

# Mixing effects and yield performance of rice varietal mixture (VarMix) in selected areas in the Philippines

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## ABSTRACT

Research around the world has established the good performance of the varietal mixture (VarMix) approach in mitigating the ill effects of biotic stresses on various crops. Thus, the efficiency of this approach against abiotic stresses is also being studied. In the Philippines, the evaluation of VarMix is limited especially in large rice areas that experience water scarcity and unpredictable occurrence of pests and diseases. In this study, yield performances of 12 VarMix combinations and their corresponding single varieties were evaluated in 12 environments nationwide that suffer from water scarcity. VarMix ADCEF yielded highest at 7.7t/ha. Analysis of mixing effects showed that 10 of the 12 combinations gained positive  $ME_{rel_{ml}}$  mean ranging from 0.25 to 17.37%. This shows that their mean yield was higher by 0.25% to 17.37% than the yield mean of their single component varieties in different environments. VarMix ABCE achieved the highest  $ME_{rel_{ml}}$  value of 175.86% in Carmen, Bohol with a 17.37% mean. ABCE also had positive  $ME_{rel_{ml}}$  (1.44% to 19.76%) in Agusan del

Norte; Aurora; Nabonton, Ligao, City Albay; Batac City, Ilocos Norte; Iloilo; Nueva Ecija; PhilRice Batac, and PhilRice Bicol. Further, ABCE had the highest positive  $ME_{max_{ml}}$  value of 100.79% (found in Bohol), indicating that its yield was 100.79% higher than the maximum yield of its corresponding variety. Batac City and Iloilo likewise had positive  $ME_{max_{ml}}$  of 1.40% and 0.90%. VarMix combinations BDE, AFE, ABCEF, and ABCDEF reached higher  $D(x)_{ml}$  with the same mean values of 0.7 across the 12 locations. The highest  $D(x)_{ml}$  of 1.9 was found in BDE and BCD. Based on the static measure of all genotype stability, BDE and single variety NSIC Rc 298 attained small  $S^2_i$  values of 2.0, where the lower the value of variability the more stable they were. In the analysis on responsiveness to environmental productivity, ADCEF was the most responsive and the largest stability ( $b_i$ ) value of 1.2 followed by ACDE, ABC and AFE with 1.1;  $b_i > 1$  means they have higher responses to a given environment. VarMix combinations that showed yield increase through the mixing effects analysis in actual field conditions will facilitate the selection of elite VarMix for future deployment in areas with similar environment. Results from this study highlighted the potential of VarMix as a stop-gap approach for increasing yield in rice production areas with

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limited source of water and unpredictable occurrences of pests and diseases.

## INTRODUCTION

Varietal mixture as an approach refers to the combination of the seeds of two or more cultivated varieties for planting. It is well-documented in different parts of the world as an effective means of controlling the ill effects of biotic stresses (Gold et al. 1989; Manthey et al. 1993; Finckh et al. 2000; Mundt 2002; Kiaer et al. 2012; Reddy 2012; Raboin et al. 2012; Gallet et al. 2014). The extended utilization of the approach for evaluating its capability in other problematic crops was demonstrated in barley for yield and competitive ability in weeds disease (Locmele et al. 2017); bean for decreasing fly damage (Ssekandi et al., 2016); banana for controlling nematodes (Quénéhervé et al. 2011); barley for malting quality (Swanston et al. 2006). Increasing yield stability using varietal mixture (VarMix) in different crops was also demonstrated in various investigations (Castilla et al. 2003; Creissen et al. 2016; Tooker and Frank 2012; Kiaer et al. 2012). VarMix in rice was found to decrease the incidence and severity of panicle blast; glutinous (>90%), non-glutinous (40%) (Zhu et al. 2005); and traditional (~10% incidence, ~5% severity), modern rice varieties (~10% incidence, ~4% severity) (Han et al. 2016), as compared to their single component varieties. Revilla-Molina et al. (2009) also observed that the total grain yield in varietal mixtures of hybrid rice was close to the yield of single varieties. In other researches, VarMix demonstrates its productivity in limited source of water in wheat (Adu-Gyamfi et al. 2015); wheat and barley (Kiaer et al. 2009); barley (Kiaer et al. 2012); and rice (Montazeaud et al. 2017).

Performance of rice VarMix in Philippine farms has yet to be determined particularly in actual rice field areas with biotic and abiotic stresses. And only in recent years was its potential explored in areas with limited source of water, and with problems in pests and diseases (Pacada et al. 2014). Predicting VarMix combinations that perform better in selected trial environments is still a challenge, but for evaluating mixture efficiency the actual field environments evaluation increases technical precision to select best varietal combinations (Newton et al. 1997; Mille et al. 2006; Kiaer et al. 2012).

This study determined the yield performance of 12 VarMix combinations in comparison to their single varieties in 12 environments by using actual yield and analyzed VarMix mixing effects, inter-varietal diversity, and measured stability.

## MATERIALS AND METHODS

### Selection of sites nationwide

Experimental areas were selected based on the occurrences of pests and diseases, and those with limited source of water. Data were gathered from available information on PhilRice, DA-Regional Field Offices (RFO), and official government websites.

### VarMix entries and establishment of setups

VarMix combinations that showed yield stability with 1:1, and 1:1:1 ratios identified by Pacada et al. (2014), and six untested VarMix ratios with 1:1:1:1:1; 1:1:1:1:1:1; 1:1:1:1:1:1:1, were used (Table 1). Phenotypic and genetic variations of six varieties used in establishing VarMix combinations are shown in Table 2. Rough and milled rice images of six single variety is shown in Figure 1.

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). All single varieties were 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 differed in the experimental areas, hence some of the entries were established from June to December 2015; the rest from January to July 2016.

### Data gathering and analysis

To document the environmental conditions in each experimental area, water table below ground was recorded every two days using three piezometers distributed throughout the setup while rainfall amount was measured using a rain gauge.

Insect pests and diseases were also monitored during vegetative, reproductive, and ripening stages by randomly selecting 10 hills in each plot. Symptoms of diseases and injuries from insect pests were observed in tillers/leaves/panicles of each hill and were assessed following the Standard Evaluation System (SES).

