Assessing yield variation in rice varietal mixtures (VarMix®) and single varieties across 12 environments using AMMI analysis

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ABSTRACT

Traditionally, rice cultivation has heavily relied on single varieties tailored for specific traits, yet these approaches have demonstrated limitations in resilience and stability. Embracing variety mixtures (VarMix) allows us to leverage genetic diversity, thereby enhancing yield stability, bolstering pest and disease management, and optimizing resource efficiency, ultimately fostering more sustainable and resilient rice production systems. This study investigates the performance of 12 rice varietal mixtures and single varieties across 12 distinct environments using the Additive Main Effects and Multiplicative Interaction (AMMI) approach, integrating variance and principal component analyses (PCA). The analysis reveals environmental factors as the predominant driver of genetic variation, with significant contributions to rice yield dynamics. Notably, genotypes such as NSIC Rc298 (A), NSIC Rc298: NSIC Rc214: NSIC Rc216 (ABC), and NSIC Rc214: PSB Rc82: NSIC Rc238 (BDE) consistently exhibited high yield across multiple environments. Furthermore, varietal mixtures, particularly BDE, demonstrated both high yield and stability, highlighting the potential of mixed genotypes in enhancing agricultural resilience. The findings underscore the importance of tailored breeding strategies and environmental considerations in maximizing rice yield and stability, contributing to sustainable crop production practices and food security initiatives.

INTRODUCTION

Varietal mixtures have gained recognition as an effective approach for disease management (Finckh et al., 2000; Mundt, 2002; Kiaer et al., 2012; Reddy, 2017; Raboin et al., 2012; Gallet et al., 2014). This innovative method has also been explored to address the challenges posed by water scarcity, particularly in regions with limited water resources, impacting wheat (Adu-Gyamfi et al., 2015), wheat and barley (Kiaer et al., 2009), barley (Kiaer et al., 2012), and rice production (Pacada and Francia, 2023; Montazeaud et al., 2017). In the Philippines, the development of varietal mixture or VarMix, is based on the mixture of seeds of selected varieties with closely similar agronomic traits but with a defined genetic dissimilarity (Pacada

KEYWORDS

VarMix, AMMI, GxE, abiotic stress

et al., 2022). This novel approach for identifying single varieties to be combined or mixed, enhances inter-varietal diversity, thus, the mixing outcome of each identified combination triggers the mechanism for disease reduction, such as barrier effect, and mechanism for increasing yield and yield stability, like complementarity and compensation effect, that happen particularly in the below ground environment or in the roots (Pacada and Francia, 2023; Pascua et al., 2020).

Despite its potential in mitigating both biotic and abiotic stresses, VarMix technology is still in its early stages of achieving yield stability. Consequently, there is a critical need for comprehensive genotype by environment (GxE) data to identify the performance of specific genotypes across multiple environments (Yan et al., 2000; Yan and Rajcan, 2002).

To investigate genetic variation, researchers have frequently employed the AMMI (Additive Main Effects and Multiplicative Interaction) analysis. For instance, AMMI analysis highlighted genotypic variability as predominant, which in the process identified IR22 as superior under unfavorable N-levels and revealed MTU1010 as stable across various environments among the 55 rice genotypes evaluated (Abdelrahman et al., 2022). The AMMI model effectively analyzed genotype × nitrogen interactions, helping to identify superior rice genotypes AMMI not only elucidates sources of variation but also provides a metric for stability measurement, which is crucial for comprehending genotypic rankings and identifying the best-performing genotypes (Falconer, 1952; Fernandez, 1991). Cantila et al. (2020) endorsed five rice lines from hybrid rice varieties for national cooperative testing in the Philippines based on AMMI. Additionally, through an AMMI1 biplot analysis, IR83142-B-7-B-B, Binuhangin, IR77298-14-1-2-13, IR70215-70-CPA-3-4-1-3, and IR77298-14-1-2 emerged as the genotypes

showcasing the highest and most stable grain yields in both well-watered and mild to moderately severe drought stress environments (Torres and Henry, 2018).

This study aims to determine significant variations in yield among 18 genotypes, comprising VarMix and single rice varieties, across 12 diverse environments, in order to identify which genotypes consistently yield well and remain stable. The assessment will utilize the Additive Main Effects and Multiplicative Interaction (AMMI) model, employing variance (ANOVA) and principal component analyses (PCA). Additionally, yield/stability rankings (AMMI stability index), combined selection index (CSi), and biplot analyses will be employed to provide a comprehensive understanding of the yield performance and stability of the tested genotypes.

