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Genotype by Environment Interaction in Rice Under High Temperature Stress Condition in Cagayan

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ABSTRACT

Twelve rice genotypes were planted in four municipalities of Cagayan (Iguig, Peñablanca, Solana, and Tuguegarao). It aimed to assess genotype by environment interaction (GEI) of rice cultivated by farmers in Cagayan as well as their stability. This study was also designed to identify genotypes suited to high temperature-prone areas and the most discriminating environment to screen genotypes for high temperature tolerance. During anthesis, the recorded maximum temperature ranged from 33.6-42.4°C in Iguig, 32.9-44.80C in Peñablanca, 32.1-40.5°C in Solana, and 32.6-41.1°C in Tuguegarao. Panicle and canopy temperature were lower than air temperature in Iguig and Peñablanca but higher in Solana and Tuguegarao. Variance due to genotype, environment and GEI were found significant ($p \leq 0.05$) for yield. Environment contributed the greatest proportion to yield which was 43.1% of the total variance, while genotype contributed 35.5%, and GEI 21.5%. Spikelet fertility, panicle length, number of grains per panicle, and grain weight were not significantly affected by GEI. However, panicle length and spikelet fertility were significantly affected by genotype and environment, while number of grains per panicle and grain weight were significantly affected only by environment. NSIC Rc 152, Rc 218, Rc 222, PR40330, and PR42026 were found high-yielding with wide adaptation to high temperature conditions. NSIC Rc 152 and PR40330 were the most outstanding genotypes. These genotypes can be used as parents for development of new heat-tolerant lines. Iguig was the best location for screening and selecting generally adapted genotypes under high temperature condition.

Keywords:
high temperature, relative humidity, rice, GxE, GEI, stability, adaptability, AMMI, CGE

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INTRODUCTION

Earth's energy budget is altered due to increase in anthropogenic substances, mostly greenhouse gases such as carbon dioxide, methane, and nitrous oxide (IPCC 2013). If emission of these greenhouse gases continues, it will cause further warming and long-lasting changes in all components of the climate system, and the impacts might be severe, pervasive and irreversible for people and ecosystems (IPCC 2014). Based on report released by National Oceanic and Atmospheric Administration (NOAA) National Centers for Environmental Information in January 2021, year 2020 ranked the second warmest year in the 141-year record, with a global land and ocean surface temperature departure with average of +0.98°C. The climate instabilities observed year after year are becoming larger.

High temperature is considered one of the major constraints in rice production. Rice grown beyond critical temperature threshold from 28 to 35°C during flowering can cause pollen and spikelet sterility leading to yield losses (Guittap et al. 2020; Prassad et al. 2006; Zhong et al. 2005). In South China, yield decreased from 1.5 to 9.7% due to post-heading heat stress (Shi et al. 2015). In Japan, temperature greater than 40°C concurred during flowering stage caused 25% yield losses (Hasegawa et al. 2009). The same occurrence were also recorded in Pakistan, India, Bangladesh, Thailand, Australia, and United States. (Jagadish et al. 2015; Matsui et al. 2015; Hasegawa et al. 2009; Teixeira et al. 2013). Different crop models like CERES, that predicted yield losses as influenced by increase in temperatures revealed that rice production in South Asia could decline up to 10% by 2030; 10-25% by 2080; and 20-40% by 2100 (Lobell et al. 2008). The continuous rise in global temperature calls for the development of new rice varieties that can withstand high temperature (Manigbas and Sebastian 2007; Redona et al. 2009; Manigbas et al. 2013).

Trait for heat-tolerance is quantitative which is highly affected by environment and it complicates screening and introgression of trait (Ye et al. 2021). Genotype by environment interaction (GEI) is about the variation in performance due to different sensitivities of genotypes to various environmental conditions. It affects the efficiency of selection in a breeding program (Osei 2018), because it reduces the association between the genotypic and phenotypic values (Manigbas 2016). Determining the contributions of genotype and environment to agronomic traits can aid in cultivar selection for a given environment (Snider et al 2013). Evaluation of genotypes is a regular part of plant breeding activities, with the aims not just to identify stable genotypes across locations and seasons but also to identify germplasm for further improvement to produce new varieties (Krishnamurthy 2021).

There are several statistical analyses used to study GEI, and the most effective are Additive Main Effects and Multiplicative Interaction (AMMI), and Genotype by Genotype by environment (GGE) (Samonte et al. 2005; Yan et al. 2000; Asfaw et al. 2009; Noriel et al. 2009).

An AMMI biplot representation was obtained to explore the pattern produced by GxE interactions. According to Kandus et al. (2010) and Asfaw et al. (2009), AMMI model provided the relative magnitude and importance of the effects of GEI and its interaction terms related with genotype and environment effects. GGE biplot, on the other hand was done to determine the relative performance of genotypes in a specific environment and at different environments. In addition, it was used to identify the most discriminating and representative environment as well as location of specific and ideal genotypes. A discriminating environment was very informative in providing information on the genotypes. A representative environment was the most suitable environment for screening genotype for a given conditions. Both analyses used biplots to evaluate results. The only difference between these models is in the initial and final steps of the analysis (Neisse et al. 2018). In the initial step, GGE analyzes G plus GE (or GEI), while AMMI separates G from GE. For final step, the only difference is the place wherein biplots for the interpretation are built. Despite the above-mentioned differences,

both AMMI and GGE analysis complement each other and can be used simultaneously to study GEI. According to Miranda et al. (2009), AMMI biplots provided relatively simple analysis which draws out conclusions concerning phenotypic stability, genotype behavior, genetic divergence between genotypes, and environments with optimal performance. GGE biplots complement on these AMMI Biplot's environmental stratification through delineating mega-environments and genotypes with optimal performance in such groups (Miranda et al. 2009).