During harvesting, the grains were weighed and yield was computed based on the PhilRice field operations manual (Gergon et al. 2017). VarMix and single variety mean, minimum, and maximum yields, standard deviation (SD), and coefficient of variance (CV) of the 12 locations were determined using the Microsoft Excel program. Gathered data from this computation were used in the analysis for determining mixing effects ( $ME_{rel_{ml}}$ ;  $ME_{max_{ml}}$ ), inter-varietal diversity ( $D(x)_{ml}$ ), and yield stability ( $S_i^2$ ;  $\hat{\mu}_{ml}$ ;  $s(d_i)^2$ ). The formula used by Kier et al. (2012) was adopted with modifications to customize the design of the experimental setup. Customized formulas used in this study are the following:

#### Relative mixing effect

$$ME_{rel_{ml}} = \frac{\hat{\mu}_l^m - \hat{\mu}_{ml}^c}{\hat{\mu}_{ml}^c}$$

where  $\hat{\mu}_l^m$  is the estimated mean yield mixture  $m$  in environment  $l$  and  $\hat{\mu}_{ml}^c$  mean of the estimated mean yields of the single varieties of VarMix  $m$  in environment  $l$ .

#### Maximum mixing effect

$$ME_{max_{ml}} = \frac{\hat{\mu}_l^m - \hat{\mu}_{ml}^{max}}{\hat{\mu}_{ml}^{max}}$$

where  $\hat{\mu}_l^m$  estimated mean yield of VarMix  $m$  in the environment  $l$  and  $\hat{\mu}_{ml}^{max}$  maximal estimated mean yield among the single varieties of VarMix  $m$  in environment  $l$ .

#### Inter-varietal diversity of yield

$$D(x)_{ml} = \sqrt{\frac{\sum_{i=1}^k (x_{mli} - \bar{x}_{ml})^2}{k-1}}$$

where  $i = 1, \dots, k$  and  $2, \dots, 6$ ;  $k = 2, \dots, 6$ , the number of single variety components of VarMix  $m$  in environment  $l$ ;  $l = 1, \dots, 12^{\text{th}}$  environment;  $x_{mli}$  is the value of component variety  $i$  of VarMix  $m$  in environment  $l$ ;  $\bar{x}_{ml}$  is the mean of all components of VarMix  $m$  in environment  $l$ .

#### Yield stability across environments

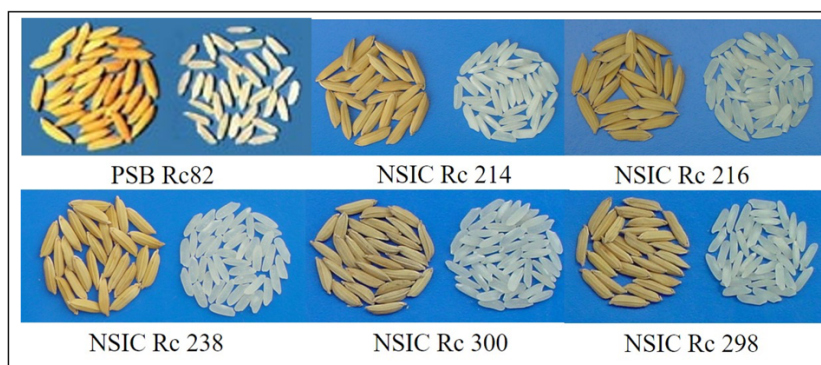
Static measure of genotype stability analyzed the environmental variance for each genotypic entity  $i$  was computed as the variance of yields of genotypic entities across the 12 environments:

$$S_i^2 = \frac{\sum_{l=1}^{12} (\hat{\mu}_{il} - \bar{\mu}_i)^2}{12-1}$$

where  $\hat{\mu}_{il}$  = estimated grain yield of genotypic entity  $i$  in trial environment  $l$ ;  $\bar{\mu}_i$  = mean yield of the genotype entity across the

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

Ratio	Single Variety/VarMix Combination	Code	Remarks
0	NSIC Rc298	A	Single variety source used by Pacada et al. (2014), for establishing VarMix combinations
	NSIC Rc214	B	
	NSIC Rc216	C	
	PSB Rc82	D	
	NSIC Rc238	E	
	NSIC Rc300	F	
1:1	NSIC Rc298 : PSB Rc82	AD	Tested ratios of Pacada et al. (2014)
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	Untested ratios
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	

**Figure 1: Photographic images of rough and milled rice of six single varieties used in this study.**

12 trial environments;  $i$  = refers to a genotypic entity. Genotypic entities are the single component varieties and the VarMixes. There is a total of  $6 + 12 = 18$  static measures of genotype stability calculated (6 singles and 12 VarMix).

Dynamic measure of genotypic stability describes the adaptability of genotypes through a linear regression model using the expected mean yield of all genotypic entities and this was calculated by:

$$\hat{\mu}_{ml} = a_m + b_m \cdot \hat{\mu}_l + d_{ml}$$

where  $\hat{\mu}_{ml}$  is the estimated grain yield of genotypic entity  $i$  in trial environment  $l$ ;  $a_m$  is intercept;  $b_m$  is the regression coefficient of genotypic entity  $i$  and the ability of genotype entity to respond to environmental productivity;  $\hat{\mu}_l$  is the expected mean yield of all genotypic entities grown in trial environment  $l$ ;  $d_{ml}$  is the deviations from the fitted regression line.

Sensitivity of genotypic entities,  $s(d_i)^2$ , to biotic and abiotic environmental factors apart from those defining general environmental productivity was computed by variance of deviations from the regression model in static measure of genotype stability analysis.

## RESULTS AND DISCUSSION

### Experimental areas and VarMix entries

The 12 experimental locations are: Purok II, RTRomualdez, Agusan del Norte (Loc1); San Mateo, Aleosan, North Cotabato (Loc2); Sitio Malacauyan, Umiray, Dingalan, Aurora (Loc3); Batang, Ligao City, Albay (Loc4); Sitio Buga, Nabonton, Ligao City, Albay (Loc5); Monte Surte, Carmen, Bohol (Loc6); San Mateo, Batac City, Ilocos Norte (Loc7); Binangkilan, Sta. Barbara, Iloilo (Loc8); Comitang, Sto. Domingo, Nueva Ecija (Loc9); PhilRice Agusan, Basilisa, RTRomualdez, Agusan del Norte (Loc10); PhilRice Batac, MMSU Campus, Batac City, Ilocos Norte (Loc11); PhilRice Bicol, Batang, Ligao City, Albay (Loc12). Most of the locations are rainfed lowland areas, while some places pumped water. Sizes of experimental areas ranged from 700-3,115 m<sup>2</sup>, depending on the available area of farmer-cooperators and PhilRice branch stations.