METHODOLOGY

Set up establishment
A total of 18 entries were used, consisting of 12 VarMix and their six single component varieties (PSB Rc82, NSIC Rc214, Rc216, Rc238, Rc298, Rc300) (Table 1). All single varieties are registered seeds, and seeds of the VarMix combinations were pre-mixed before sowing. All areas transplanted 21-day-old seedlings at 20 x 20cm between rows and hills; laid out in randomized complete block design (RCBD) with two replicates each. Farmer practice was used in cultural and fertilizer management. No chemical pesticides were used to monitor the performance of all entries for pest and disease reactions. Planting calendars differ among experimental areas, hence, some of the entries were established from June to December 2015; the rest from January to July 2016.

Table 1: Ratios of single varieties to form VarMix entries and their codes

Ratio	Single Variety/VarMix Combination	Code
0	NSIC Rc298	A
	NSIC Rc214	B
	NSIC Rc216	C
	PSB Rc82	D
	NSIC Rc238	E
	NSIC Rc300	F
1:1	NSIC Rc298 : PSB Rc82	AD
1:1	NSIC Rc298 : Rc238	AE
1:1:1	NSIC Rc298 : Rc214 : Rc216	ABC
1:1:1	NSIC Rc298 : Rc300 : Rc238	AFE
1:1:1	NSIC Rc214 : Rc216 : PSB Rc82	BCD
1:1:1	NSIC Rc214 : PSB Rc82 : Rc238	BDE
1:1:1:1	NSIC Rc298 : Rc214 : Rc216 : Rc238	ABCE
1:1:1:1	NSIC Rc298 : Rc216 : PSB Rc82 : Rc238	ACDE
1:1:1:1	NSIC Rc298 : Rc216 : Rc238 : Rc300	ACEF
1:1:1:1:1	NSIC Rc298 : Rc214 : Rc216 : Rc238 : Rc300	ABCEF
1:1:1:1:1	NSIC Rc298 : PSB Rc82 : Rc216 : Rc238 : Rc300	ADCEF
1:1:1:1:1:1	NSIC Rc298 : Rc214 : Rc216 : PSB Rc82 : Rc238 : Rc300	ABCDEF

Twelve environments were selected based on the occurrences of pests and diseases and those with limited sources of water. Data information of evaluated incidence of biotic stresses, and actual data of water table level below ground, and amount of rainfall in 12 trial locations, were presented in a published paper of Pacada and Francia (2023). The characteristics of these 12 environments are presented in Figure 1.

Statistical analysis
The model used for the Analysis of Variance (ANOVA) in a

randomized complete block design is $Yijk = \mu + Gi + Ej + GEij + Bij + \varepsilon ijk$, where $Yijk$ is the grain yield observation for the *i*-th genotype, *j*-th environment, and *k*-th replication. μ is the overall mean of the grain yield across all locations, Gi represents the effect of the *i*-th genotype, Ej denotes the effect of the *j*-th environment, $GEij$ accounts for the interaction effect between the *i*-th genotype and the *j*-th environment, Bij is the effect of the *k*-th replication within the *j*-th environment, εijk is the random error term associated with the *i*-th genotype, *j*-th environment, and *k*-th replication. Index

definitions are the following: i: Index for genotypes, values range from 1 to n_g, where $n_{g=18}$ is the total number of genotypes; j: index for environments, values range from 1 to n_e, where