Cagayan, which is located in the northeastern part of mainland Luzon, approximately 17° 30' north and 121° 15' east, is one of the highest rice producers in the Philippines with annual rice production of 895,580 metric tons (BAS 2014). Most rice fields in Cagayan are irrigated, however temperature with more or less than 35°C occurred in Cagayan annually, specifically during the months of April and May (Manigbas 2007). High temperature causes heat-induced spikelet sterility which can possibly result to 10-15% yield loss. Farmers use rice varieties with no known reported tolerance to heat stress, and under this condition, their crops are subsequently affected. At Philippine Rice Research Institute (PhilRice), new genotypes were being developed to address this problem using Nagina 22, Dular, WAB 56-125, and Giza-178 as tolerant parents.

Considering the problem, the study was conducted to assess genotype by environment interaction of 12 rice genotypes being cultivated by farmers in Cagayan, evaluate their stability, identify genotypes suited to high temperature-prone areas, and identify the most discriminating environment to screen high temperature tolerance of genotypes.

MATERIALS AND METHODS

Characteristics of genotypes

Eight popular rice varieties in Cagayan, two high temperature-tolerant elite lines, tolerant and susceptible checks obtained from Plant Breeding and Biotechnology Division (PBBD), PhilRice were used in this experiment (Table 1).

Table 1. List of genotypes and important agronomic traits.

VARIETIES	AVE. YIELD (T/HA)	MAX. YIELD	MATURITY (DAS)	NO. OF PROD. TILLER
NSIC Rc 152	6	8.7	109[b]	15
NSIC Rc 160	5.6	8.2	107[b]	14
NSIC Rc 216	6	9.7	112[b]	14
NSIC Rc 218	3.8	8	120[a]	14
NSIC Rc 222	6.1	10	114[b]	14
NSIC Rc 238	6.4	10.6	110[b]	15
NSIC Rc 298	5.3	8.2	118[a]	14
PSB Rc 10	4.8	7.5	106[b]	16
*PR42026	6.8	9.9	116[a]	17
*PR 40330	5.8	7.1	110[b]	17
NSIC Rc 240 (susceptible check)	6.4	10.6	115[a]	12
*N22 (tolerant check)	6.1	7.6	103[c]	17

Note: Data were taken from the records of National Cooperative Test (from different irrigated lowland environments across the country)

* Data was derived from Plant Breeding and Biotechnology Division, PhilRice

a - 1st Batch with days to 50% flowering of ≥85 DAS

b - 2nd Batch with days to 50% flowering of 75-84 DAS

c - 3rd Batch with days to 50% flowering of 64-74 DAS

Description of test sites and experiment

The experiment was conducted in four municipalities of Cagayan namely: Iguig (17° 44' 59.82 N lat., 121° 45'13.46 E long., 25.09m elevation); Peñablanca (17° 39' 45.65 N lat., 121° 56' 50.32 E long., 289.44m elevation); Solana (17° 39' 10.21 N lat., 121° 39' 4.05 E long., 57.14m elevation) and; Tuguegarao (17° 36' 47.45 N lat., 121° 43' 37.27 E long., 30.82m elevation).

A total of 250 g of seeds were prepared for each genotype per site. Sowing was done on different dates to synchronize flowering time of all genotypes during high temperature months (April-May). Schedule of sowing was based on days to 50% flowering obtained from database of National Cooperative Testing (NCT) and PBBD, PhilRice (Table 1). Fourteen-day old seedlings were planted and laid out in a randomized complete block design (RCBD) with three replications. Plot size was 25 m2 consisting of 625 plants per plot with 20x20 cm spacing. Appropriate cultural management practices were implemented with fertilizer rate based on soil analysis.

Temperature and relative humidity

Temperature, percent relative humidity (RH), and dew point were taken using thermometer-hygrometer with USB data logger. It was installed in the middle of the field, one meter above the canopy on April 12, 2017, logger was also set to record every three minutes and downloaded every two weeks.

At flowering, panicle and canopy temperature were taken using IRT thermometer, Apogee MI-230. Canopy and panicle temperature were measured twice per replication per genotype, while panicle temperature was measured three times per replication per genotype. Measurement was performed during the time of peak anthesis which was between 9 to 11 am, precise start and end time of measurement were also noted. Panicle and canopy were measured in Iguig on May 1, 5 and 7, in Peñablanca on May 2, 8 and 11, in Solana on May 3, 6 and 9, and in Tuguegarao on May 4, 10 and 12.