### Water table level below ground and amount of rainfall data

Monitoring of available water during the trial saw water scarcity from establishment to ripening stage (Figure 2). Rice plants need 13.95mm to 15.95mm water/day particularly under rainfed lowland conditions during panicle initiation to flowering stage (Aryal 2012; Materu et al. 2018; Dianga et al. 2021). Nabonton in Albay (Loc5) received no rainfall during this critical stage while PhilRice Batac (Loc11) received as low as 0.3 mm averaging only 4.7 mm of rainfall throughout the entire course of the trial. Agusan del Norte (Loc1) got much rainfall with an average of 13.1mm; other locations recorded limited or erratic water source during the critical stage. Monitoring of water table below ground demonstrated different soil types. Areas that showed poor water retention are: Aleosan, North Cotabato (Loc2); Batang, Albay (Loc4); Nabonton (Loc5); Batac City,

**Table 2: Summary of genetic (Pacada et al, 2022) and phenotypic variations (Pinoy Rice Knowledge Bank, PhilRice) of six selected varieties.**

Variety (Code)	Grouping based on genetic similarities	Agronomic Characteristics		Grain Quality		Reactions to Pests and Diseases																												
						BLAST (Induced)					BLB (Induced)				Tungro (Induced)			Tungro (Modified)			BPH	G L H	DH (WH)	Stemborer						YSB				
		Ave Yld. (t/ha)	Mat. (DAS)	Plt. Ht. (cm)	Grain Shape (mm)	AC	A	B	C	D	G	A	B	C	G	A	D	G	A	C				G	WSB	Y S B	WSB	YSB						
																												C	B		H	A	D	I
NSIC Rc214 (B)	I	10.2	116	106	3.5 S	21.9 I	-	-	-	-	I	-	-	-	I	-	-	S	-	S	MR	MR	-	-	-	-	-	-	-	R				
PSB Rc82 (D)	II, 2.1a	12.0	110	100	3.2 S	21.5 I	-	-	-	-	R	-	-	-	I	-	-	S	-	-	I	MS	I	-	-	-	-	-	-	-				
NSIC Rc238 (E)	II, 2.1b.1.2	10.6	110	104	3.2 S	21.0 I	S	R		I	I	I	I	I	-	S	S	-	S	S	I	MR	-	MR	R	MR	MS	R	MR	-				
NSIC Rc300 (F)	II, 2.1b.1.2	10.4	115	98	2.9 I	20.4 I	S	R	R	I	I	S	I	I	-	S	S	-	S	S	MR	MR	-	-	-	S	I	-	-	-				
NSIC Rc298 (A)	II, 2.1b.2.1	8.2	104	93	3.2 S	19.5 I	S	S	S	S	I	I	I	I	-	S	S	-	S	S	MR	I	-	-	-	S	MR	-	-	-				
NSIC Rc216 (C)	II, 2.1b.2.2	9.7	112	96	3.2 S	20.5 I	S	-	-	-	-	I	-	-	-	S	-	-	S		MR	MR	-	-	-	-	-	-	-	MR				

*S-susceptible; MS-moderately susceptible; I-intermediate; MR-moderately resistant; R-resistant; A – PhilRice CES; B – PhilRice Isabela; C – PhilRice Midsayap; D – UPLB; E - DA-CVIARC; F – VSU; G – IRRI; H – PhilRice Agusan; I – BLARC; J – WESVIARC*

*Genetic similarities sub-clustering is based on the  $\approx 0.39$  R and T genetic similarity coefficient, same number and letter belong to the same cluster group; AC-Amylose Content; Grain shape: S-Slender; I-Intermediate*

*Note: Grain Shape (L/W) = Slender (S)-more than 3.0; Intermediate (I)-2.0-3.0; Bold (B)-less than 2.0; AC= Waxy/Glutinous (W)-<2.0%; Very low (VL)-2.1-10.0%; Low (L)-10.1-20.0%; Intermediate (I)-20.1-25.0%; High (H)-25.1 and above*



Note: Loc1-Purok II, RTRomualdez, Agusan del Norte; Loc2-Brgy. San Mateo, Aleosan, North Cotabato; Loc3-Sitio Malacauyan, Brgy. Umiray, Dingalan, Aurora; Loc4-Batang, Ligao City, Albay; Loc5- Nabonton, Ligao City; Loc6-Brgy. Monte Surte, Carmen, Bohol; Loc7-Brgy. 28, San Mateo, Batang City, Ilocos Norte; Loc8-Brgy. Binangkilan, Sta. Barbara, Iloilo; Loc 9-Brgy Comitang, Sto. Domingo, Nueva Ecija; Loc10-PhilRice Agusan, Basilisa, RTRomualdez, Agusan del Norte; Loc11-PhilRice Batac, MMSU Campus, Batac City, Ilocos Norte; Loc12-PhilRice Bicol, Batang, Ligao City, Albay; PI-Panicle Initiation

**Figure 2: Water table level below ground and amount of rainfall data recorded in all trial locations.**

Ilocos Norte (Loc7); and PhilRice Bicol (Loc12)-where there was rainfall but water table below ground was very low as shown in Figure 2 (gray filling indicates no water below; white shows presence of water). Areas that could hold water longer are: Dingalan, Aurora (Loc3); Carmen, Bohol (Loc6); Sta. Barbara, Iloilo (Loc8); Sto. Domingo, Nueva Ecija (Loc9); and PhilRice Agusan (Loc10) -where whenever there was rainfall, there was also high water table below ground.

