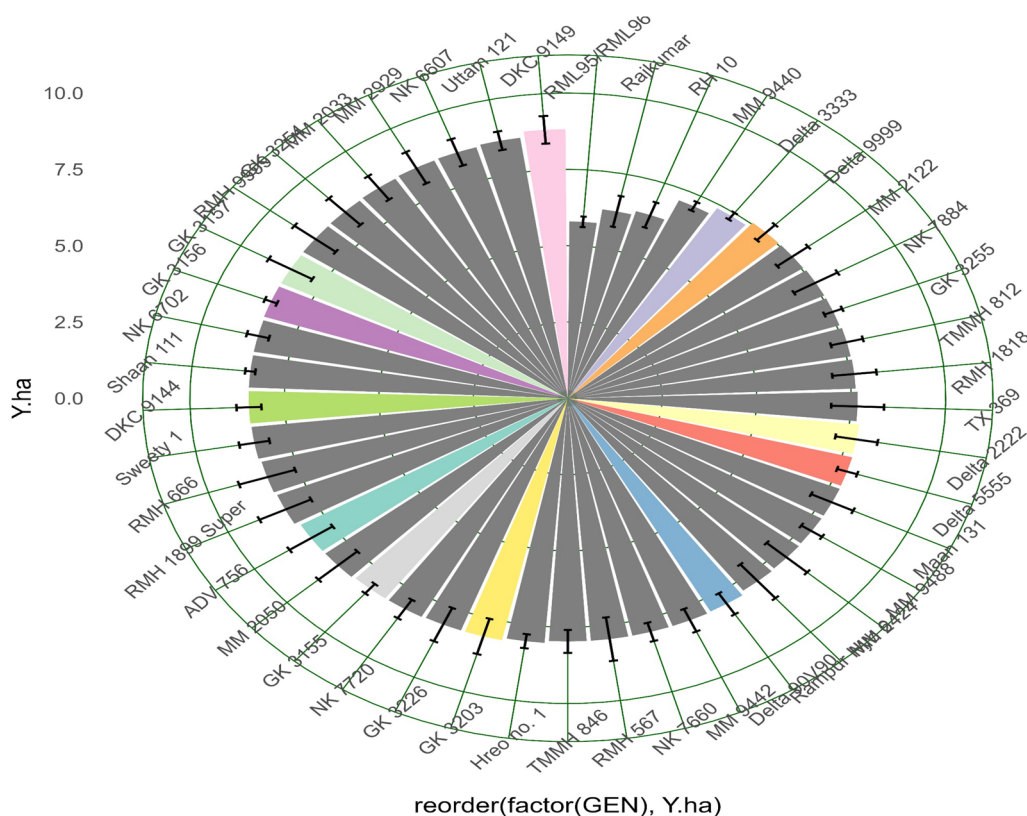


Fig. 3 Circular bar diagram depicting the Yield/ha with standard error for the tested Hybrids in 2020–2022

Table 1 Combined analysis of AMMI and ANOVA of maize hybrids evaluated for grain yield across locations in 2020–2022

Source	df	Sum Sq	Mean Sq	F value	Pr(>F)	Proportion	Accumulated	Significance
ENV	3	28.418	9.472617	75,400	0.0001	NaN	NaN	***
REP(ENV)	8	0.001	0.000126	0.0002	1.00	NaN	NaN	ns
GEN	42	392.27	9.33975	11.4	0.000174	NaN	NaN	***:
GEN:ENV	126	368.599	2.925387	3.56	0.000384	NaN	NaN	***
PC1	44	90.28	2.05181	2.5	0.0012	49	49	**
PC2	42	52.324	1.2458	1.52	0.00196	28.4	77.4	**
PC3	40	41.696	1.0424	1.27	0.125	22.6	100	ns
Residuals	852	700.023	0.821624	NA	NA	NA	NA	

All variables with significant (p < 0.05) genotype-vs-environment interaction were analyzed. To minimize the blocking effect, the mean of each year was applied during the analysis. Df: degree of freedom, ENV: Environment, REP: Replication, GEN: Genotype, PC: Principal components. NA: Not available, ns: none significant

environments (Yan and Tinker 2006). Similarly, y-axis of the biplot represents the IPCA1 score, which measures the contribution of the GEI effect to the overall yield variation. By the same token, Hybrids and environments that are farther away from the origin of the biplot have a stronger GEI effect, meaning their yield performance varies more across different environments (Gebreselassie et al. 2022). Emphasizing selection of superior genotypes in crop breeding is primarily focused on the pivotal trait of yield (Gauch 2013). AMMI₁ plot illustrates that hybrids such as NK6607, GK3157, GK-3203, RMH-666, and RMH1819-super exhibit resilience and high productivity for yield. In contrast, Rajkumar, TX-369, and Delta-5555 are stable for yield but fall below the threshold, indicating their unsuitability for selection as major phenotypes aimed at maximizing yield. Furthermore, MM2033, DKC9141, DKC9144, RMH-9999, and NK6702 demonstrate high yields but are positioned far from the origin, rendering them unstable and, consequently, unsuitable for commercial cultivation. In AMMI Plot 1, the vector length of an environment indicates the degree to which it contributes to and influences the genotype by environment interaction (GEI) captured by the first principal component axis (IPCA1) (Krishnamurthy et al. 2017). Thus location Tarahara have larger vector length indicates that the higher the discriminatory ability of the environment, indicating that the environment has a stronger impact on the genotype-by-environment

interaction (highlight the variations in genotypic performance more clearly) followed by Parwanipur and Nepalgunj. Whilst Rampur have shorter vector length indicates lower the discriminatory ability of the environment, suggesting that the environment has a weaker impact on the genotype-by-environment interaction. Rampur is regarded as the perfect index for selection of consistent and high yielding genotypes.

The AMMI2 biplot, depicting principal component axes (IPCA1 and IPCA2), illuminates the nature and magnitude of genotype-environment interactions (GEIs) in the study (Gebreselassie et al. 2022; Singh et al. 2019). With 48.9% and 28.4% of the total variance explained by IPCA1 and IPCA2, respectively, the cumulative contribution reaches 77.3% (Fig. 4). Notably, Nepalgunj exhibits the greatest distance from the origin, followed by Parwanipur and Tarahara, indicating their stronger influence on GEIs. In contrast, Rampur displays the shortest vector, suggesting a relatively weaker impact on interaction patterns. Genotype performance also reveals distinct patterns across environments. DKC9149 emerges as the top-yielding variety in Parwanipur, while RMH-567 dominates in Rampur. Tarahara showcases Uttam 121 and RH-10 as the highest performers, and Nepalgunj witnesses Rampur Hybrid 6, NK7884, and MM2033 achieving the highest yields. These observations highlight the crucial role of environment-specific adaptation in determining genotype performance. In Graphics, the Genotype Environment Index (GEI) is represented in Fig. 5

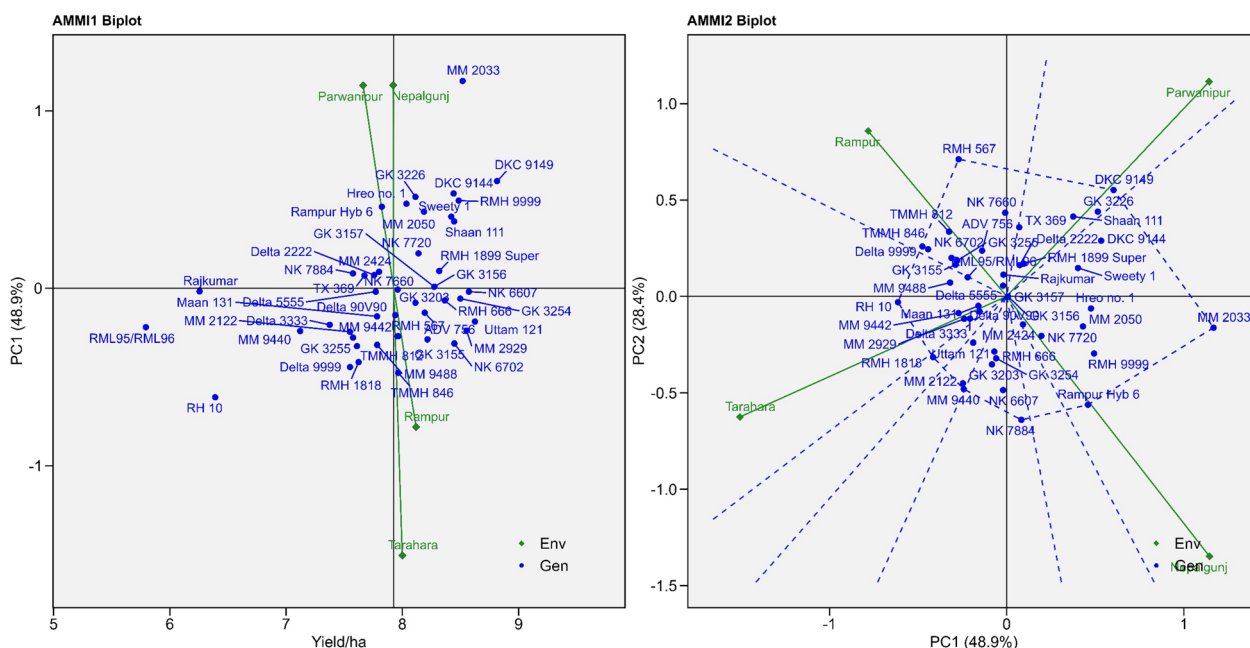


Fig. 4 AMMI 1 and 2 Biplot depicting the performance of multinational companies' maize varieties for grain yield/ha across four diverse environments