Agronomic characteristics

Time of anthesis was observed only under cloudless day. Beginning, peak, and end anthesis were observed by tagging three primary panicles from three replications of each genotype. Traits such as panicle length, number of spikelet per panicle, 1000 grain weight, spikelet fertility and yield were determined using Standard Evaluation System for Rice (IRRI 2014).

Additive main effects and multiplicative interaction (AMMI)

Data on grain yield and spikelet fertility were used and combined analysis of variance across environments was also performed. AMMI method as described by Zobel et al. (1988) was used to investigate stability and GxE interaction using statistical model:

$$Y_{ij} = \mu + g_i + e_j + \sum_{k=1}^{t} \lambda_k + \alpha_{ik} + \gamma_{jk} + r_{ij} + \varepsilon_{ij}$$

Where: Y_{ij} is the mean response of genotype i in the environment j; μ is the overall mean; g_i is the fixed effect of genotype i (i=1, 2, …g); e_j is the average experimental error; the GxE interaction is represented by the factors; λ_k a unique value or singular value of kth Interaction Principal Component Analysis (IPCA), (k= 1, 2, … t, where t stands for the maximum number of estimable main components); α_{ik} is a singular value for the i^{th} genotype in the kth IPCA, γ_{jk} is a unique value of the jth environment in the k^{th} IPCA; r_{ij} is the error for the GxE interaction or AMMI residue; and k is the characteristic non-zero root, k=[1, 2, …min(G-1, E-1)].

AMMI stability value (ASV)

Modified AMMI stability value (MASV) was calculated in order to rank genotypes in terms of stability using the formula:

$$MASV = \sqrt{\sum_{k=1}^{N'-1} \left(\frac{SSIPCA_n}{SSIPCA_{n+1}}\right) (IPCA_n)^2 + (IPCA_{N'})^2}$$

Where:

SS = Sum of squares

IPCA1 = interaction principal component analysis axis 1

IPCA2 = interaction principal component analysis axis 2

Modified AMMI stability value was based on AMMI stability value as described by Purchase in 1997. The only difference between ASV and MASV was that in MASV, only significant IPCAs were used.

In interpreting MASV, higher IPC score either positive or negative means genotypes are more specifically adapted to a certain environment. Smaller ASV indicates a more stable genotype across environments.

The yield stability index was calculated as:

YSI = RASV + RY

Where RASV is the rank of AMMI stability value and RY is the rank of the mean grain yield of genotypes across environments.

GGE analysis

The GGE biplot was based on the (multiplicative) model (Cornelius et al. 1996) with formula:

$$\gamma_{ij} = \mu + \sum_{k=1}^{t} \lambda_k + \alpha_{ik} + \varepsilon_{ij}$$

Where: Yij is the mean response of genotype i in the environment j; μ is the overall mean; ε_ij is the average experimental error; the GEI is represented by the factors; λ_(k) is a unique value or singular value of kth Interaction Principal Component Analysis (IPCA), (k= 1, 2, … t, where t stands for the maximum number of estimable main components); α_(ik)is a singular value for the ith genotype in the kth IPCA; and k is the characteristic non-zero root, k=[1, 2, …min(G-1, E-1)].

Statistical software

Statistical analysis, including correlation, AMMI stability and biplot analysis, and GGE biplot analysis were done with software language R v3.4.2.

RESULTS AND DISCUSSION

Description of test locations

All test sites are located in Cagayan province specifically in the northeastern part of mainland Luzon, approximately 17° 30' north and 121° 15' east. The tests sites were in Minanga Norte, Iguig (17°45’45”N lat, 121°44’05”E long), Callao, Peñablanca (17°41’14”N lat, 121°48’04” E long), Solana (17°38’45”N lat, 121°40’52”E long) and Tuguegarao (17°37’14”N lat, 121°45’03”E long). These sites are located at different elevations. Iguig is 24 m above sea level, Peñablanca 46 m, Solana 27 m and Tuguegarao 30 m. To determine the distance of test sites from each other, the location of PAGASA Field Operations Center in Capitol Hills Tuguegarao City was used as reference point. Hence, Minanga Norte, Iguig is 16 km north, Callao, Peñablanca is 18 km west, Lingu, Solana is 15 km east and Capatan, Tuguegarao is 6.8 km south.

All of the test locations were under irrigated lowland condition where water was supplied by the National Irrigation Association (NIA). Cagayan River supplied irrigation water to Iguig and Solana, while Pinacanauan River supplied Peñablanca and Tuguegarao. However, water supply in Solana and Tuguegarao was limited due to emergency irrigation canal repair as a result, water requirements were supported by water pump.

Varying temperature and relative humidity during reproductive and grain-filling stages

Daily temperature and relative humidity (RH) from panicle initiation to ripening stage (April 12-June 6, 2017) were obtained in four sites and presented in Figure1A-D. Based on the results, temperature higher than 35°C occurred in all experimental sites. Maximum temperature across sites ranged from 32.1 to 46.1°C. RH on the other hand, ranged from 38.7 to 100%.