$n_{e=12}$ is the total number of environments (locations); and k: index for replications, values range from 1 to n_r, where $n_{r=3}$ is the number of replications per environment.
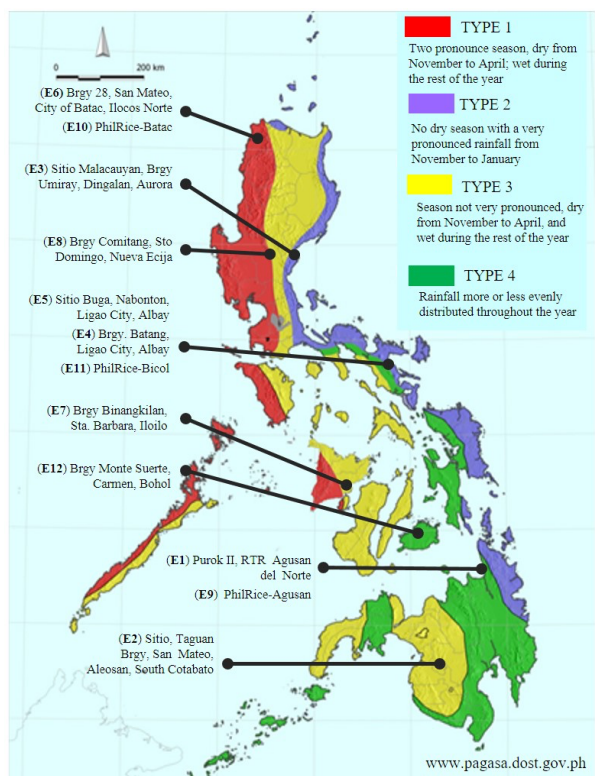

Figure 1: Climatic zones based on modified coronas classification (MCC) and the actual location of 12 experimental set up.

Additive Main Effects and Multiplicative Interaction (AMMI) model was used for genotype × environment (G × E) interaction analysis. Formula used is of

$y^N_{ij}= \mu + g_i + e_j + \Sigma\lambda k Y_{ik}\alpha_{jk} + \xi_{ij}$ where y_{ij} is the yield of the i-th genotype in the j-th environment, N is the number of principal components in the AMMI model, μ is the overall mean of the genotypes, g_i is the genotype effect for the i-th genotype, and e_j is the environment effect for the j-th environment. $\Sigma\lambda k Y_{ik}\alpha_{jk}$ represents the interaction effect captured by the principal components of the AMMI model, where $\Sigma\lambda k$ is the eigenvalue of the k-th principal component axis, Y_{ik} is the genotype principal component score for axis k, α_{jk} is the environment principal component score for axis k, Σ_{ij} is the residual value after accounting for the main effects and interactions. Index definitions are the following i: index for genotypes, ranging from 1 to n_g, j: index for environments, ranging from 1 to n_e, k: index for principal components, ranging from 1 to N, where N is the number of principal components included in the AMMI model.

The AMMI stability index (ASi) following Purchase's (1997) formula: ASi= [[(IPCA1 SS/IPCA2 SS) x IPCA1 scores2] + IPCA2 scores2]1/2. All biplot analyses were conducted using PB Tools version 1.4 (http://bbi.irri.org/products), while analysis of variance, descriptive statistics, and correlation analysis were performed using STAR version 2.0.1 (2014).

RESULTS

AMMI with ANOVA and PCA

The AMMI analysis, integrating both ANOVA and PCA, revealed substantial significance in various sources of variation, including environments, replications within environments, single variety/varietal mixtures (G), and GxE interactions (Table 2). Environment emerged as a predominant factor, contributing to 89.7% of the explained variance, exerting a significant impact on the performance of genotypes. Within each environment, replications contributed a smaller but still statistically significant portion (1.2%) to the variability (Table 2). The choice of single variety or varietal mixtures accounted for 1.3% of the overall variance (Table 2), underlining that while genotype selection is influential, it is not the primary factor determining yield in this study.

Table 2: AMMI-Analysis of variance of single variety/varietal mixtures of yield in 12 locations with interaction components based on principal component analysis.

Source of variation	DF		SS	MS	Explained Variance (%)
Environment (E)	11		1030.927	93.721**	85.7
REP within E	12		13.751	1.146**	1.1
Single variety/Varietal mixtures (G)	17		14.471	0.851**	1.2
GXE (IPCA)	187		90.587	0.484**	7.5
IPC1		27	35.995	1.333**	3.0
IPC2		25	17.823	0.713**	1.5
IPC3		23	9.062	0.394 ns	0.8
IPC4		21	8.685	0.414 ns	0.7
IPC5		19	7.293	0.384 ns	0.6
IPC6		17	3.234	0.19 ns	0.3
IPC7		15	2.581	0.172 ns	0.2
IPC8		13	2.396	0.184 ns	0.2
IPC9		11	2.227	0.202 ns	0.2
IPC10		9	0.962	0.107 ns	0.1
IPC11		7	0.329	0.047 ns	0.0
Pooled Error		204	53.607	0.263	
Total		413	1203.343		