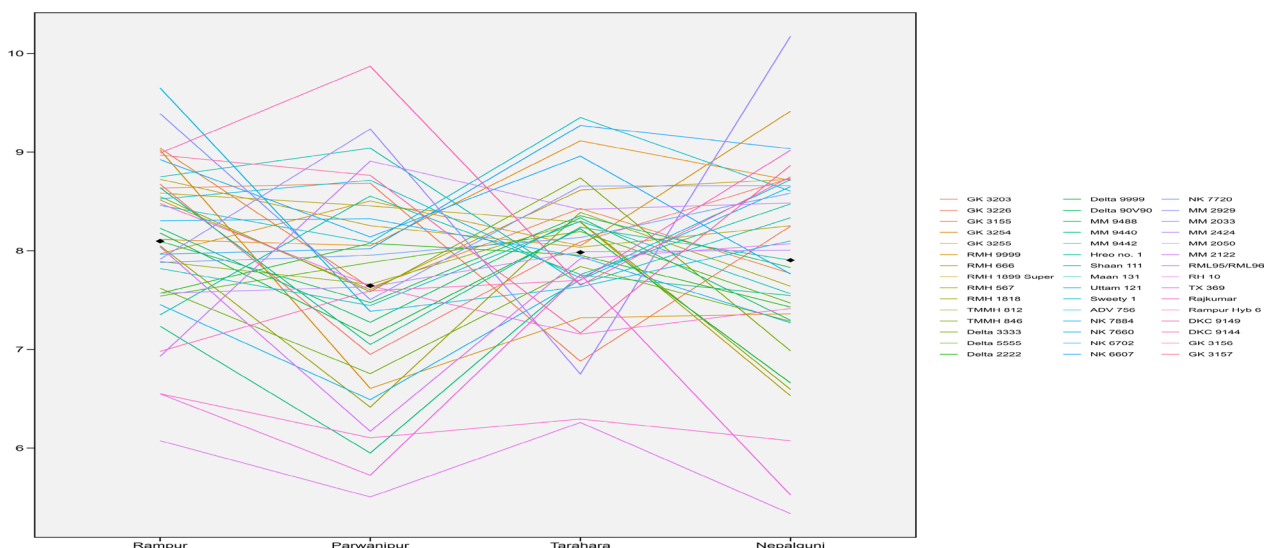


Fig. 5 Genotype environment index (GEI) for yield across different hybrids and research stations (interaction) from 2020 to 2022

Which won where biplot

Seven vertex hybrids, namely MM2929, NK6702, TMMH-846, RH-10, RML-95/96, MM-2033, DKC-9149 were connected to form a polygon, representing their grain yield performance. Likewise, application of a "which-won-where" approach in a sorghum investigation revealed the presence of three mega-environments characterized by different optimal cultivars (Rakshit et al. 2012). Six lines extend from the biplot's origin, intersecting the sides of the polygon perpendicularly, effectively dividing the biplot into eight regions. These divisions categorize environments into two sectors based on their yield similarities, suggesting a potential partition into two mega-environments. The genotype exhibiting the highest responsiveness is characterized by the vertex farthest from the biplot origin, indicating superior yield performance across environments within its sector. The first mega-environment comprises two environments: Parwanipur and Nepalgunj. Notably, hybrids MM2033 and DKC-9149 emerge as the top performers in this mega-environment. Similarly, MM2929 and NK-6702 stand out as winners for Rampur and Tarahara, respectively, possessing the longest vector lengths within their respective mega-environments (Fig. 6).

GGE biplot for specific location winner hybrids

Principal component analysis reveals that the first and second components account for 58.51% and 17.24% of the total variance, respectively, culminating in a cumulative total of 75.75%. In Nepalgunj, genotypes such as Delta 3333, TMMH-812, MM2122, Mann 131, Delta 3535, NK7720, Shaan 111, Sweety 1, DKC9144, and DKC9149

emerged as winners in terms of both stability and yield. Meanwhile, Rampur Hybrid 6, TX-369, MM2424, Delta 2222, and NK7884 exhibited stability in Rampur. Transitioning to Parwanipur, MM9440, Delta 3333, MM2122, Maan-131, Shaan 111, DKC 9149, and Sweety 1 demonstrated stability. Finally, in Tarahara, hybrids NK7884, MM2424, Delta 2222, TX.369, and Delta 5555 achieved both stability and high performance (Fig. 7). These findings offer valuable insights into the selection of suitable genotypes for different Maize-growing regions in Nepal.

Discriminateness vs representativeness of the environments over genotypes

The discriminate versus representativeness biplot, showcased in (Fig. 8), serves as a valuable GGE biplot for assessing test environments (Gebreselassie et al. 2022; Adham et al. 2022). Environments with high discriminating ability are adept at distinguishing between hybrids based on their performance. Environments in this study were carefully chosen to effectively separate maize hybrids with different yield potentials, leading to precise identification of high-performing ones. But how representative are these environments? Representativeness refers to how similar a test environment's results are to other environments. Here, the angle between a test environment and the "average environment" serves as a dual measure of both representativeness and discriminating ability (Esan et al. 2023). A small angle indicates high representativeness (similar to the average) and low discriminating ability (hybrids perform similarly), while a larger angle suggests the opposite: low representativeness (unique environment) and high discriminating ability

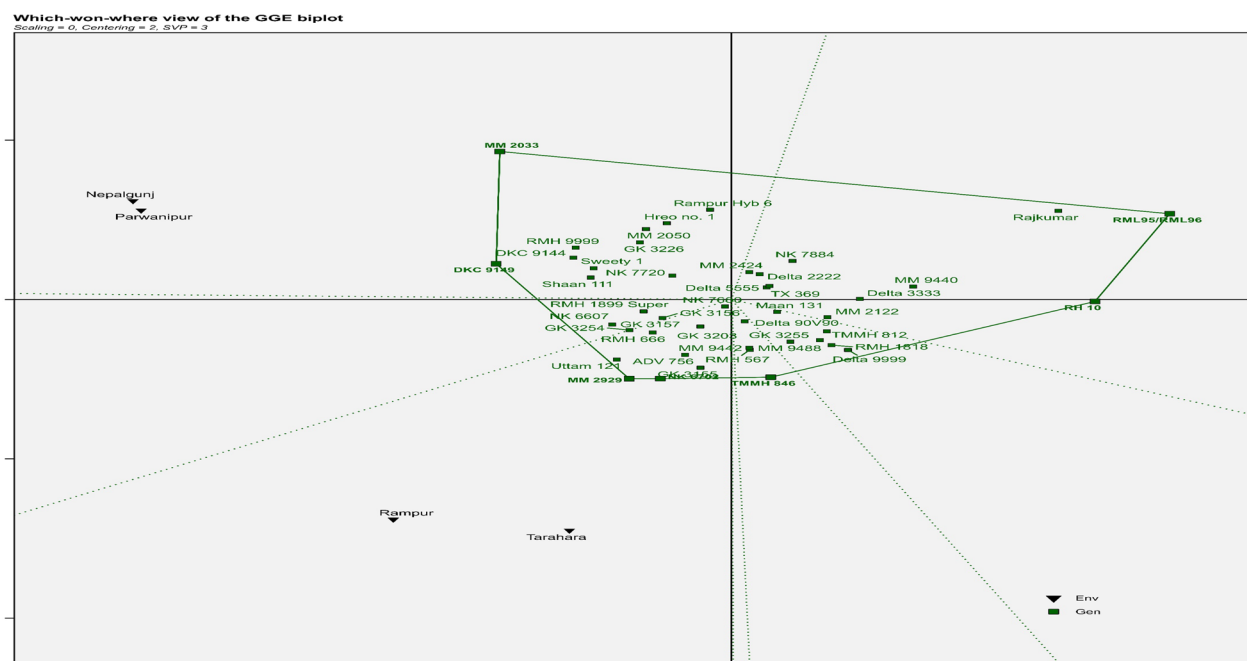


Fig. 6 "Which-Won-Where" biplot for Genotype × Environment Interaction for yield/ha in 2020–2022

(hybrids perform distinctly) (Khan et al. 2021). Rampur and Parwanipur stand out as more representative environments compared to Nepalgunj and Tarahara, while the latter pair, along with Parwanipur, are the most discriminating. This relationship flips for representativeness, with DKC-9149 and Shann-111 taking the reins for discriminating ability, while GK3157, RMH-666, MM2929, and GK3203 shine in representativeness. Highly discriminating environments are crucial for identifying hybrids with stable yields and specific adaptations. This explains why the acute angles between all environments in this study suggest positive correlations: Nepalgunj and Parwanipur show the strongest positive correlation, followed by Rampur and Tarahara. In other words, genotypes tend to perform similarly across these environments.