During flowering, minimum and mean temperature in all sites were not significantly different (Table 2). However, varying maximum temperature was recorded. Peñablanca had the highest maximum temperature of 41.6°C followed by Iguig with 39.7°C. Comparatively, Solana had the lowest with 0.5°C lower than in Tuguegarao. In terms of RH, difference between sites was not significant with range between 33.4 and 100%. During this period, when temperature was ≥35°C, the average RH was 59.2% ranging between 38.7 and 95.1%.

During the grain-filling to ripening stage, varying minimum and maximum temperature were observed (Table 2). During this stage, Solana recorded the highest minimum temperature of 25.6°C and lowest maximum temperature of 37.4°C. Peñablanca on the other hand, had the lowest minimum temperature of 24.2°C and highest maximum temperature of 40.6°C.

The optimum temperature threshold for normal development of rice ranged from 27 to 32°C (Shah et al. 2011; Yin et al. 1996). Temperature higher than threshold affects almost every growth stage of rice. However, the most sensitive stage to high temperature stress is booting, and flowering stages (Jagadish et al. 2007). Temperature higher than 35°C during flowering phase caused spikelet sterility and reduce yield (Manigbas and Sebastian 2007).

Table 2. Average minimum, maximum and mean temperature and percent relative humidity on different experimental sites during flowering stage.

Temperature (°C)/ Relative Humidity (%)	Iguig		Peñablanca		Solana		Tuguegarao	
	Flowering	Grain-filling	Flowering	Grain-filling	Flowering	Grain-filling	Flowering	Grain-filling
Minimum	23.4±0.67	24.6±0.56	23.0±0.68	24.2±0.61	23.4±0.85	25.6±2.41	23.4±0.62	24.5±0.55
	(22.5-24.6)	(23.6-25.6)	(22.0-24.1)	(23-25.6)	(22.0-24.7)	(22.9-30.2)	(22.0-24.2)	(23.5-25.5)
	73.6±17.9	49.7±7.3	71.7±18.9	49.0±8.2	73.0±18.3	54.6±8.6	75.2±17.3	51.6±7.8
	(33.5-100)	(36.9-66)	(36.6-100)	(36.8-64.8)	(33.4-98.0)	(33.4-69.1)	(36.2-98.3)	(40.9-69.3)
Maximum	39.7±2.49	39.2±2.0	41.6±3.38	40.6±3.10	37.4±2.26	36.9±2.13	37.9±2.27	38.3±2.07
	(33.6-42.4)	(35.0-42.2)	(32.9-44.8)	(34.4-46.1)	(32.1-40.5)	(32.8-40.9	(32.6-41.1)	(32.5-41.5)
	78.6±15.9	96.1±3.0	77.8±16.5	96.9±2.8	79.1±16.3	91.3±10.1	80.8±15.2	97.3±1.7
	(38.9-100)	(87.5-100)	(41.1-100)	(91.7-100)	(38.7-99.3)	(70.5-99.8)	(44.1-98.7)	(94-99.8)
Mean	29.9±1.07	29.9±1.32	30.1±1.21	30.3±1.61	29.0±1.00	29.6±1.78	29.0±0.85	29.4±1.27
	(27.1-30.9)	(27.1-32.5)	(27.1-31.4)	(27.3-33.4)	(26.8-30.4)	(27.0-32.8)	(27.2-30.5)	(27.2-31.8)
	76.2±16.9	79.0±6.4	74.8±17.6	77.1±7.6	76.2±16.9	78.1±9.2	77.8±16.3	80.4±6.3
	(36.8-100)	(67.8-91.1)	(38.8-100)	(64.1-90.2)	(36.0-98.3)	(62.2-90.3)	(39.6-98.5)	(69.5-90.90)

Phenotypic plasticity in overcoming high temperature stress-induced damage across hot tropical rice-growing regions was predominantly governed by relative humidity (Jagadish 2014). Effect of high temperature stress was compounded by 85-90% RH during flowering stage (Yan et al. 2010), which induced complete grain sterility in rice (Abeysiriwarden et al. 2002).

All test locations had high temperature (>35°C) from flowering to ripening stage confirming that all genotypes were exposed to high temperature stress. RH was also high in all sites. When temperature was ≥35°C, RH was 38.7 to 95.1%, a state which is very detrimental to flowering and grain-filling.

Panicle and canopy temperature

Canopy and panicle temperature are important data in evaluating genotypes in response to high temperature stress. Among the four sites, Solana had the highest panicle and canopy temperature of 35.4°C and 34.9°C, respectively (Table 3). It was followed by Tuguegarao with 30.3°C canopy temperature and 32.1°C panicle temperature. Location with the lowest canopy and panicle temperature was Peñablanca. In Peñablanca and Iguig, panicle temperature was higher compared to canopy temperature in all genotypes. This is contrary in Solana wherein most genotypes had higher canopy temperature than panicle temperature.

Table 3. Canopy and panicle temperature in four sites.