### Biotic stress evaluation

Each growth stage underwent its respective occurrence of pests and diseases. For vegetative stage, biotic stresses were monitored in Dingalan, Aurora (Loc3); Batang, Albay (Loc4); and PhilRice Bicol (Loc12) – where whorl maggot was dominant (Loc3 had a scale rating of 3). During reproductive stage, biotic stresses were profiled in: Nabonton, Albay (Loc5); San Mateo, Ilocos Norte (Loc7); Sta. Barbara, Iloilo (Loc8); and Bicol (Loc12)- where tungro, leaf streak, brown spot, narrow brown leaf spot (NBLS), bacterial leaf blight (BLB), dead hearts, leaf folder, whorl maggot, false smut, and sheath blight were evaluated as pests and diseases. High incidence of false smut was recorded in Loc8 with 6% to 25% infected florets in most of the entries; whorl maggot was observed in Loc7 with 2 or more leaves/hill affected in all entries. Tungro was also observed in Loc5 and Loc8 with a scale rating of only 2 and 1, respectively.

In the ripening stage, 11 locations were profiled in terms of rat damages in Loc1, BLB in Loc4, NBLS in Loc5; panicle blast and sheath blight occurred in Loc6, and rice bug was monitored in Loc12 (Table 3). Some VarMix combinations had significantly lower infections than their corresponding variety. Single variety D (PSB Rc 82), for example, had >6% to 25% NBLS; but VarMix combination with variety D had 1% to 6% only in Loc11(Figure 3a). The same trend was observed in Loc12 where rice bug and tungro damages were monitored. Rice bug damage of 8-15% was noticed on grain panicles of variety D; but in VarMix BCD, BDE, ADCEF, and ABCDEF, the damage was only >8% (Figure 3b). Also, high tungro infestation was recorded in PhilRice Bicol, where varieties A (NSIC Rc 298) and D had 23% and 18% damages. However, their combination with variety A had only 0-18%; with variety

D, only 0-10% damage. Variety NSIC Rc 300 (F) was identified as highly susceptible to tungro; but when mixed with the other five single varieties, reduction was by 25% to 41% (Figure 3c).

Numerous investigations have explained mechanisms possibly involved in this disease and pest reduction. One is the dilution effect where disease was reduced through the presence of some resistant varieties (Chin and Wolfe 1984). Second is the barrier effect where the resistant variety shields susceptible components, thus preventing the spread of pests and diseases (Browning and Frey 1969).

### Yield performance

Yields of VarMix and their corresponding single varieties varied across the 12 locations. VarMix ADCEF yielded highest at 7.7t/ha and single variety NSIC Rc 298 had the lowest at 5.4t/ha. VarMix ABCE yielded lowest at 2.0 t/ha and single varieties NSIC Rc 214 (B) and Rc298 (A) both had only 0.5 t/ha (Table 4). Highest mean yield was attained in six of the 12 locations: PhilRice Agusan (Loc10); Sta. Barbara, Iloilo (Loc8); Batac City, Ilocos Norte (Loc7); Sto. Domingo, Nueva Ecija (Loc9); Agusan del Norte (Loc1); and Nabonton in Albay (Loc5). Actual monitoring for water availability showed that during the critical growth stage, only Loc10 and Loc1 received considerable amounts of water; Loc8 and Loc9 had limited or erratic water source but retained water below ground for a long time; and poor water retention was observed in Loc7 and Loc5 (Figure 2). Lowest mean yield was in Loc12, Loc2, and Loc6, which suffered from water scarcity and significant pest and disease infestations (Figure 3).

### Relative and maximum mixing effects

Results showed that 10 out of 12 VarMix entries gained positive  $MER_{el_{mi}}$  mean ranging from 0.25% to 17.37% indicating that their mean yield was higher than that of their single component varieties in different environmental conditions (Figure 4a). The combination ABCE achieved the highest  $MER_{el_{mi}}$  in Carmen, Bohol (Loc6) (175.86%) with a mean of 17.37% (Table 5). Other locations where ABCE had positive  $MER_{el_{mi}}$  (1.44% to 19.76%) are: Agusan del Norte (Loc1); Dingalan, Aurora (Loc3); Nabonton in Albay (Loc5); Batac City, Ilocos Norte



Code	Loc1										Loc2					Loc3					Loc4					Loc5					Loc6						
	Brown Spot	NBLS	Sheath Blight	GD	Defoli a-tor	White heads	Rat Damage	Panicle Blast	Brown Spot	Sheath Blight	GD	GD	Leaf folder	White heads	Brown Spot	NBLS	Sheath Blight	GD	GD	Leaf folder	White heads	Brown Spot	NBLS	Sheath Blight	GD	GD	Leaf folder	White heads	Brown Spot	NBLS	Sheath Blight	GD	GD	Leaf folder	White heads	Panicle Blast	Sheath Blight
A	0	1	3	1	0	0	2	6	0	4	3	1	1	3	3	1	2	4	1	0	3	8	0	0	0	5	2	4	2	0	0	0	0	0	0	9	9
B	0	0	0	1	1	0	0	0	0	4	3	1	1	2	3	1	3	2	1	0	3	7	0	1	2	1	0	3	1	0	1	0	0	9	6		
C	0	0	0	3	1	1	1	0	0	3	3	1	2	3	2	0	2	2	1	0	2	7	1	0	0	3	1	4	3	0	0	0	9	9			
D	0	0	0	2	1	0	0	2	0	2	3	1	3	3	1	0	1	3	1	1	6	7	0	1	0	5	0	3	4	0	0	0	8	5			
E	0	0	0	2	1	1	0	2	0	4	4	1	3	3	2	0	2	2	0	0	3	7	0	1	0	5	1	3	4	0	0	0	9	8			
F	0	0	0	2	1	1	1	0	3	3	2	0	1	3	3	1	1	3	1	0	1	7	1	0	1	3	1	4	0	1	0	0	3	6			
AD	0	0	0	2	1	0	1	2	0	4	3	1	2	3	2	1	2	3	1	1	4	7	0	1	0	4	2	3	3	1	0	0	8	8			
AE	0	0	1	2	1	1	0	3	0	3	3	1	2	3	3	1	3	3	0	0	3	8	0	2	1	3	1	6	1	0	1	1	9	9			
ABC	0	0	0	1	1	0	0	3	0	3	3	1	1	2	3	0	3	2	0	0	2	8	0	1	0	5	1	4	2	0	1	0	8	6			
AFC	0	0	0	3	1	1	1	2	0	3	3	1	1	2	3	0	2	2	0	0	3	8	0	1	1	5	0	3	4	0	0	0	9	9			
BCD	1	0	0	2	1	1	0	1	0	3	3	1	1	3	2	0	3	2	0	0	2	8	0	1	1	3	0	4	3	0	0	0	8	8			
BDE	0	0	0	2	1	0	1	2	0	3	3	1	1	3	3	0	2	2	0	0	2	7	0	2	1	3	1	4	3	0	0	0	9	7			
ABCE	0	0	0	1	1	0	0	0	0	3	3	1	1	3	2	0	3	2	1	0	3	7	0	1	0	4	1	4	2	0	0	0	9	7			
ACDE	0	0	0	2	1	0	1	1	1	3	3	1	1	2	2	0	1	4	2	1	3	7	1	1	0	3	3	3	3	0	0	0	9	6			
ACEF	0	0	0	3	1	1	1	0	0	0	3	2	2	2	3	0	3	3	0	0	4	8	0	0	1	3	0	3	2	0	0	0	9	7			
ABCEF	0	0	0	2	1	1	0	0	0	3	3	1	1	2	3	0	2	2	0	0	1	7	1	2	1	3	1	4	2	0	0	0	7	5			
ADCEF	0	0	0	3	1	0	1	1	0	3	3	3	2	2	3	0	3	3	1	0	5	7	0	0	1	4	0	4	4	0	0	0	7	6			
ABCEDEF	0	0	0	3	1	0	1	2	0	3	2	1	1																								