*** highly significant at p ≤ 0.01, ns= not significant.*

GxE interactions explained 7.5% of the variance, with IPCA revealing specific interactions contributing to this variability. Principal components, IPC1 and IPC2, with explained variances of 3.1% and 1.6% respectively (Table 2), emerged as substantial contributors, highlighting key interactions influencing

difference to the yield of the G (Figure 2). Nevertheless, other IPCs still played a role in explaining additional variance in GXE (Table 2).
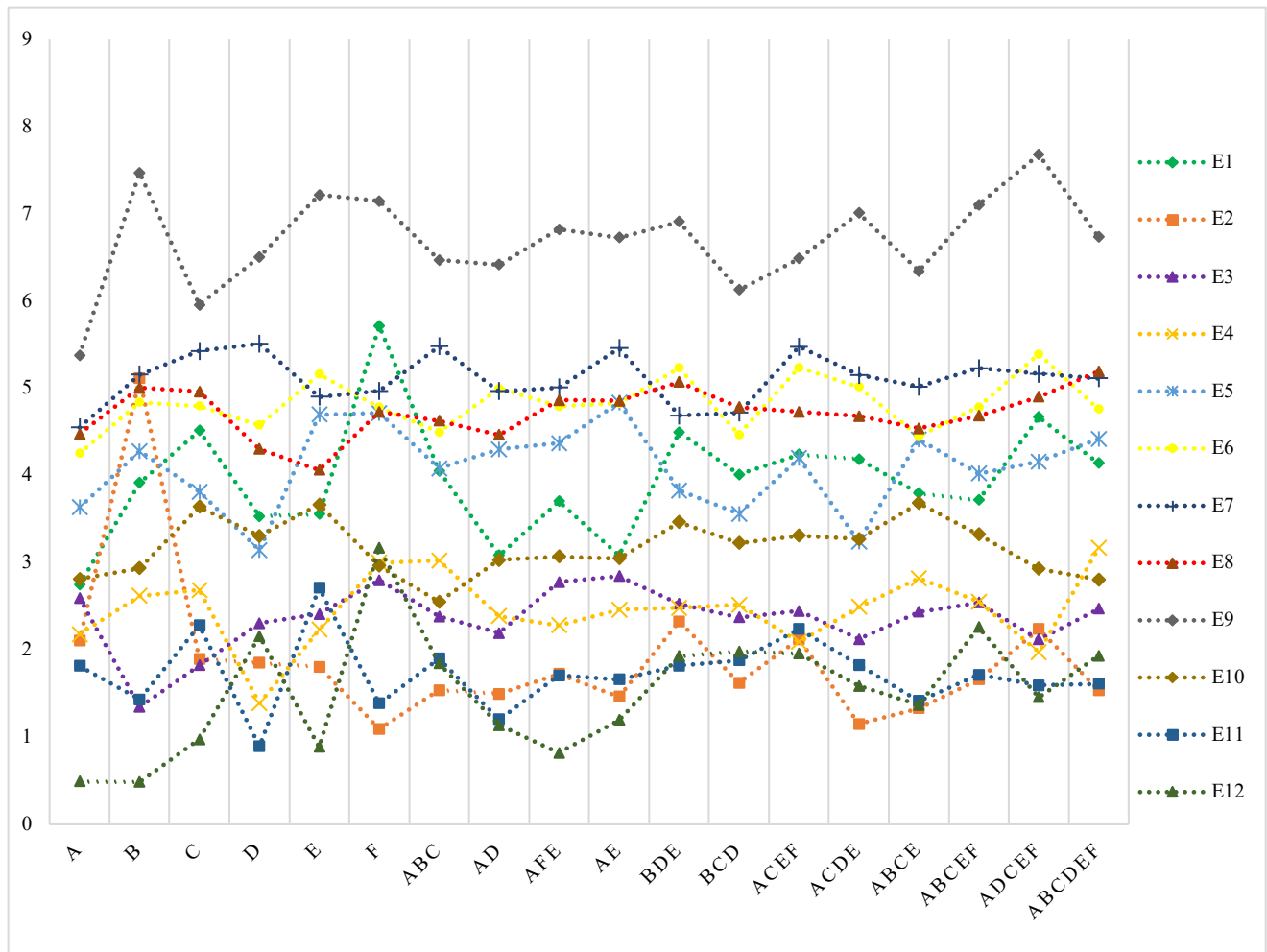

Figure 2: The mean yield of single variety/varietal mixtures in each environment. X axis=yield (t/ha), Y axis= genotypes (single variety/varietal mixtures), E=environment.