Stability and average yield performance of the hybrids

To assess the stability and average yield performance of the 45 Maize Hybrid hybrids, the average environment coordination (AEC) approach was employed. The stability axis, represented by a double-headed arrow axis that intersects the biplot origin at a right angle to the AEC on the basis of singular value partitioning (SVP=1), serves as a crucial indicator of genotype stability. The vertical axis (ordinate) of the biplot divides genotypes into two groups based on their average yield performance: those above and below the average. Genotypes located closest to the vertical stability axis displayed the most stable yield performance. These stable performers

included RML-95/96, RH-10, Rajkumar, MM9440, Delta 333, Delta555, NK7660, RMH1899 super, Shan 111, and NK7660 (Fig. 9). Conversely, genotypes further away from the stability axis exhibited more unstable yield, characterized by significant fluctuations across environments. Examples of these unstable genotypes include MM2920, TMH846, MM2033, RH-6, Hero no 1, Delta 9999, and MM2050. However, it’s crucial to note that stability alone does not guarantee success. Among the stable genotypes, several achieved both stability and high average yields; these high-performing, stable hybrids included RML95/96, RH-10, MM9440, Delta-333, Tx-369, and Delta 5555. Other stable genotypes, such as NK7660, RMH1899 super, and GK3203, demonstrated stable but lower average yields.

Ranking hybrids relative to the ideal hybrids

An analysis of 45 maize hybrids grown in four different environments using a GGE biplot revealed that hybrids closer to the center of the concentric circles had higher average yields. By the same token, GK3157, NK6607, RMH1899 Super, GK3254, RMH666, Shan 111, DKC9149 and Sweety-1 were identified as the most desirable hybrids due to their high yields and lies first concentric circle proximity to the ideal genotype. Besides, NK6702, GK3155, MM944, Mann 131, Delta 5555, NK7720, MM2424, RMH999, DKC9144 located in the second concentric circle near the ideal genotype, exhibited both good yields and stability. Hybrids MM9440, Hero no 1

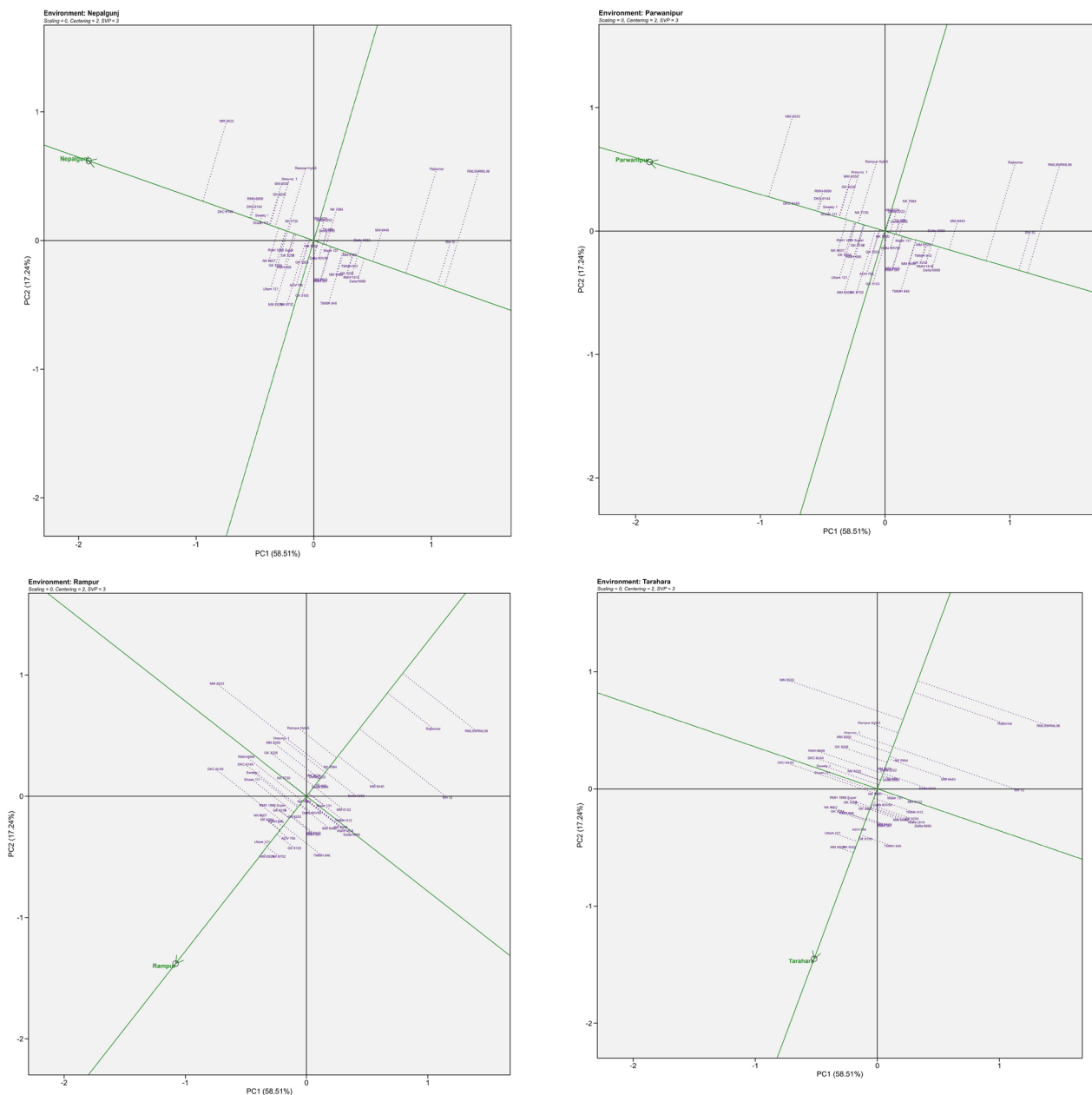


Fig. 7 GGE biplot illustrating genotype-by-environment interactions for winner genotypes across Nepalgunj (I), Rampur (II), Parwanipur (III), and Tarahara (IV) for the 2020–2022 growing seasons

positioned further from the ideal genotype, showed lower stability. Hybrids RH10, Rajkumar, MM2033, RML95/96, RH-6, located outer side the concentric circles, had low yields and were considered unsuitable for further breeding cycles (Fig. 10).

Best linear unbiased prediction (BLUP)

A substantial proportion of the traits examined displayed significant genotype-by-environment (GxE) interactions

at a 5% significance threshold, as evaluated using the Likelihood Ratio Test (LRT) implemented in the gamem_met function of the metan R package. Variables with nonsignificant Genotype effect, Pant aspects, ear aspects, insect Score, Final plant Stands/ha Ear height, Plant height. This implies that these traits exhibit consistent expression across varying environmental conditions. Consequently, within this context, employing the BLUP approach is likely to yield more precise and dependable