**A**

NBS rating (%)

VarMix Code

Legend: Narrow brown spot (NBS), Average

VarMix Code	NBS rating (%)
A	2.1
B	1.5
C	1.6
D	5.2
E	3.2
F	1.5
AD	3.3
AE	3.3
ABC	2.9
AFE	2.8
BCD	3.2
BDE	4.2
ABCE	3.2
ACDE	3.7
ACEF	3.8
ABCEF	2.7
ADCEF	3.5
ABCDEF	3.2

**B**

Rice bug rating (%)

VarMix Code

Legend: Rice bug, Average

VarMix Code	Rice bug rating (%)
A	4.2
B	1.8
C	2.9
D	5.0
E	3.8
F	3.2
AD	5.8
AE	3.4
ABC	1.9
AFE	5.3
BCD	3.8
BDE	3.8
ABCE	3.2
ACDE	5.8
ACEF	3.8
ABCEF	3.2
ADCEF	3.0
ABCDEF	3.4

**C**

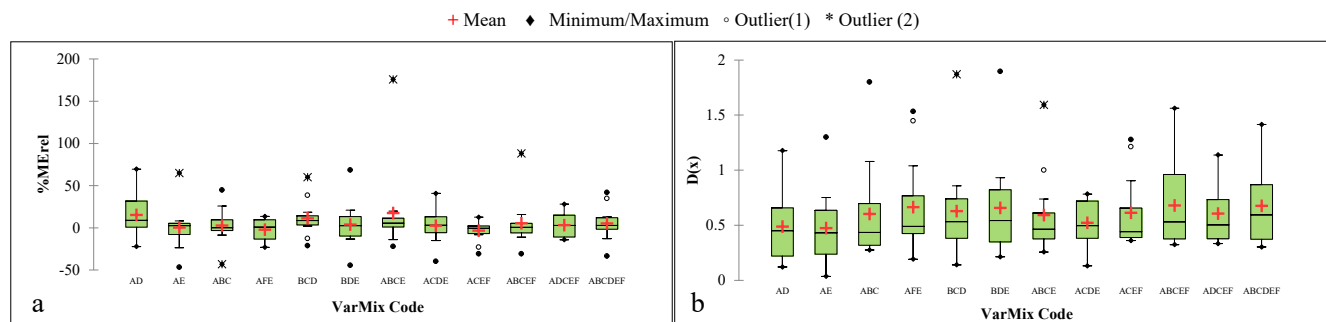
% Incidence of Tungro /plot

VarMix Code

Legend: Vegetative, Reproductive, Maturity, Average

VarMix Code	Vegetative (%)	Reproductive (%)	Maturity (%)
A	0.5	7.5	22.5
B	0.5	0.5	0.5
C	0.5	0.5	0.5
D	0.5	12.5	17.5
E	0.5	2.5	2.5
F	0.5	27.5	42.5
ABC	0.5	7.5	7.5
AD	1.0	7.5	10.0
AFE	0.5	12.5	17.5
AE	0.5	2.5	2.5
BDE	0.5	2.5	2.5
BCD	0.5	2.5	2.5
ACEF	0.5	2.5	2.5
ACDE	0.5	7.5	10.0
ABCE	0.5	0.5	0.5
ABCEF	0.5	2.5	5.0
ADCEF	0.5	2.5	2.5
ABCDEF	0.5	2.5	2.5

**Figure 3: Incidences of (A) narrow brown spot (NBS) in PhilRice Batac (Loc11), and (B) rice bug and (C) tungro in PhilRice Bicol (Loc12).**



Note: **AD** (NSIC Rc 298:PSB Rc 82), **AE** (NSIC Rc 298:Rc 238), **ABC** (NSIC Rc 298:Rc 216), **AFE** (NSIC Rc 298:Rc 300: Rc 238), **BCD** (NSIC Rc 214:Rc 216:PSB Rc 82), **BDE** (NSIC Rc 214:PSB Rc 82:NSIC Rc 238), **ABCE** (NSIC Rc 298:Rc 214:Rc 216:Rc 238), **ACDE** (NSIC Rc 298:Rc 216:PSB Rc 82:NSIC Rc 238), **ACEF** (NSIC Rc 298:Rc 216:Rc 238:Rc 300), **ABCEF** (NSIC Rc 298:Rc 214:Rc 216:Rc 238:Rc 300), **ADCEF** (NSIC Rc 298:PSB Rc 82:NSIC Rc 216:Rc 238:Rc 300), **ABCDEF** (NSIC Rc 298:Rc 214:Rc 216:PSB Rc 82:NSIC Rc 238:Rc 300).