Temperature (°C)	Iguig	Peñablanca	Solana	Tuguegarao
Canopy	30.3±1.28 (28.2-32.8)	29.6±0.73 (28.4-30.6)	35.4±0.78 (33.9-36.5)	32.4±1.33 (29.6-34.2)
Panicle	32.1±0.56 (30.8-33.4)	31.3±0.17 (30.6-31.8)	34.9±0.53 (33.8-36.0)	32.8±0.56 (32.0-34.3)

Peñablanca and Iguig had the highest air temperature but with lowest panicle and canopy temperature due to water in the paddy that supported the cooling process of the canopy. In all sites, when air temperature was ≥33°C, RH was between 33.4 and 71.2% (Fig. 1). The combination of high air temperature and low relative humidity will induce transpiration and significantly cool the canopy and panicle. The phenotypic plasticity to avoid or escape high temperature stress, enables the rice plant to have high yields even under critical threshold temperature condition (Jagadish et al. 2015). However, this heat-avoiding mechanism of rice plant is highly dependent on soil moisture which sustains the evaporative cooling of the plants (Luan and Vico 2020). Transpiration in a water-deficit soil is less due to partial opening of the stoma (Rizhsky et al. 2002). Thus, Solana and Tuguegarao had higher panicle and canopy temperature despite lower air temperature. In addition, absence of water in the paddy similar to the case in Solana and Tuguegarao, removed the safeguard to reduce canopy temperature by absorbing heat.

Genotype by environment interaction (GEI) analysis

Flower opening time

All genotypes began to flower between 8:05 and 9:10 AM. Peak of flower opening time as indicated by simultaneous opening of more than ten spikelets per panicle, occurred between 8:45 and 10:07 AM. End-time of flowering between 9:10 and 10:55 AM was recorded when half of the opened spikelets were already closed. Based on the average performance of the 12 genotypes across four locations, the earliest to flower was PSB Rc 10 and the latest was NSIC Rc 218. The earliest genotype to reach peak flowering was NSIC Rc 238 and the latest was NSIC Rc 298. Genotypes with the longest duration of flowering time were NSIC Rc 160 and Rc 216 with 1 h and 41 min, while the earliest was PR40330 with

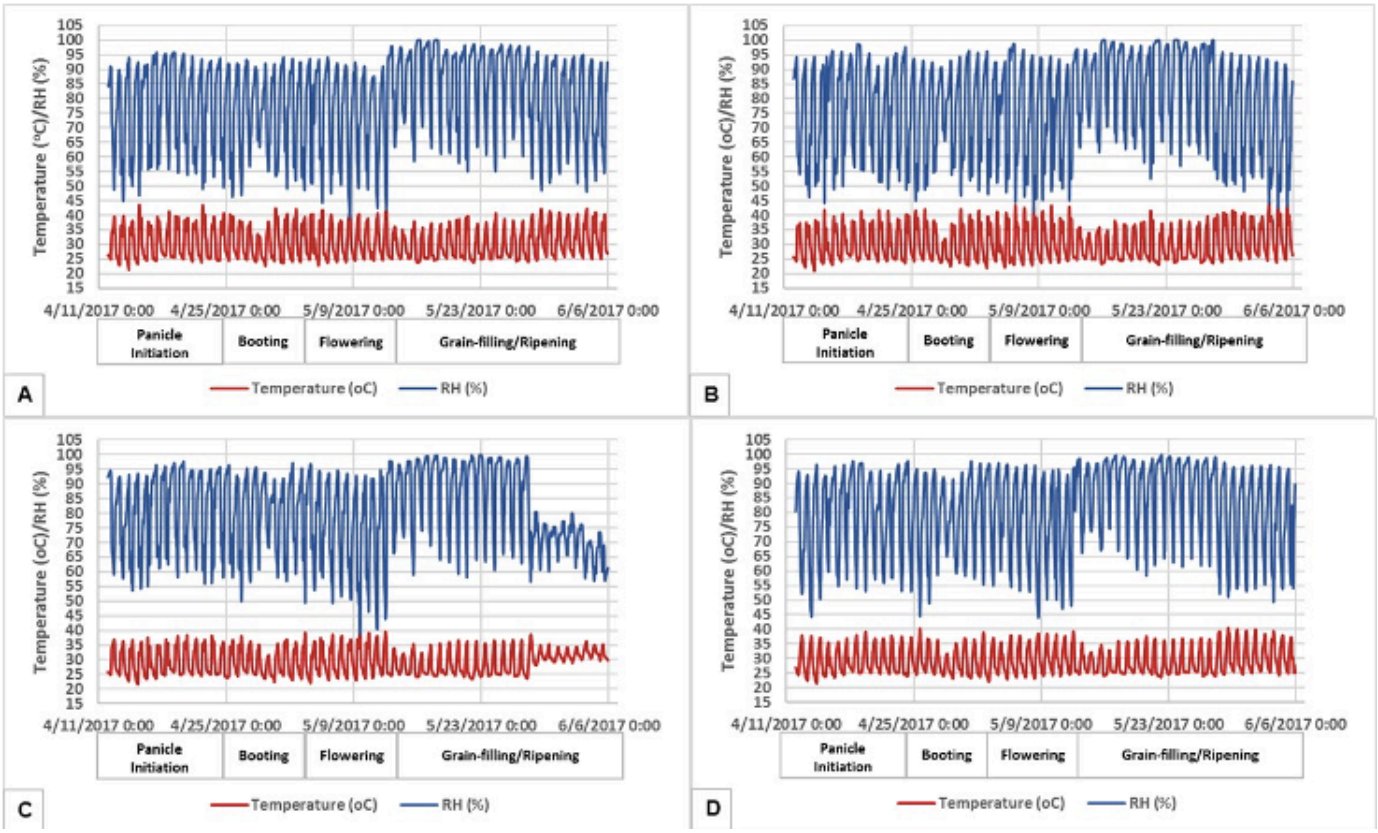

Figure 1. Temperature (°C) and percent relative humidity (RH) in A. Iguig, B. Peñablanca, C. Solana, D. Tuguegarao, Cagayan during reproductive stage (April 12- June 6, 2017).

only 1 h and 10 min. Duration of flower opening time showed no significant differences in four locations.