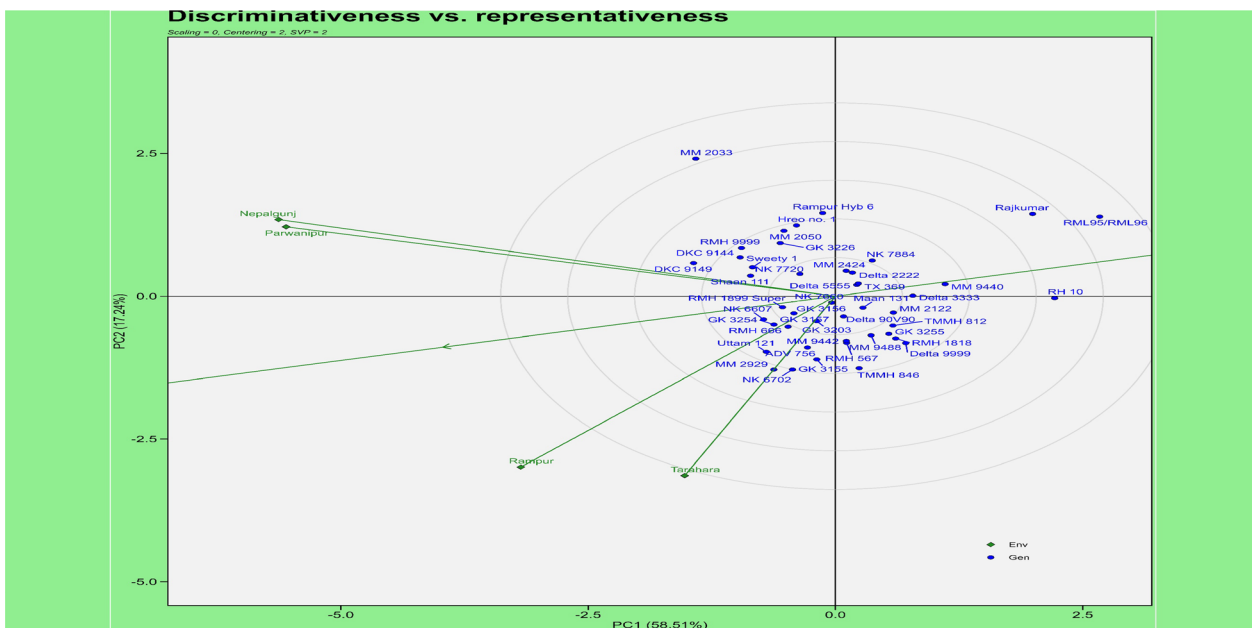


Fig. 8 GGE Biplot illustrating the discriminativness and representativeness of 45 maize hybrids across four locations over a two-Season (2020–2022)

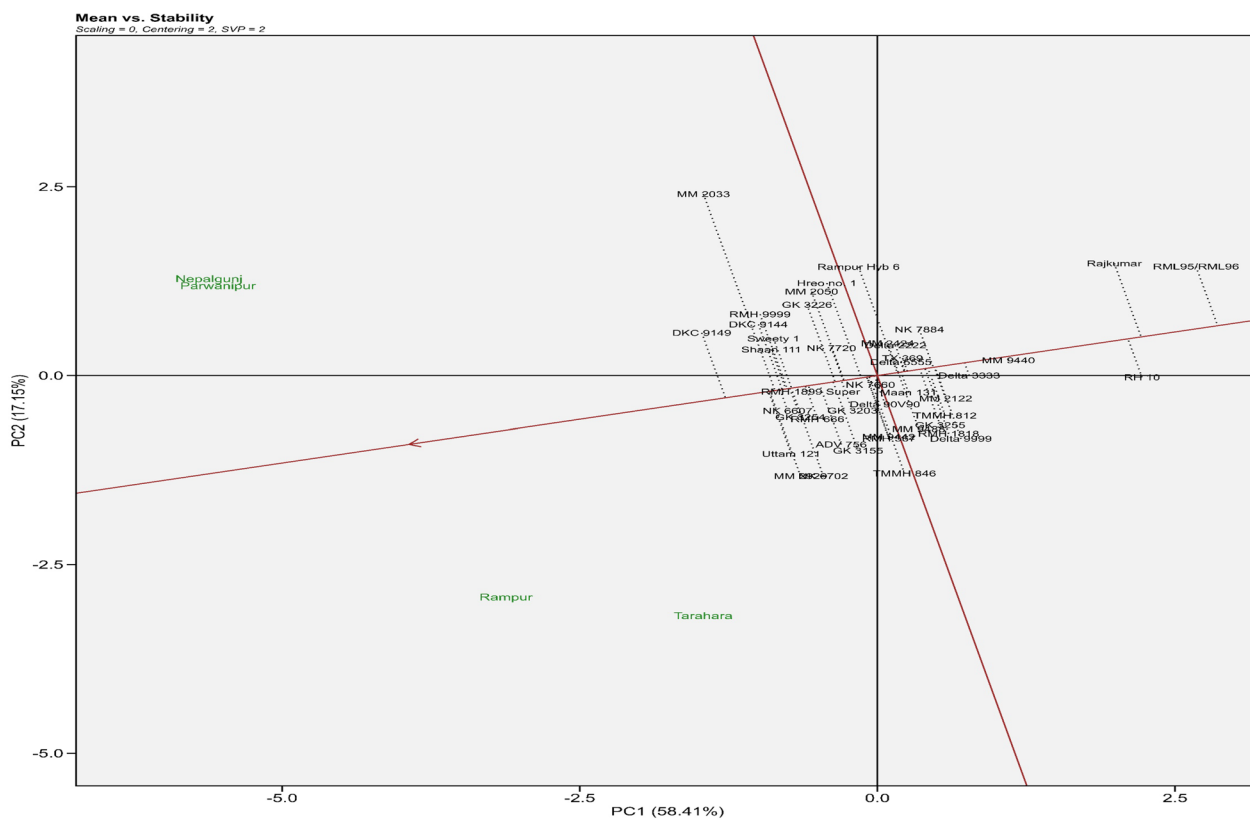


Fig. 9 A GGE biplot illustrating the stability and yield performance of 45 maize hybrids across four locations in 2020–22

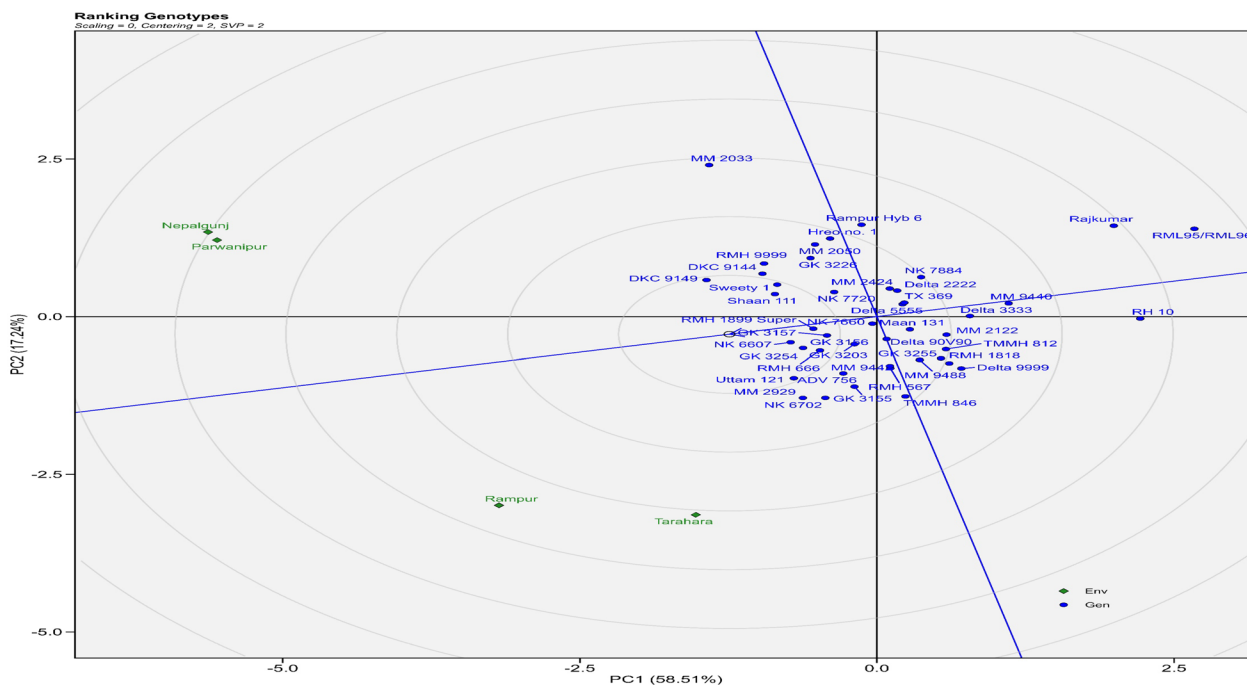


Fig. 10 GGE plot depicting the comparison and ranking of genotypes based on the ideal genotype of maize hybrids over 2 season 2020–2020

predictions of trait performance across diverse environments. The predicted mean values of the hybrids for each of the significant Gx E interaction studied traits are presented in Figs. 1 and 2. BLUP plot delineates the superior performers and stabilized hybrids for yield, with DKC9141 (8.81 tons/ha), Uttam 121 (8.62 tons/ha), NK6607 (8.57 tons/ha), MM2929 (8.55 tons/ha), and MM2033 (8.51 tons/ha) emerging as top contenders. MM9442 (7.95 tons/ha) exhibits a performance just above the yield threshold, indicating its potential. In contrast, RML-96, Rajkumar, RH-10, and Rampur Hybrid six display poor and unstable genotypes, rendering them unsuitable for further selection (Fig. 11). A similar discernment is observed in the test grain weight domain, where NK7720 (384.85 g), RMH66 (382 g), and GK3255 (372.98 g) stand out with the largest test grain weight, reaching up to 350 g. Notably, NK6607 (4.93 cm) followed by DKC9144 (4.87 cm), NK7660 (4.85 cm) boosts the largest cob diameter, and ADV756 just surpassed the threshold line (4.60 cm) and 25 hybrids lies below the BLUP threshold while GK3255 leads in cob length (16.32 cm) followed by ADV756, Sweety 1, MM2050, and NK7884 (15.45 cm) just touch BLUP threshold. On the contrary, TX369 to Shann 111 exhibit poor cob length, and MM24 to Delta 333 fall below the threshold value, warranting their exclusion from further evaluation.