**Figure 4: Boxplot of (a) relative mixing effect (MErel); and (b) inter-variatal diversity of VarMix entries.**

**Table 4: Summary statistics of yield performances of VarMix combinations.**

Entries	Mean	Min	Max	SD	CV %
A	3.1	0.5	5.4	1.4	45.4
B	3.7	0.5	7.5	2.0	54.1
C	3.6	1.0	6.0	1.6	45.2
D	3.3	0.9	6.5	1.7	51.6
E	3.6	0.9	7.2	1.7	48.2
F	3.9	1.1	7.1	1.8	45.9
AD	3.5	1.5	6.5	1.6	44.4
AE	3.3	1.1	6.4	1.7	51.8
ABC	3.5	0.8	6.8	1.7	49.8
AFE	3.5	1.2	6.7	1.8	49.9
BCD	3.7	1.8	6.9	1.6	42.5
BDE	3.4	1.6	6.1	1.4	41.1
ABCE	3.7	2.0	6.5	1.6	42.1
ACDE	3.5	1.2	7.0	1.8	50.7
ACEF	3.5	1.3	6.3	1.6	46.4
ABCEF	3.6	1.7	7.1	1.6	44.8
ADCEF	3.7	1.5	7.7	1.9	52.4
ABCDEF	3.7	1.5	6.7	1.7	45.2

(Loc7); Sta. Barbara, Iloilo (Loc8); Sto. Domingo, Nueva Ecija (Loc9); PhilRice Batac (Loc11); and PhilRice Bicol (Loc12) (Table 7).

However, VarMix combinations AFE recorded a negative  $ME_{rel_{ml}}$  mean in the following locations: North Cotabato (Loc2); PhilRice Bicol (Loc12); Agusan del Norte (Loc1); Batang, Albay (Loc4); Carmen, Bohol (Loc6); and PhilRice Batac (Loc11). Likewise, VarMix ACEF had negative  $ME_{rel_{ml}}$  mean in: North Cotabato; Batac City, Ilocos Norte (Loc7); PhilRice Bicol; Agusan del Norte; Bohol; Sto. Domingo, Nueva Ecija (Loc9); and PhilRice Agusan (Loc10). Unlike other locations, Nueva Ecija provided a positive  $ME_{rel_{ml}}$  mean in 11 VarMix combinations; only ACEF had a negative  $ME_{rel_{ml}}$  mean, indicating that the mean yield of their corresponding single varieties was higher (Table 5). Four VarMix combinations outyielded their corresponding single varieties in PhilRice Bicol and PhilRice Batac. In North Cotabato, only ADCEF surpassed the mean yield of its corresponding single varieties (Table 7).

Among the combinations, ABCE had positive and the highest  $ME_{max_{ml}}$  value of 100.79% (found in Bohol) indicating that its yield was 100.79% higher than the maximum yield of its corresponding single varieties. Other locations that got positive  $ME_{max_{ml}}$  are Batac City, Ilocos Norte (Loc7) at 1.40%, and Sta.

**Table 5: Relative mixing effects according to VarMix combination and location.**

Code	Number of positive ME_rel%	Mean ME_rel%	Lowest (minimum) ME_rel%	Highest (maximum) ME_rel%
VarMix combination				
ABCE	9	17.37	-22.20	175.86
ABCEF	8	5.41	-30.91	88.13
AD	9	15.19	-22.16	69.56
BDE	6	3.88	-44.49	68.47
AE	7	0.25	-46.74	65.00
BCD	10	11.32	-21.18	60.08
ABC	6	2.78	-43.19	44.76
ABCDEF	9	5.25	-33.49	41.97
ACDE	7	2.51	-39.84	40.84
ADCEF	7	3.26	-14.07	28.04
AFE	6	-2.30	-23.08	13.52
ACEF	5	-3.29	-30.89	12.58
Location				
Loc9	11	5.38	-0.39	13.18
Loc7	10	3.46	-8.06	14.38
Loc8	9	1.96	-12.67	13.52
Loc5	9	3.10	-15.14	20.48
Loc6	9	48.21	-21.00	175.86
Loc10	9	4.26	-13.23	19.23
Loc3	8	10.44	-12.34	44.76
Loc4	8	11.44	-14.07	69.56
Loc1	7	5.02	-23.08	29.08
Loc12	4	-4.84	-46.74	40.22
Loc11	4	-2.77	-16.59	12.58
Loc2	1	-24.03	-44.49	28.04

Barbara, Iloilo (Loc8) at 0.90 %. VarMix ACDE and ABCEF did not exceed the maximum yields of their corresponding single varieties in any location, hence had poor yield performance everywhere and negative  $ME_{max_{ml}}$  as well (Table 6). Nueva Ecija (Loc9) had the most VarMix combinations that recorded positive  $ME_{max_{ml}}$  (AD, AFE, BCD, ABCDEF). Detailed information on locations and VarMix combinations that obtained positive  $ME_{max_{ml}}$  are shown in Table 8.

#### Inter-variatal diversity

VarMix combinations AFE, BDE, ABCEF, and ABCDEF reached higher  $D(x)_{ml}$  mean values of 0.7 across 12 locations (Figure 4b). These combinations demonstrated the effectiveness of inter-variatal diversity performance in each given environment with respect to their phenotypic and genetic characteristics in comparison to their corresponding single varieties; the higher the value of  $D(x)_{ml}$  the more diverse are the means of their corresponding single varieties. Highest  $D(x)_{ml}$  value of 1.9 was in BDE and BCD; VarMix ABC had 1.8 while

**Table 6: Maximum mixing effects according to VarMix combination and location.**

Code	Number of positive ME_max%	Mean ME_max%	Lowest (minimum) ME_max%	Highest (maximum) ME_max%
<b>VarMix combination</b>				
ABCE	3	-3.79	-58.47	100.79
AD	5	-0.10	-26.84	38.77
AE	3	-9.75	-55.54	28.27
AFE	4	-17.41	-62.23	9.82
BCD	3	-9.32	-54.47	9.59
ABC	2	-13.92	-66.25	7.21
ADCEF	3	-16.17	-54.06	6.51
ABCDEF	2	-19.49	-69.94	5.54
BDE	1	-16.38	-68.26	2.51
ACEF	1	-20.16	-56.80	0.47
ACDE	0	-16.39	-45.26	-2.88
ABCEF	0	-19.87	-67.54	-3.70
<b>Location</b>				
Loc9	4	-1.96	-8.51	3.79
Loc3	3	-7.36	-24.20	9.59
Loc7	3	-3.66	-13.91	8.18
Loc8	3	-4.73	-14.98	9.82
Loc4	3	-6.51	-34.22	38.77
Loc5	3	-8.91	-30.96	12.34
Loc1	2	-15.66	-46.07	14.73
Loc6	2	-15.62	-62.23	100.79
Loc2	1	-45.57	-69.94	6.51
Loc12	1	-31.85	-55.54	4.68
Loc10	1	-7.41	-17.94	6.41
Loc11	1	-13.50	-23.48	0.47

ABCE and ABCEF reached 1.6 (Table 9). ABCE, AD, and BCD had the highest positive  $ME_{max_{ml}}$  mean compared to seven other VarMix combinations that also had positive  $ME_{max_{ml}}$ .