Yield and yield components

Combined analysis of variance showed that grain yield of 12 genotypes across four locations were significantly ($p \leq 0.05$) affected by genotype (G), environments (E) and GEI. Yield components such as panicle length, number of grains per panicle, and grain weight were not significantly ($p \geq 0.05$) affected by GEI. However, panicle length was significantly affected by genotype and environment while the number of grains per panicle and grain weight were significantly affected by environment.

Partitioning of the sum of squares of the components using AMMI analysis of variance (Table 4) for yield showed that environments contributed 43% of the total variation, 35.5% due to genotype and 21.5% due to GEI. Results showed that most variation was due to environment (43%). Environments (E) were characterized by the average performance of genotypes in the particular environment, and significant results indicated that the four environments were diverse (Malosetti et al. 2013) with large differences among environmental means. Large contribution of environment was also reported by Ikmal et al. (2020) under drought conditions, and Manigbas et al. (2016) under irrigated lowland conditions. Highly significant genotypic effect indicated that genotypes varied in their average performance across environments (Table 4). GEI significant effect, on the other hand, demonstrated differences in genotypes' response to variation in environmental conditions (Manigbas et al. 2016). However, the sum of squares of genotypes was 1.7 times higher compared to GEI (Table 5), which indicated broad adaptation of genotypes used (Gauch 2015). This was to the level of heterozygosity at the population level and amount of genetic heterogeneity within the individual (Baye et al. 2011). The magnitude of GEI was influenced by the genetic structure of the genotype, and in the absence or at minimum GEI, the variance between individuals was expected to

be homogenous or less heterogenous. GEI was high (21.5%), but G was higher (35.5%) which indicates that genotypes were adapted but the degree of their adaptation and interaction to environments were significantly different.

Significance in GEI denotes interactions that resulted from the changes in the magnitude of differences between genotypes from one environment to another (Fig. 2). It also signifies a cross-over type of GEI as revealed by changing yield rank of genotypes across environments. Further partitioning of GEI showed two IPCAs significant at 5% probability level, generating three IPCAs but only the first two were significant. IPCA1 explained 48.8% of the GEI sum of squares, and IPCA2 further explained 36.8%. Although the third IPCA was not significant, IPCA1 and IPCA2 accounted for a total of 85.6% of the interaction sum of squares, and it explained maximum interaction variation (Kilic 2014). The use of first two IPCAs were adequate for best predictive model (Gauch and Zobel 1997). Succeeding IPCAs captured mostly noise, and therefore unnecessary to predict validation observations.

Table 4. AMMI analysis of variance of grain yield (kg/ha-1) of 12 genotypes and four environments.

SOURCE OF VARIATION	df	F VALUE	PR(>)		% SUM OF SQUARES	% SUM OF SQUARES OF GEI
Environment	3	43.92	2.58E-05	***	43.1	
Blocks/Reps	8	0.92	0.50			
Genotype	11	9.12	1.17E-10	***	35.5	
GxE interaction	33	1.84	0.01	*	21.5	
PC1	13	2.28	0.01		10.5	48.8
PC2	11	2.03	0.03		7.9	36.8
PC3	9	0.98	0.46		3.1	14.5
Residuals/Error	88					

The blocks/Reps source of variation refers to blocks within environments
Significant codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.'

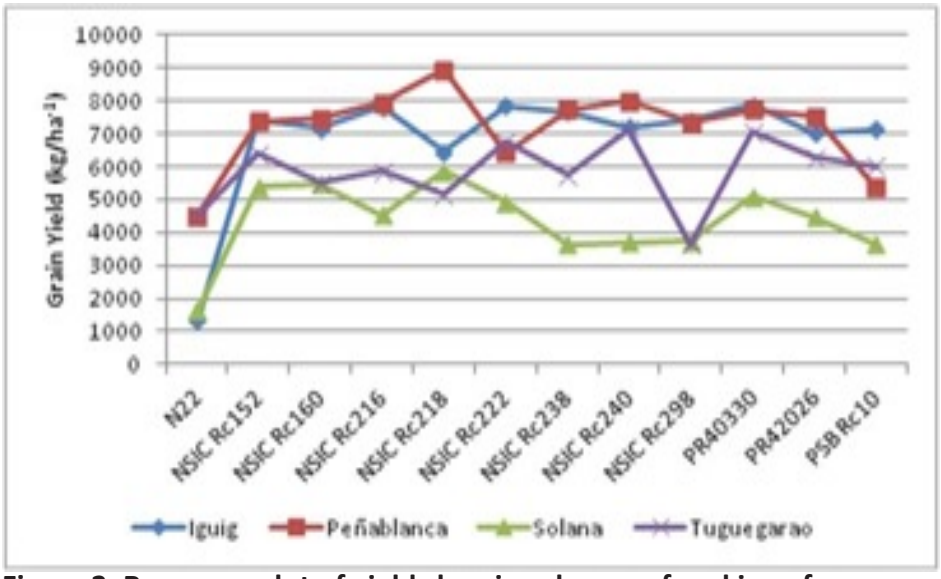

Figure 2. Response plot of yield showing change of ranking of genotypes across environments.