Assessment of anthesis duration reveals that Rampur Hybrid 6 requires more than 118 days, surpassing

ADV-756 and DKC-9149 in duration. The mean value for the Days to Anthesis across the tested hybrids is 112 days. Remarkably, Delta-9999 and Delta-5555 exhibit the shortest duration to anthesis and silking, both falling below 110 days after sowing (DAS). The BLUP mean value for the Days to Silking is approximately 114 days, providing a comprehensive understanding of the reproductive development timeline. 25 more hybrids have days to silks emergence ranges from 112 to 115 days. In terms of Shelling Percentage, the mean value across the tested genotypes stands at 79%. Notably, Uttam-121 (81.35%), NK7884 (80.97%), and GK-3226 (80.69%) showcase the highest shelling percentages (Fig. 12). However, it is essential to highlight that MM2424 (79.19%) approaches the threshold value for shelling percentage, Hero no 1, DKC have least shelling % (77.27% & 77.58%).

WAASBY model for simultaneous selections for yield and stability

This study introduces a novel quantitative stability measure called WAASB, proving its effectiveness in identifying genotypes that excel in both high productivity and broad adaptability across diverse environments. Analyzing four datasets exhibiting various GEI patterns, the researchers determined BLUP to be the most accurate predictive model for genotype performance. WAASB, coupled with yield data that means a weight of 50:50 (affording equal consideration to both yield and stability), revealed

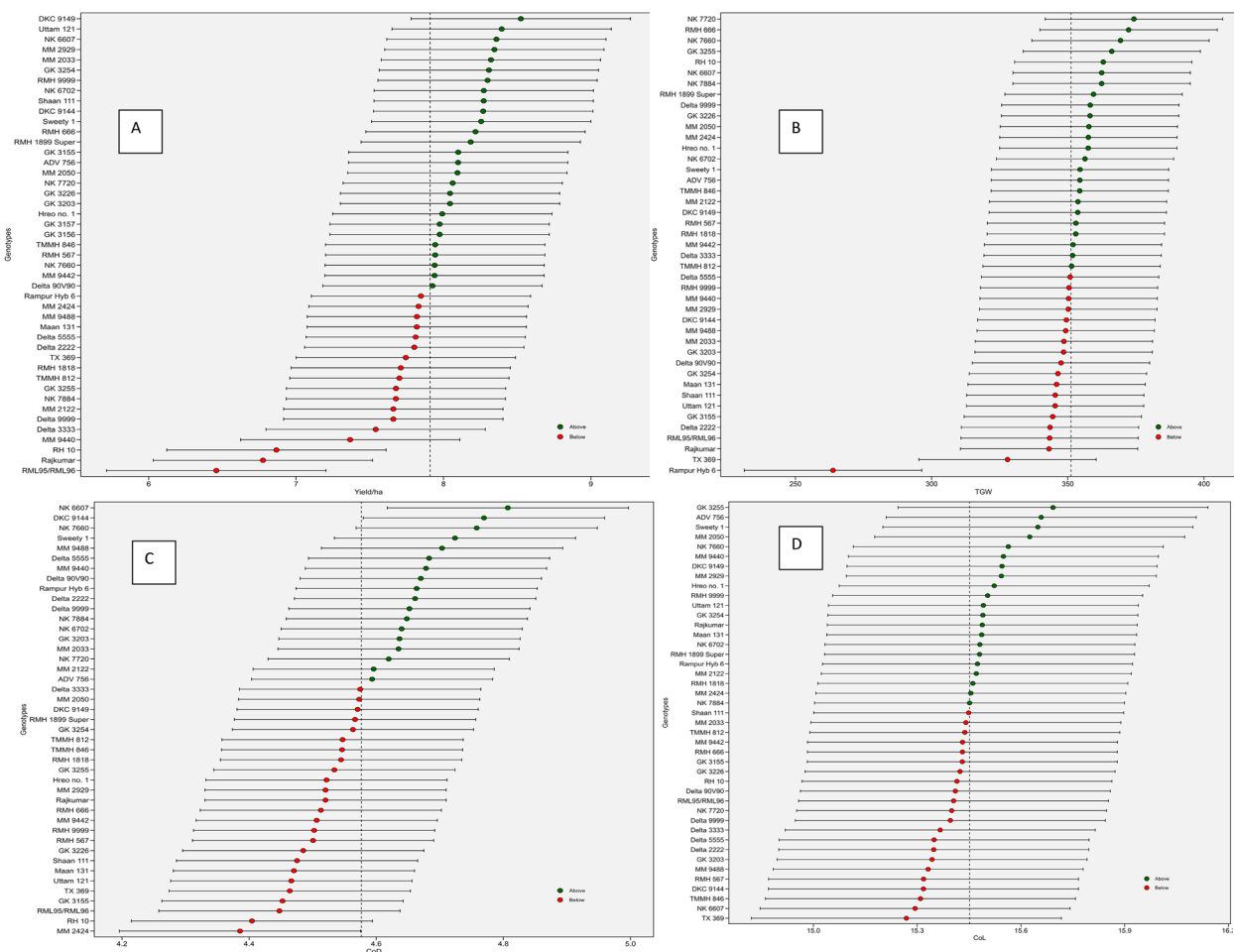


Fig. 11 BLUP Plot illustrating the mean values of **A** Yield per Hectare (Yield/ha), **B** Thousand Grain Weight, **C** Cob Diameter, and **D** Cob Length across various hybrid varieties

RMH-666, GK 3254, Uttam 121, and RMH-1899 has the highest values WAASBY, demonstrating exceptional stability and yield. Conversely, RH-10, RML96/96, MM2033, and MM9440 were identified as underperforming and unstable under the tested conditions (Fig. 13).

Plotting the heat map graphics for the WAASB index and WAASB/GY ratio

For a more precise assessment of yield and stability, the experimental genotypes underwent ranking according to their WAASBY index scores. This (Fig. 14) presents two heatmaps that explore how maize genotypes are ranked according to different factors. The first one examines how rankings change based on the number of components used for stability analysis. The second reveals how rankings vary based on the balance between stability and yield, where 100/0 favors stability and 0/100 favors yield exclusively. A combined analysis of IPCA and WAASB/GY ratio revealed four distinct clusters of maize

genotypes. The Red Cluster comprises genotypes characterized by both low productivity and instability, including DKC9144, DKC9149, Rampur Hybrid 6, MM2050, MM2033, RMH-567, and GK-3226. In contrast, the Blue Cluster encompasses genotypes that exhibit high productivity but are concurrently unstable, featuring Rajkumar, MM2122, MM9440, Delta-9999, TMMH-812, RMH-1818, and GK-3255. The Black Cluster is characterized by stable yet unproductive genotypes, such as RH-10, RML-95/96. Finally, the Green Cluster comprises genotypes marked by both high productivity and stability, exemplified by MBS-1132, MBS-1144, Trivikram to Shree Ram 9696.

Multi-trait stability index (MTSI) and strength, and weakness of the selected maize hybrids for overall conditions.