Mean Yields
The mean yields for single varieties and mixed varieties varied from 3.085 to 3.873 tons/ha, with a grand mean of 3.545 t/ha (Table 3, Figure 2). Notably, single varieties F, B, E, and C exceeded the grand mean average, while no varietal mixture with two varieties surpassed the grand mean (Figure 3). Only

varietal mixes with three (BDE) and four varieties (ACEF) recorded yields exceeding the grand mean. Among varietal mixes with five varieties or more, ADCEF, ABCDEF, and ABCEF were the only ones surpassing the grand mean.

Table 3: Single variety/varietal mixtures with their corresponding mean yield, stability index, combined selection index and ranks across 12 environments and the correlation between yield and stability index.

Code	Yield,μ (t/ha)	Rank in Yield	ASi	RanK in ASi	CSi	Rank in CSi
A	3.085	18	0.932	16	34	12
B	3.715	3	2.885	18	21	11
C	3.564	9	0.521	13	22	9
D	3.288	17	0.246	2	19	6
E	3.608	8	0.917	15	23	10
F	3.873	1	1.633	17	18	7
ABC	3.538	10	0.364	9	19	7
AD	3.306	16	0.355	8	24	10
AFE	3.495	12	0.504	12	24	10
AE	3.538	11	0.386	10	21	8
BDE	3.73	2	0.242	1	3	1
BCD	3.439	15	0.333	5	20	7
ACEF	3.713	4	0.341	6	10	2
ACDE	3.477	13	0.345	7	20	7
ABCE	3.467	14	0.284	3	17	4
ABCEF	3.632	7	0.328	4	11	3
ADCEF	3.69	5	0.784	14	19	7
ABCDEF	3.657	6	0.464	11	17	5

Pearson correlation (r) (Yield and ASi)   =0.33

A=NSIC Rc298; B=NSIC Rc214; C=NSIC Rc216; D=PSB Rc82; E=NSIC Rc238; F=NSIC Rc300; ABC=NSIC Rc298:NSIC Rc214:NSIC Rc216; AD=NSIC Rc298:PSB Rc82; AFE=NSIC Rc298:NSIC Rc300:NSIC Rc238; AE=NSIC Rc298:NSIC Rc238; BDE=NSIC Rc214:PSB Rc82:NSIC Rc238; BCD=NSIC Rc214:NSIC Rc216:PSB Rc82; ACEF=NSIC Rc298:NSIC Rc216:NSIC Rc238:NSIC Rc300; ACDE=NSIC Rc298:NSIC Rc216:PSB Rc82:NSIC Rc238; ABCE=NSIC Rc298:NSIC Rc214:NSIC Rc216:NSIC Rc238; ABCEF=NSIC Rc298:NSIC Rc214:NSIC Rc216:NSIC Rc238:NSIC Rc300; ADCEF=NSIC Rc298:PSB Rc82:NSIC Rc216:NSIC Rc238:NSIC Rc300; ABCDEF=NSIC Rc298:NSIC Rc214:NSIC Rc216:PSB Rc82:NSIC Rc238:NSIC Rc300; ASi=AMMI stability index; CSi=combined selection index.
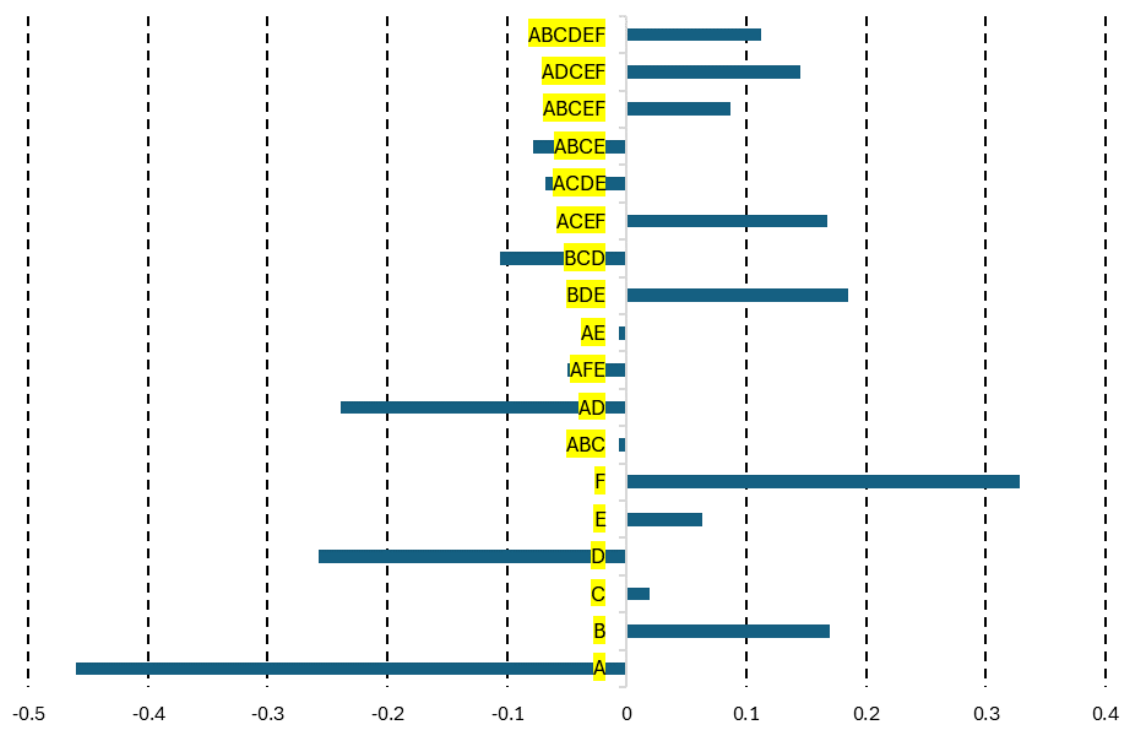