Spikelet fertility (SF)

Spikelet fertility (SF) was significantly affected by genotype and environment but not by GEI. In the presence of high temperature stress, there is an opportunity to select for stable and tolerant genotypes (Kang et al. 2004) based on spikelet fertility. SF was considered the most prominent trait that is affected by high temperature (Karwa et al. 2020). Temperature at flowering also known as dehiscent temperature was associated with high temperature tolerance (Grospe et al., 2016) as represented by panicle temperature. Solana had the highest stress received in terms of panicle temperature, while Peñablanca had the lowest. Thus, most genotypes had highest percent SF in Peñablanca with 77.4 to 92.5%. In Solana, all genotypes had the lowest with 66.6 to 83.4%. High temperature in Solana resulted to SF declined of 12.8%. According to Jagadish et al. (2007), exposure to temperatures above 33.7°C for less than one hour was enough to cause spikelet sterility which occurred mostly in Solana. High temperature stress in all test sites was compounded by high RH which was proven by Matsui et al. in 1999 where he explained the role of moisture in swelling of pollen grains which was the driving force to rupture the anthers and pollinate. Under high temperature of >35°C, dry air promotes desiccation of dehiscing anthers (Matsui et al. 1997; Shah et al. 2011), impeding swelling of anthers affecting pollination. Effects of high temperature and RH resulted to reduce shedding of pollen on the stigma (Shah et al. 2011). Spikelet fertility can also be reduced up to 47% as pollen viability and germination reduced by 20 and 44%, respectively (Thuy et al. 2020).

Average across environment showed NSIC Rc 218 as the most fertile genotype, followed by PR40330. The least fertile was the susceptible check NSIC Rc 240. Genotype with high SF also varied across environments, PR42026 was highest in Iguig, NSIC Rc 218 in Peñablanca, NSIC Rc 298 in Solana and PR40330 in Tuguegarao. The most stable genotype with the least affected spikelet fertility was PR40330 with only 0.3% decline (Fig. 3).

Correlation analysis of SF, grain yield, panicle length, number of grains per panicle, grain weight, air temperature and RH during reproductive and flowering stage along with canopy and panicle temperature showed that grain yield under high temperature was significantly ($p \leq 0.05$) correlated to SF (Table 5). Grain yield was significantly correlated to grain weight, same as SF to number of grains per panicle and panicle length. Both grain yield and SF were significantly correlated to RH during flowering stage, canopy and panicle temperatures.

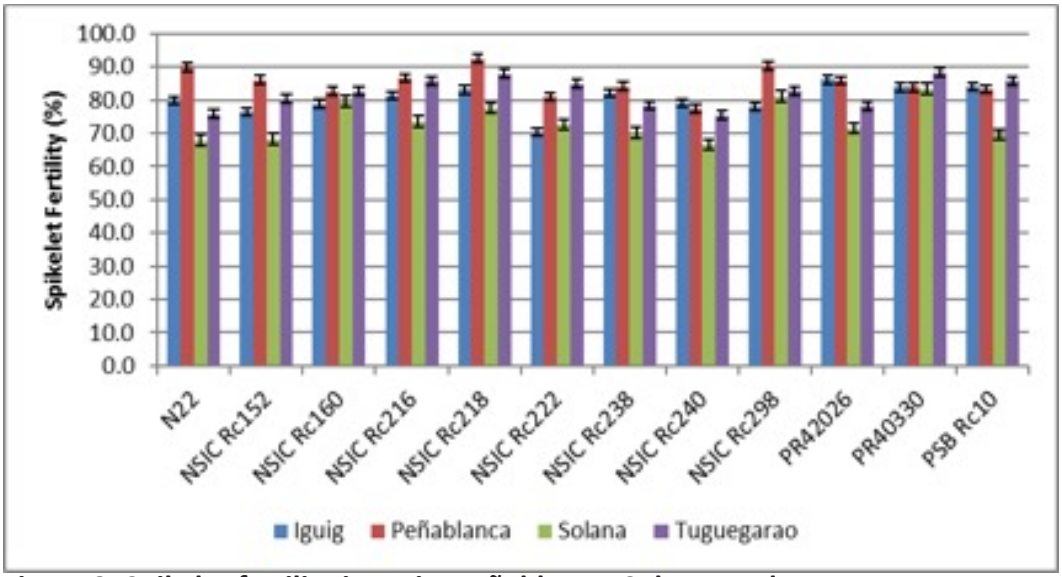

Figure 3. Spikelet fertility in Iguig, Peñablanca, Solana, and Tuguegarao.