Using the factorial analysis multi genotype ideotype index (FAGMI), a method that ranks maize hybrids based

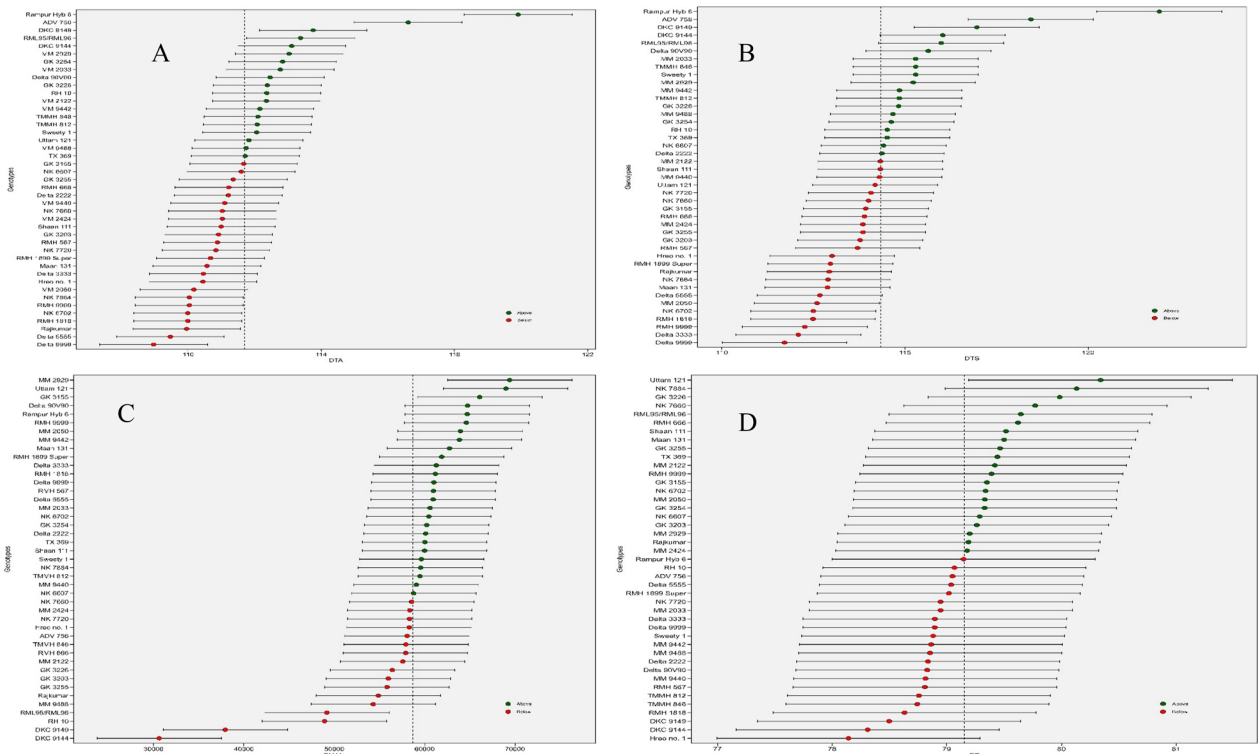


Fig. 12 BLUP Plot representing the mean values for **A** Day to Anthesis, **B** Day to Silking, **C** Ear per Hectare (Ear/Ha), and **D** Shelling Percentage across diverse varieties

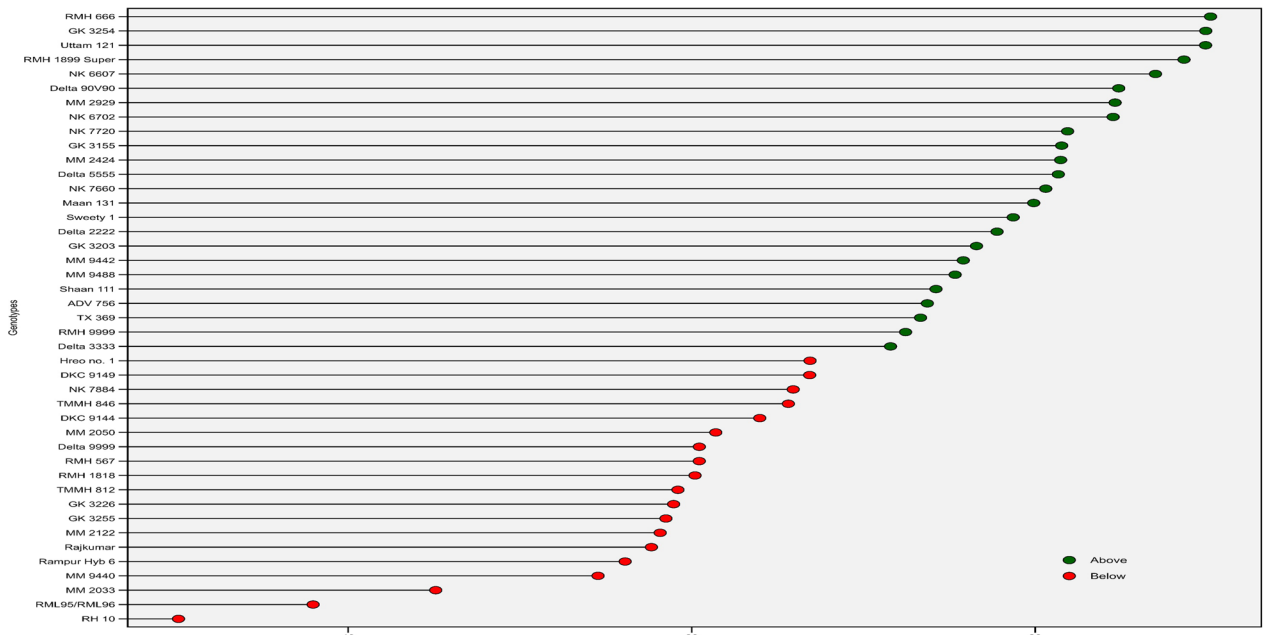


Fig. 13 This figure reveals the stability of various maize genotypes in terms of yield (ha) using the WAASB metric. Red-headed genotypes (unselected) exhibit lower WAASB values, indicating instability and potential discarding for further breeding programs

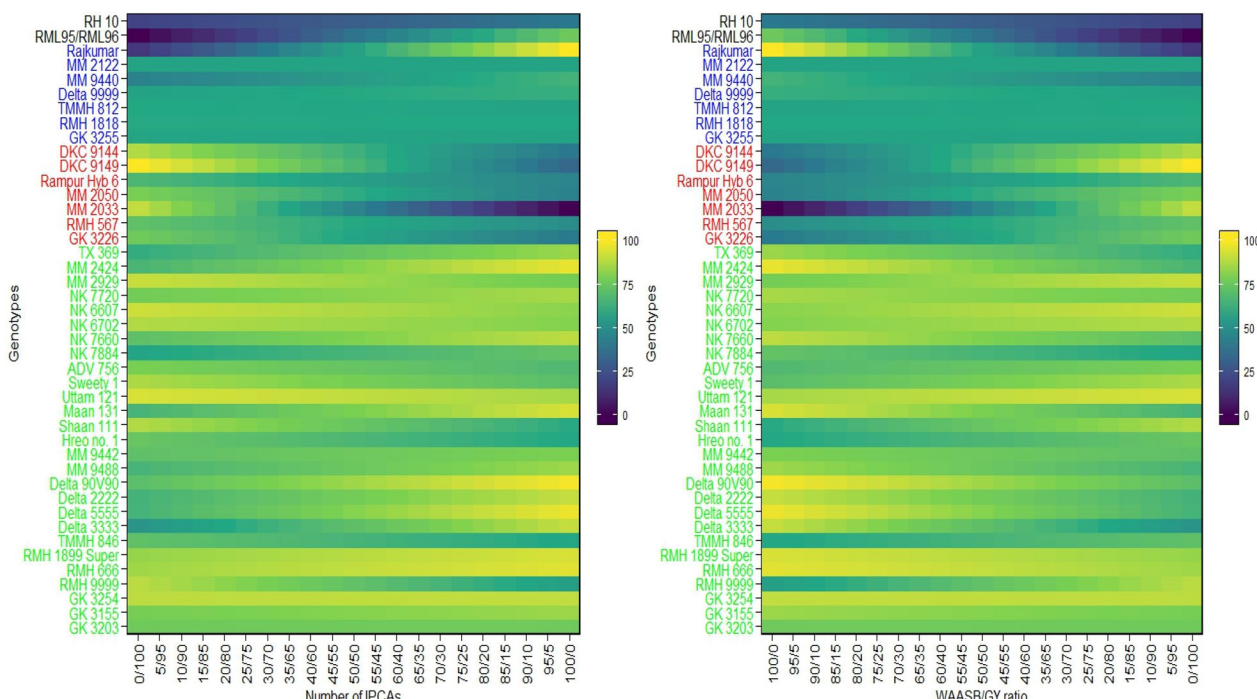


Fig. 14 Ranks of genotypes illustrated by the number of principal components (PC) utilized for estimating the weighted average of absolute scores (WAAS), and WAAS-to-grain yield (GY) ratio

on their performance and stability across multiple important traits, this study identified nine superior hybrids for farmers. Selecting the top 20%, the researchers found MM 2033, NK 6607, MM 2929, Sweety 1, NK 6702, NK 7660, Uttam 121, GK 3203, and MM 2050. These hybrids, marked with red dots in the figure, are expected to be both productive and suitable for a wide environment (Fig. 15). Moreover, the factors analyzed, located at the center of the radar plot, are the most significant

contributors to Factor Analysis (FA) and are less important for selection. The radar plot depicting strengths and weaknesses illustrates the strong and weak points of FA for each all genotype Fig. 16.