Figure 3: Yield deviation of genotypes from the grand mean (3.545 tons/hectare) across all environments. Note: Differences among genotypes were considered statistically and highly significant at p < 0.001, based on ANOVA analysis. Single letter or combined letters denote a specific genotype.

In terms of environments, the mean yields varied from 1.534 to 6.694 t/ha (Table 4). Environments with Type IV climate (E9, E5, and E1), two environments with Type I (E6 and E8), and a Type III environment (E7) exhibited yields surpassing the grand mean. Interestingly, no environment with Type II was found to exceed the grand mean yield (Type of environment from PAGASA). These results highlight the diverse performance of genotypes across different environments and underscore the significance of environmental conditions in influencing overall yield outcomes.

Table 4: Twelve environments of the study with their corresponding mean yield.

Environments	Locations (Climate type*)	Yield (t/ha)
E1	RTR, Agusan (Type IV)	3.953
E2	Aleosan, North Cotabato (Type III)	1.893
E3	Umiray, Aurora (Type II)	2.362
E4	Batang, Bicol (Type IV)	2.464
E5	Buga, Bicol (Type IV)	4.095
E6	Batac, Ilocos Norte (Type I)	4.825
E7	Iloilo (Type III)	5.110
E8	Comitang, Sto. Domingo, Nueva Ecija (Type I)	4.718
E9	PhilRice- Agusan (Type IV)	6.694
E10	PhilRice-Batac (Type I)	3.168
E11	PhilRice-Bicol (Type IV)	1.726
E12	Bohol (Type IV)	1.534

Climate types I and III have most months that are dry season while types II and IV have most months that are rainy season (PAGASA, 2014).

Stability Indices and AMMI biplots

The results clearly indicate that higher yield does not necessarily equate to greater stability, as demonstrated by the correlation coefficient of 0.33 (Table 3). For example, genotype F, which ranked 1st in yield, dropped to 17th in the AMMI Stability Index (ASi). Similarly, B ranked 3rd in yield but fell to 18th in ASi, and ADCEF, which was 5th in yield, ranked 14th in ASi. This suggests a location-specific trait expression for these genotypes, further confirmed by the AMMI2 biplot. In the AMMI2 analysis, F had a near proximity to environment E12, B was closely associated with E2, and ADCEF aligned with E9 (Figure 4), indicating that these genotypes exhibit specific adaptability to certain environments.
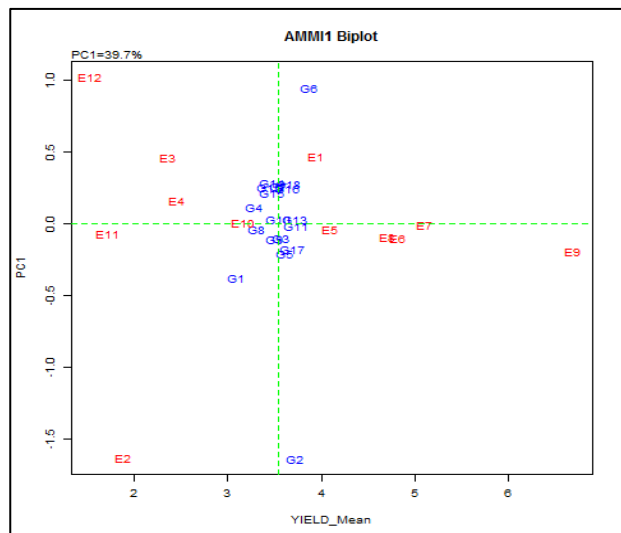