The identified positive mixing effects of some VarMix combinations despite environmental condition revealed the interactions among their single varieties with different functional diversity. Thus, the larger the yield difference of each single variety, the higher the mixing effects of a particular combination (Kiaer et al. 2009, 2012). Furthermore, variability of VarMix performance also depends on the area with soil heterogeneity (Garcia-Palacios et al. 2011). This was observed in the actual mean yield of a single variety with low or diverse yield, but when combined with another variety the yield increased (Table 4). In addition, differences of each single variety in terms of resistance to pests and diseases, and phenotypic and genotypic characteristics expressed variability in performance when combined. This was noticed in most of the VarMix with single variety NSIC Rc 214 (B), and NSIC Rc 298 (A) provided excellent mixing effects that led to better yield and performance in different environmental conditions (Table 9). The unique genetic makeup of Rc214 and the different genetic background of Rc 298 (Table 2), a direct seeding variety, and the compensation and complementation mechanisms of other single varieties when combined contributed to the overall performance of a particular combination (Pacada et al, unpublished).

#### Yield stability analysis

Based on the static measure of all genotype stability across environments, using environmental variance ( $s_e^2$ ), VarMix BDE and single variety NSIC Rc 298 attained the small  $s_e^2$  value of

**Table 7: Locations and VarMix combinations with positive  $ME_{rel_{ml}}$ .**

VarMix Combination	Location with positive $ME_{rel_{ml}}$	Location	VarMix combination with positive $ME_{rel_{ml}}$
ABCE	Loc3, 7, 8, 12, 1, 5, 6, 9, 11	Loc9	AD, AE, ABC, AFE, BCD, BDE, ABCE, ACDE, ABCEF, ADCEF, ABCDEF
ABCEF	Loc3, 7, 8, 4, 6, 9, 10, 11	Loc7	AD, AE, ABC, AFE, BCD, ABCE, ACDE, ABCEF, ADCEF, ABCDEF
AD	Loc7, 8, 12, 1, 4, 5, 6, 9, 10	Loc8	AD, AE, AFE, ABCE, ACDE, ACEF, ABCEF, ADCEF, ABCDEF
BDE	Loc3, 12, 1, 4, 6, 9	Loc5	AD, AE, ABC, AFE, BCD, ABCE, ACEF, ADCEF, ABCDEF
AE	Loc7, 8, 4, 5, 6, 9, 10	Loc6	AD, AE, ABC, BCD, BDE, ABCE, ACDE, ABCEF, ABCDEF
BCD	Loc3, 7, 12, 1, 4, 5, 6, 9, 10, 11	Loc10	AD, AE, ABC, AFE, BCD, ACDE, ABCEF, ABCDEF
ABC	Loc3, 7, 5, 6, 9, 10	Loc3	ABC, AFE, BCD, BDE, ABCE, ACEF, ABCEF, ADCEF, ABCDEF
ABCDEF	Loc3, 7, 8, 1, 4, 5, 6, 9, 10	Loc4	AD, AE, BCD, BDE, ACDE, ACEF, ABCEF, ABCDEF
ACDE	Loc7, 8, 1, 4, 6, 9, 10	Loc1	AD, BCD, BDE, ABCE, ACDE, ADCEF, ABCDEF
ADCEF	Loc2, 7, 8, 1, 5, 9, 10	Loc12	AD, BCD, BDE, ABCE
AFE	Loc3, 7, 8, 5, 9, 10	Loc11	BCD, ABCE, ACEF, ABCEF
ACEF	Loc3, 8, 4, 5, 11	Loc2	ADCEF

2.0; in this analysis, the lower the value of variability the more they were stable. For responsiveness to environmental productivity analysis, VarMix ADCEF was the most responsive in a given environment ( $b_i > 1$ ) and had the largest stability by having a  $b_i$  value of 1.2. ACDE, ABC, and AFE were next, all with the same  $b_i$  value of 1.1. Most of the corresponding varieties of these combinations had an average response to environment ( $b_i = 1$ ). Only Rc298 showed a lower value of 0.8 that is less responsive to a given environment. Variability of a single variety in the environmental regression coefficient,  $b_i$ , may be an imperative measurement of the ability of a less responsive single variety to compensate for a more responsive single variety in less productive environments (Kiaer et al. 2012). This can be seen in VarMix ADCEF where its corresponding single variety has average ( $b_i = 1$ ) to lower ( $b_i < 1$ ) response to a given environment. Lastly, the variance of deviation ( $s(d_i)^2$ ), using the value of static measure of genotype stability, a lower



**Table 8: Locations and VarMix combinations with positive MEmax<sub>ml</sub>.**

VarMix Combination	Location with positive MEmax <sub>ml</sub>	Location	VarMix combination with positive MEmax <sub>ml</sub>
ABCE	Loc7, 8, 6	Loc9	AD, AFE, BCD, ABCDEF
AD	Loc12, 1, 4, 5, 9	Loc3	ABC, AFE, BCD
AE	Loc8, 4, 6	Loc7	BCD, ABCE, ADCEF
AFE	Loc3, 8, 5, 9	Loc8	AE, AFE, ABCE
BCD	Loc3, 7, 9	Loc4	AD, AE, ABCDEF
ABC	Loc3, 5	Loc5	AD, ABC, AFE
ADCEF	Loc2, 7, 10	Loc1	AD, BDE
ABCDEF	Loc4, 9	Loc6	AE, ABCE
BDE	Loc1	Loc2	ADCEF
ACEF	Loc11	Loc12	AD
ACDE	-	Loc10	ADCEF
ABCEF	-	Loc11	ACEF