Selection of the cold wave tolerant genotype based on MTSI.

A study was conducted to identify maize hybrids with superior performance under cold stress conditions.

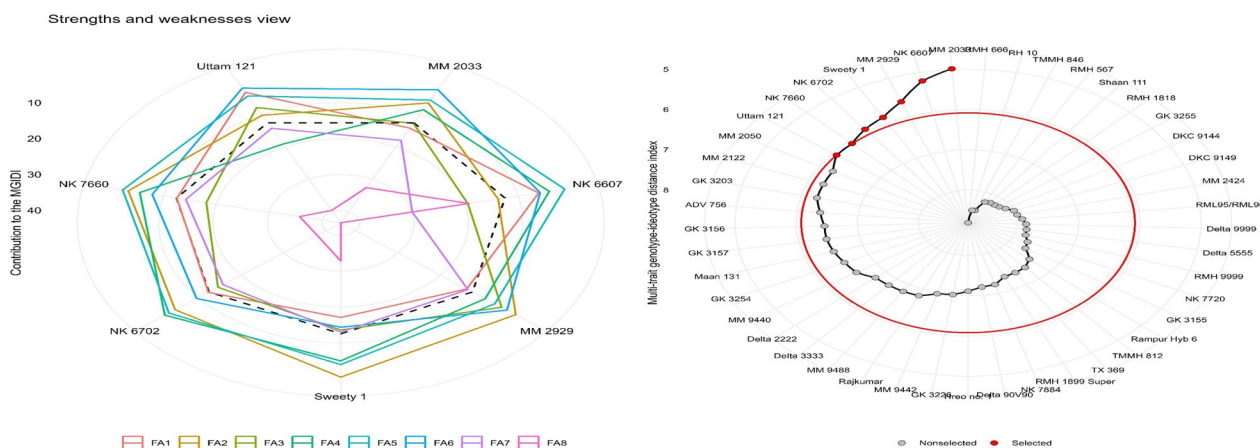


Fig. 15 Representation of multi trait stability index-genotype ranking of hybrid maize over two seasons over 4 locations in Nepal at 20% selection intensity

The strengths and weaknesses view of treatments

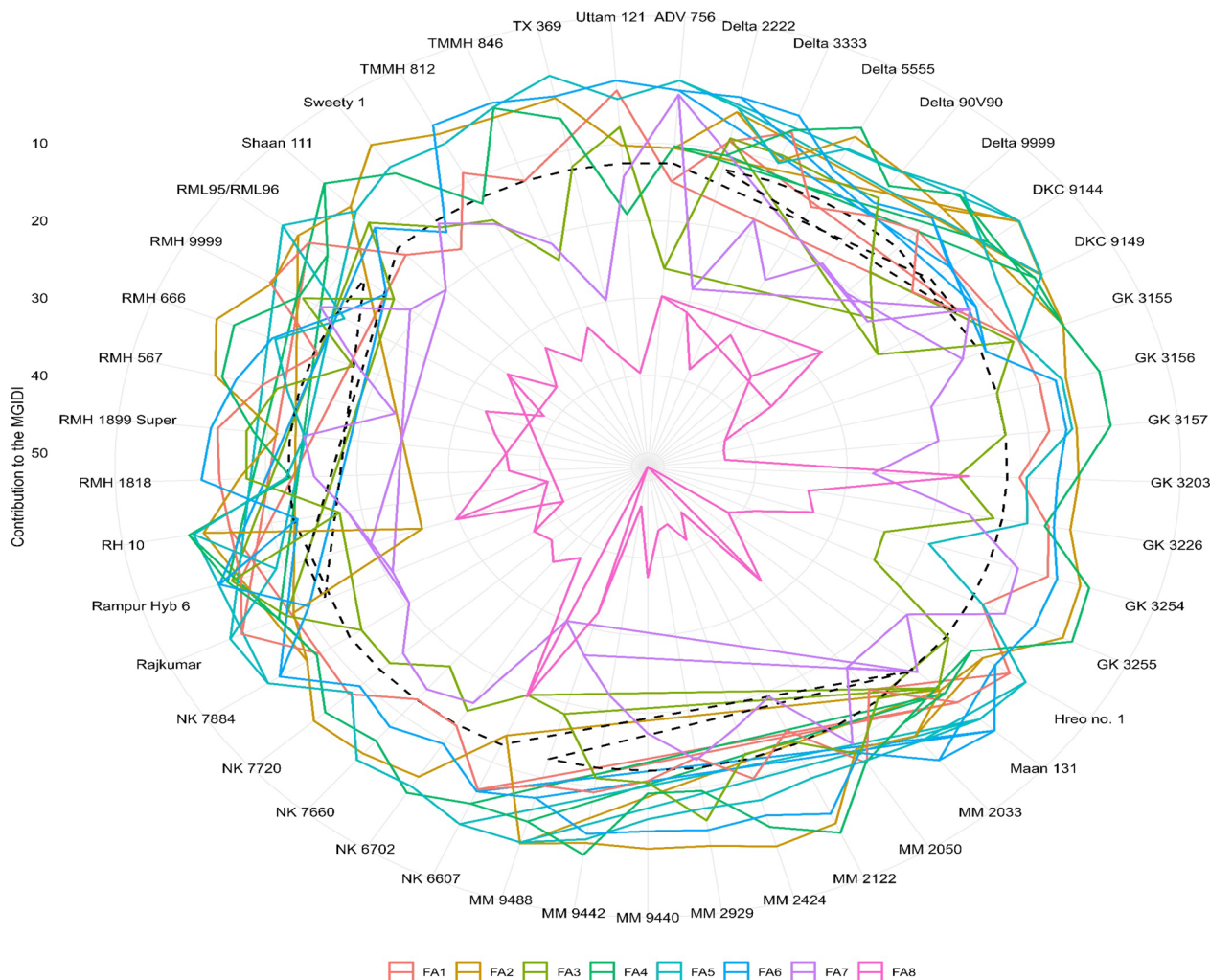


Fig. 16 Graphics depicting the strength and weakness of the all tested varieties in 2020–2022

Seventeen hybrid hybrids were initially selected based on their differential tolerance analysis (DTA) data, indicating tolerance to day temperatures of ≤ 10 °C. Further evaluation using a multi-genotype ideotype index led to the selection of only three hybrids (denoted by red dots) that demonstrated exceptional performance and stability. At 17% selection intensity, these superior hybrids, namely GK3254, TMMH-846, and MM-9442, were chosen for their suitability for cultivation in environments with frequent cold waves (Fig. 17). Interestingly, genotypes with days to anthesis exceeding 108 days exhibited an escape mechanism, as their flowering period coincided with a period of higher minimum day temperatures (13–15 °C). Coefficient value for the FA1 is 0.87 and Community coefficient is 0.76 which contain the both variable Yield/

ha and Days to anthesis. Elaborating the value of SD for yield/ha is 0.93 and days to anthesis is 2.86 Table 2

Discussion

This investigation into the stability and superior performance of hybrid maize under cold stress and stress escape condition revealed distinct patterns in their performance over specific and wide location. By using the protocol of AMMI, BLUP, GGE Biplot, and MTSI, enabled a comprehensive analysis of the impact of cold wave escape and cold-wave conditions on the selected maize hybrids sought to identify resilient maize hybrids. This study unveils The AMMI1 plot highlights that hybrids such as NK6607, GK3157, GK-3203, RMH-666, and RMH1819-super exhibit both resilience and high productivity

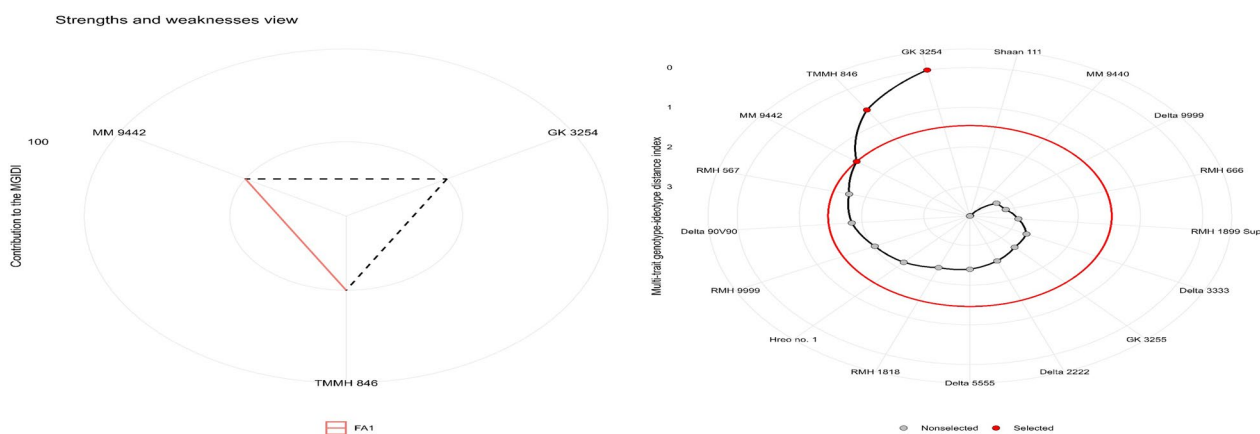


Fig. 17 Impact of cold waves on winter maize hybrid selection (2020–2022) based on days to anthesis that exposed to cold waves and yield/ha