Figure 4: Biplot using mean yield and PC1 distributed 18 genotypes and 12 environments (AMMI1). Legend: G1=A; G2=B; G3=C; G4=D; G5=E; G6=F; G7=ABC; G8=AD; G9=AFE; G10=AE; G11=BDE; G12=BCD; G13=ACEF; G14=ACDE; G15=ABCE; G16=ABCEF; G17=ADCEF; G18=ABCDEF.

Genotypes BDE, D, ABCE, ABCEF, and BCD achieved the highest ranks in ASi, indicating their greater stability. Notably, BDE's IPCA scores were located near the origin in the AMMI2 biplot (Figure 5), reflecting its minimal interaction with environments and thus greater stability. The Comprehensive Selection Index (CSi), which combines both yield and stability (ASi), identified BDE, ACEF, and ABCEF as the top-ranking varietal mixtures, placing them 1st, 2nd, and 3rd, respectively. This highlights their combined superiority in both yield and stability, making them the most desirable genotypes.
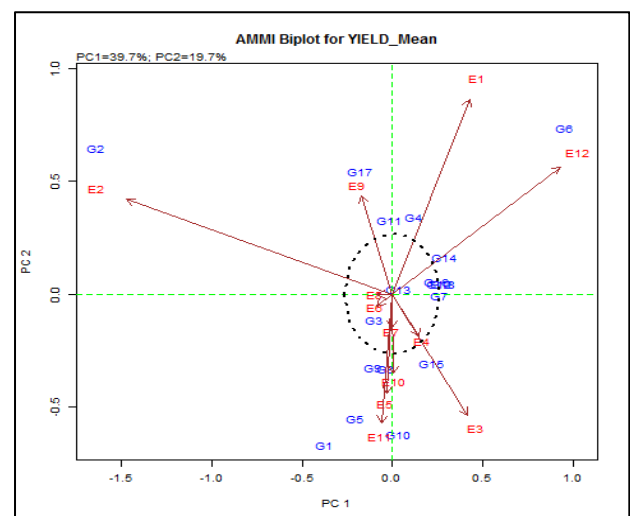

Figure 5: Biplot using the two main IPCA axes (IPCA1 and IPCA2) showed the location-specific and stable genotypes (AMMI2). Legend: G1=A; G2=B; G3=C; G4=D; G5=E; G6=F; G7=ABC; G8=AD; G9=AFE; G10=AE; G11=BDE; G12=BCD; G13=ACEF; G14=ACDE; G15=ABCE; G16=ABCEF; G17=ADCEF; G18=ABCDEF.

The AMMI1 biplot showed these top-performing genotypes located to the right of the mean, which further supports their high yield potential. Meanwhile, the proximity of these genotypes to the origin in the AMMI2 biplot, with low PC1 and PC2 scores, underscores their stability across environments. The simultaneous consideration of ASi and CSi not only justifies the selection of these genotypes but also aligns with their visual representation in the AMMI biplots, confirming their adaptability and consistent performance across diverse environments."