**Table 9: Inter-varietal diversity (D(x)<sub>ml</sub>) of VarMix combinations.**

VarMix code	Mean D(x)	Lowest (minimum) D(x)	Highest (maximum) D(x)
ABCEF	0.7	0.3	1.6
ABCDEF	0.7	0.3	1.4
AFE	0.7	0.2	1.5
BDE	0.7	0.2	1.9
BCD	0.6	0.1	1.9
ACEF	0.6	0.4	1.3
ADCEF	0.6	0.3	1.1
ABC	0.6	0.3	1.8
ABCE	0.6	0.3	1.6
ACDE	0.5	0.1	0.8
AD	0.5	0.1	1.2
AE	0.5	0.0	1.3

**Table 10: Parameters for yield and yield stability of VarMix combinations.**

VarMix Code	$S_t^2$	$b_i$	$s(d_i)^2$
A	1.96	0.82	4.44
B	4.04	1.05	9.57
C	2.60	0.96	5.24
D	2.88	1.00	5.74
E	3.03	1.03	5.56
F	3.15	0.99	6.34
AD	2.47	0.95	5.22
AE	2.93	1.05	6.30
ABC	3.03	1.07	6.88
AFE	3.12	1.06	6.54
BCD	2.51	0.97	5.81
BDE	2.00	0.87	4.17
ABCE	2.44	0.96	5.31
ACDE	3.11	1.07	5.97
ACEF	2.59	0.98	5.05
ABCEF	2.65	0.99	5.23
ADCEF	3.74	1.18	8.85
ABCDEF	2.73	1.00	6.08

value was found in VarMix BDE with 4.2. The lower the value of  $s(d_i)^2$ , the more they are sensitive to biotic and abiotic stresses. But the corresponding single varieties (B, D, and E) demonstrated high sensitivity to both stresses with  $s(d_i)^2$  values of 9.6, 5.7, and 5.6, respectively (Table 10).

Yield stability can be explained by the complementation mechanism which involves increasing rates of plant productivity (Loreau and Hector 2001; Hooper et al. 2005). It also includes the facilitation mechanism where every component variety benefits another component that is more resilient to both abiotic and biotic stresses (Garcia-Barrios 2002).

Analysis of genotypic responsiveness of VarMix combinations and their corresponding single varieties across 12 locations provided basic information on their different levels of complementarity and compensation mechanism. These can be observed in the identification of VarMix combinations with the largest stability ( $s_t^2$ ), responsiveness to different environments ( $b_i$ ), and less sensitive to biotic and abiotic stress ( $s(d_i)^2$ ). In another investigation, it was revealed that the best-performing mixture for yield stability was found in three or more mixtures than in a mixture with only two in controlling fungus (Newton et al. 1997), disease infestation (Mille et al. 2006), and low-input crop management (Kiaer et al. 2012) evaluation. Furthermore, Wang et al. (2016) asserted that yield stability attributed to diversity and compensatory effects of a varietal mixture under limited irrigation condition increases water-use efficiency in winter wheat.

Uncovering the VarMix mechanism for limited source of water was revealed in below-ground investigations of Pascua et al. (2020). In the present study, VarMix ADCEF and ABCDEF yielded higher than their corresponding single varieties due to the complementation mechanism of NSIC Rc 298 (A) root traits. The total lateral root length plasticity of Rc298 played an important role during water-stress conditions in the degree of branching in response to the stress. The presence of Rc298 in some VarMix stimulated the increased root plasticity in response to control or cycles of alternate wetting and drying. Rc298 traits enhance the VarMix characteristics such as expressing high root plasticity by increasing root water uptake from a deeper soil layer through hydraulic lift; redistributing the water taken up by its roots to the upper drier soil and taken up by neighboring roots with less plasticity; and the redistributed water may have been used by other component varieties that improved their root systems (Pascua et al. 2021).

## CONCLUSIONS

In this study that used no chemical inputs, VarMix combinations demonstrated their capability to lessen pest and disease infestations in the field. The occurrence of pests and diseases is unpredictable, thus planting VarMix in vulnerable rice production areas not only prevents yield losses but also promotes farmer productivity. In addition, the actual field performance of VarMix specifically in areas with limited source of water showed its potential in combatting water scarcity. While a single multi-trait local rice variety well-adapted to areas with both biotic stress and water scarcity has yet to be developed, planting VarMix looms as a novel approach.

The six varieties used in establishing the subject VarMix combinations can still be improved upon by formulating untested combinations and ratios. Meanwhile, data gathered from field observations and controlled environments can help expedite the selection of superior combinations in the future toward having more resilience to water scarcity.

The identification of six locations – PhilRice Agusan (Loc10); Sta. Barbara in Iloilo (Loc8); Batac City, Ilocos Norte (Loc7); Sto. Domingo, Nueva Ecija (Loc9); Agusan del Norte (Loc1); and Nabonton, Ligao City, Albay (Loc5) where the combinations attained highest yields- can be used as reference for future deployment of VarMix in areas with similar environmental conditions. In addition, VarMix combinations that were scrutinized through the mixing effects (NSIC Rc298 : Rc214 : Rc216 : Rc238), inter-variety diversity (NSIC Rc214 : PSB Rc82 : Rc238; NSIC Rc298 : Rc300 : Rc238; NSIC Rc298 : Rc214 : Rc216 : Rc238 : Rc300; NSIC Rc298 : Rc214 : Rc216 : PSB Rc82 : Rc238 : Rc300) , and yield stability analyses (NSIC Rc214 : PSB Rc82 : Rc238; NSIC Rc298 : PSB Rc82 : Rc216 : Rc238 : Rc300; NSIC Rc298 : Rc216 : PSB Rc82 : Rc238; NSIC Rc298 : Rc214 : Rc216; NSIC Rc298 : Rc300 : Rc238), (Tables 5 to 10) using actual field environment conditions can facilitate the selection of elite VarMix combinations in a specific location. Results of this research thus far point to the potentials of VarMix as a stop-gap approach for rice production in unpredictable environments where yield loss is likely to occur.

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## CONFLICT OF INTEREST

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

## CONTRIBUTION OF INDIVIDUAL AUTHORS

Main Author conceptualized, designed, analyzed and interpreted the data, and drafted the manuscript. Second Author initially drafted the manuscript and assisted in organizing the tables and figures.

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