Table 2 Prediction of selection differential for studied traits based on the MTSI index

Variable	Factor	Xo	Xs	SD	SD%	h ²	SG	SG%	sense	goal
DTA	FA1	104.4559	107.3187	2.862745	2.740625	1	2.862745	2.740625	increase	100
Yield.ha	FA1	8.846482	9.776678	0.930196	10.51487	1	0.930196	10.51487	increase	100

DTA: Days to anthesis, Xo: Population mean, Xs: Sample population mean, SD: Selection differential, SD%: Selection differential %, h²: heritability broad sense, SG: Selection Gain, SG%: Selection gain percent

in terms of yield. Similar results was obtained for the grain yield of maize by Ndhlela et al. (2014); Kunwar et al. (2024). On the other hand, Rajkumar, TX-369, and Delta-5555 demonstrate stability for yield but consistently fall below the established threshold, indicating that they are not suitable as major phenotypes for maximizing yield. Likewise, AMMI biplot analysis revealed that certain cassava genotypes and environments performed well in terms of yield (Wodebo et al. 2023). Elaborating this result showed that PCA captured a substantial portion of yield variation, with PC1 and PC2 explaining 48.9% and 28.4%, respectively, for a cumulative contribution of 77.3%. This finding aligns with previous studies on bread wheat in Turkey, where PCA explained around 61% of yield variation (Ilker et al. 2011). Thus, AMMI biplot analysis highlighted the importance of location-specific adaptation for optimizing maize yield under cold escape. Moreover, DKC9141 (8.815 tons/ha) thrived in Parwanipur’s conditions, while RMH-567 (7.965 ton/ha) excelled in Rampur. Tarahara proved to be suitable for Uttam 121 (8.625 ton/ha) and RH-10 (6.39 tons/ha), and Nepalgunj offered diverse options with Rampur Hybrid 6 (7.82 ton/ha), NK7884 (7.575 tons/ha) and MM2033 (8.51 ton/ha) all demonstrating superior performance. Another similar study on Maize that support this study hypothesis for wide performance Combining performance data from multiple locations, KWM-91×KWM-93 (8.89 t ha⁻¹) and KWM-92×KWM-93

(8.60 t ha⁻¹) reigned supreme in terms of grain yield (Shrestha et al. 2022). Based on which won where biplot analysis, across diverse environments, maize hybrids MM2033 and DKC-9149 emerged as top performers in the first mega-environment. Mean-while, MM2929 and NK-6702 reigned supreme in the specific conditions of Rampur and Tarahara. Another study on sorghum, Of the three mega-environments defined in a similar Indian research station, the largest yielded top performance was SPH 1606/1609 (Rakshit et al. 2012; Enyew et al. 2021). In the same way, GGE biplot analysis unveil that In Nepalgunj, genotypes like Delta 3333, TMMH-812, and MM2122 not only delivering impressive yields but also demonstrating remarkable stability across diverse conditions. Meanwhile, Rampur favored stability, with Rampur Hybrid 6 and TX-369 reigning supreme. Finally, Parwanipur found superior for MM9440 and Delta 3333, who showcased their unwavering performance in this unique environment. This regional patchwork of winners highlights the importance of tailored recommendations for maize hybrids in Nepal, ensuring optimal yields and resilience for farmers facing varied ecological challenges. Another maize hybrid stability via GGE biplot analysis documented revealed that G9 and G10 emerging as the most reliable options in Africa (Ruswandi et al. 2021). What’s more, discriminate versus representativeness biplot shows that Rampur and Parwanipur stand out as more representative environments compared to

Nepalgunj and Tarahara, while the latter pair, along with Parwanipur, are the most discriminating. Ironically, this relationship flips for representativeness, with DKC-9149 (8.441) and Shann-111 (8.44 tons/ha) taking the reins for discriminating ability, while GK3157 (8.47 ton/ha), RMH-666 (8.365 ton/ha), MM2929 (8.55 ton/ha), and GK3203 (8.11 ton/ha) shine in representativeness.

Utilizing BLUP and WAASBY, a maize field trial identified superior performers for both yield and stability: DKC9141 (8.57 t/ha), Uttam 121 (8.55 tons/ha), NK6607 (8.51 t/ha), MM2929 (8.55 t/ha), and MM2023 (8.51 t/ha). Over and above, WAASBY-identified Green Cluster highlighted stable, high-yielding genotypes like MBS-1132 and Shree Ram 9696. MTSI analysis pinpointed MM 2033 (8.53 t/ha) and others as promising for escape to cold wave, while GK-3254 (8.49 t/ha), TMMH-846 (7.96 t/ha), and MM-9442 (7.95 t/ha) demonstrated strong adaptability and stability under cold waves. Similar Protocol is used by various researcher for the study of stability and superior yield performance of various crops. For instance, lentils ideal genotypes (Hossain et al. 2023), rhizome and essential oil stability of Turmeric (Munda et al. 2022), Sugar quality of the sugar beet (Taleghani et al. 2023), grain yield of finger millet (Anuradha et al. 2022), Potato (Sood et al. 2020), Barley superior hullness (Ajay et al. 2021), wheat (Verma and Singh 2021), common Bean (Santos et al. 2019), castor (Memon et al. 2023), ashwagandha for at once stability parameter and root yield (Pratibha et al. 2023). Another investigation into sweetcorn maize that supports this study, employing a linear mixed model, revealed that various genotypes demonstrate greater phenotypic stability and mean performance across interactive traits (Patel et al. 2023). Since today's sequencing techniques result in a high number of genetic markers, many scholars expect a high number of accessions in combination with a high number of genetic markers. For Plant-Environment Interactions there might be content to see the description of genetic resources presented using the population genetics owing to advancement in molecular study like genome wide association study for yield and stress tolerance can uncover quantitative trait loci (QTLs) and single nucleotide polymorphisms (SNPs) that contribute to the genetic architecture of these complex traits such as in interspecific population of chickpea (Kalve et al. 2022), flowering time prediction in chrysanthemum (Su et al. 2024). GWAS can identify candidate genes and variants controlling stability and stress tolerance, while stability analyses validate the phenotypic effects of these variants. Therefore, such molecular study has further scope on such populations to uncover the underlying genetic architecture.

Conclusion

In conclusion, this study conclusively emphasizes the crucial role of Genotype-Environment interaction (GEI) in breeding resilient and productive winter maize hybrids for diverse Nepalese environments. Employing cutting edge statistical tools, the research identified superior hybrids exhibiting both high average yields and adaptability across locations. Empirical model based selected hybrids such as GK3157 (8.47 tons/ha), NK6607 (8.57 tons/ha), and DKC9141 (8.815 tons/ha) emerged as top performers across most environments, while others like Delta 3333 (7.375 tons/ha) and Rampur Hybrid-6 (7.82 tons/ha) displayed region-specific suitability. Based on multi genotypes ideotype distance indexing, MM2033 identified as most stable and superior performer for interactive traits. Additionally, promising hybrids maize that tolerate to cold waves snaps are GK3254 (8.49 tons/ha), TMMH-846 (7.965 tons/ha), and MM-9442 (7.95 tons/ha) were identified, highlighting the potential for targeted recommendations based on regional needs. Thus, implementing these findings can substantially optimize winter maize yield at the farmer level, contributing to food security and economic well-being in Nepal.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s43170-024-00266-7>.

Supplementary Material 1. Mean of tested hybrids for all parameter that were evaluated.

Supplementary Material 2. Factorial rank of the all genotypes based on MTSI value.

Supplementary Material 3. Contribution factor rank of the selected genotypes based on MTSI.

Supplementary Material 4. Factorial analysis of all phenotypic marker based on Factorial loading value.

Supplementary Material 5. Descriptive statistics of all the variables measured of Hybrid maize.

Supplementary Material 6. Mean of Hybrids across the Environment.

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Author contributions

C.B. K: Investigation, writing—original draft preparation, project administration; Supervision; B.B: Investigation, Methodology, software, validation, data curation; writing—review and editing. S.S: investigation; D.N.M: investigation; R.C: investigation; J.U: investigation; P.P: investigation. All authors reviewed the manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations**Ethics 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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