DISCUSSION

By employing AMMI and PCA, researchers can gain valuable insights into the genetic variation underlying crop performance across different environments. This study, emphasizing the environment as a predominant factor for genetic variation in rice yield, aligns with research that highlights the significant role of the environment in variation (Samonte et al., 2005; Mostafavi et al., 2014). In contrast, it diverges from earlier rice studies where the environment is not the primary contributor to variation (Sharifi et al., 2017; Saied, 2010; Tariku et al., 2013). The environment as a major factor influencing genetic variation in rice yield in this study can be attributed to the significant variability in soil dynamics within the rice-growing areas of the Philippines (Magahud et al., 2015). Previous research has also identified soil as a significant environmental factor for rice yield (Cantila et al., 2020). Rainfall patterns and temperature have also contributed to variability in studying rice genotypes in the Philippines (Stuecker et al., 2018; Cantila and Quitel, 2017). Notably, the Type IV climate condition was found to impact the yield of hybrid rice (Cantila and Boholano, 2021).

The presence of additional IPCs (IPC3-IPC11) underscores the intricate interplay of environmental conditions and genotype interactions in shaping rice yield. The interaction between genetics and the environment introduces greater dissimilarity among the genotypes, influencing the genetic mechanisms regulating physiological processes associated with yield stability, and consequently impacting genotypic performance across test environments. This approach also aids in determining which genotypes have performed better in the study.

The identification of top-performing single varieties and varietal mixtures in each environment provides insights into their unique adaptability and resilience. In line with a previous study, the VarMix BDE (top performing in this study) combination has demonstrated stability in yield compared to other combinations and its single varieties evaluated (Pacada and Francia, 2023). This stability can be attributed to the diverse genetic backgrounds of the single varieties used (Pacada et al., 2022) and their mixing effect, triggering inter-varietal diversity when established in environments with biotic and abiotic stress (Pacada and Francia, 2023). The presence of biotic trait-based SNP markers, including *Xa23, Xa4, xa5, Pi-ta, id9001699*, and *id11010994*, highlights disease resistance in single varieties B (NSIC Rc214), D (PSBRc82), and E (NSIC Rc238) (Pacada et al., 2023). The above scientific basis is the overall advantage of VarMix compared to single varieties, specifically when planted in rice environments having biotic and abiotic stresses.

Pacada and Francia (2023) highlighted that three VarMix combinations - BDE, ABCEF, and ABCDEF - identified among the top five VarMix genotypes in this study and achieved a higher inter-varietal diversity mean of 0.7. This suggests that the phenotypic and genotypic traits of each individual variety (Pacada et al., 2022) contributed to expressing inter-varietal diversity performance among VarMix combinations, despite the presence of biotic stresses during the ripening stage and water scarcity during the growth stages of plants in 12 environments. The mixing effect and yield performance of VarMix combinations and their individual varieties are discussed in detail in the published research by Pacada and Francia (2023) and the VarMix mechanism under limited water resources by Pascua et al. (2020).

The findings on stability indices and AMMI biplots encourage a deeper exploration of genotype performance across diverse environments. The observed discrepancy between high yield and stability index positions for certain genotypes, such as F, B, and ADCEF, suggests location-specific responses that warrant further verification. This location specificity is supported by the AMMI2 analysis, revealing proximity in IPCA scores between genotypes and environments. The complexity of genotype behavior in different contexts emphasizes the importance of tailoring breeding and cultivation strategies based on the specific conditions of each location. Consequently, the AMMI stability indices serve as valuable tools not only for ranking genotypes but also for deciphering their adaptability and stability—crucial considerations for sustainable and resilient crop production.

CONCLUSION

This study provides valuable insights into the genetic variation, influencing rice yield across diverse environments. The complex interplay of genotype-by-environment interactions underscores the dominance of environmental factors on rice genotypic performance. The identification of top-performing genotypes, exemplified by VarMix BDE, highlights the importance of harnessing diverse genetic backgrounds and their interactive effects to enhance yield stability. These findings underscore the need for further exploration of genotype responses under varying environmental conditions. They emphasize the necessity for developing tailored breeding strategies that are specifically adapted to the unique challenges and opportunities presented by different geographical locations and agricultural environments. The application of AMMI stability indices emerges as pivotal in assessing genotype adaptability and stability, thereby promoting sustainable and resilient crop production practices. Overall, this study contributes significantly to refining breeding strategies aimed at enhancing rice cultivation in dynamic agricultural landscapes.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest related to this publication.

CONTRIBUTIONS OF INDIVIDUAL AUTHORS

The main author is the project leader of the "Varietal Mixture of Rice to Enhance Yield and Mitigate Effects of Climate Change in Stress-Prone Areas". She integrated the vital informations obtained from the result of VarMix experimental research. The second author conceptualized, designed, analyzed and interpreted the data.

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