Table 5. Correlation analysis associated with SF and yield.

Traits	SF			Yield		
	Pearson r	P-value		Pearson r	P-value	
SF	1			0.7	0.01	*
Yield	0.7	0.01	*	1		
Panicle Length	-0.33	0.02	*	0.26	0.40	ns
No. of Grains Per Panicle	-0.22	0.03	*	0.3	0.32	ns
1000 Grain Weight	-0.3	0.32	ns	-0.73	0.00	***
RAT	0.83	0.00	***	0.87	0.00	***
RRH	-0.81	0.00	***	-0.86	0.00	***
FAT	0.76	0.00	***	0.85	0.00	***
FRH	-0.69	0.01	**	-0.82	0.00	***
CT	-0.86	0.00	***	-0.89	0.00	***
PT	-0.87	0.00	***	-0.87	0.00	***

Correlation is significant at 5% level (2-tailed) in LSD
Significant codes of P-value: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.'
RAT – reproductive air temperature, RRH – reproductive relative humidity, FAT – flowering air temperature, FRH – flowering relative humidity, CT – canopy temperature, PT – panicle temperature

Stability of rice genotypes under high temperature

AMMI 1 biplot showed the main and interaction effects (PC1) of both genotypes and environments on grain yield with a model fit of 89.1% accounted from 78.6% of the total variation in main effects, and 10.5% from PC1 variation in GEI sum of squares. In AMMI 1 biplot, the horizontal dotted line shows the interaction score of zero and the vertical dotted line indicates the grand mean yield (Fig. 4). Displacement along the horizontal axis indicates difference in genotype and environment main effects, while vertical axis specifies interaction differences between genotypes and between environments (Asfaw et al. 2009). Most genotypes were high-yielding except N22 (G1), NSIC Rc 298 (G9) and PSB Rc 10 (G12). Among high-yielding genotypes, NSIC Rc 152 (G2) and NSIC Rc 222 (G6) were the most stable, while PSB Rc 10 (G12) was the most stable among low-yielding genotypes. Peñablanca (E2) was the highest-yielding and the most stable environment. Solana (E3), although stable it was also the lowest-yielding environment. Yield in Tuguegarao (E4) was below average while Iguig (E1) was considered high yielding but both were highly interactive with genotypes.

For adaptability of genotypes, AMMI2 biplot was used (Fig. 5), which accounted for 48.8% PC1 and 36.8% PC2 with a model fit of 85.6%. It was clarified that the closer the genotypes score to the center of the biplot, the more stable and adapted they are (Purchase 1997; Kilic 2014). Genotypes that are far from the center has high GEI, thus, consider unstable. The same principle applies to environment, whereof those nearer to the center of origin of biplot are considered stable and contribute little to interaction. But those that are far, are considered to have high GEI and contributes more to interaction yet showed specific adaptation to environment near them. Other genotypes showed negative and/or positive interaction to environments. Samonte et al. (2005) described that genotype with PC1 scores greater than 0, responded positively (i.e. adapted) to environments with PCA1 scores of greater than 0, but responded negatively (i.e. not adapted) to environments that had PCA1 scores of less than 0.

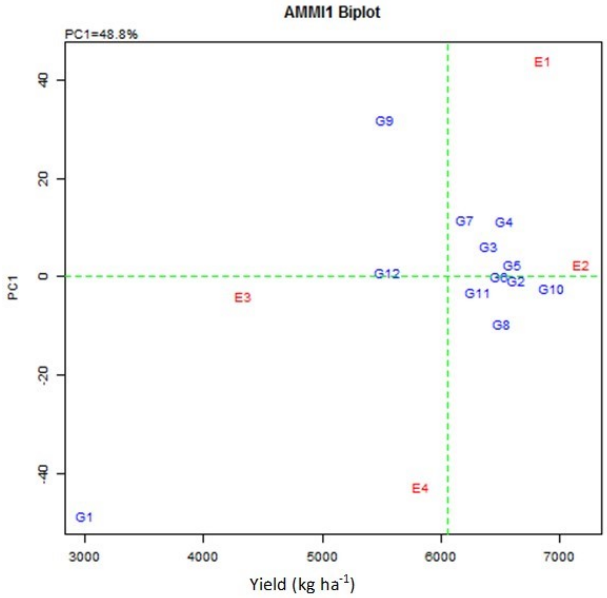

Figure 4. AMMI 1 biplot showing the main and interaction effects (PC1) of both genotypes and environments on grain yield with a model fit of 89.1% accounted from 78.6% of the total variation in main effects and 10.5% from PCA1 variation in GEI sum of squares.

Legend:

G1 - N22	G5 - NSIC Rc 218	G9 - NSIC Rc 298	E1 - Iguig
G2 - NSIC Rc 152	G6 - NSIC Rc 222	G10 - PR40330	E2 - Peñablanca
G3 - NSIC Rc 160	G7 - NSIC Rc 238	G11 - PR42026	E3 - Solana
G4 - NSIC Rc 216	G8 - NSIC Rc 240	G12 - PSB Rc10	E4 - Tuguegarao

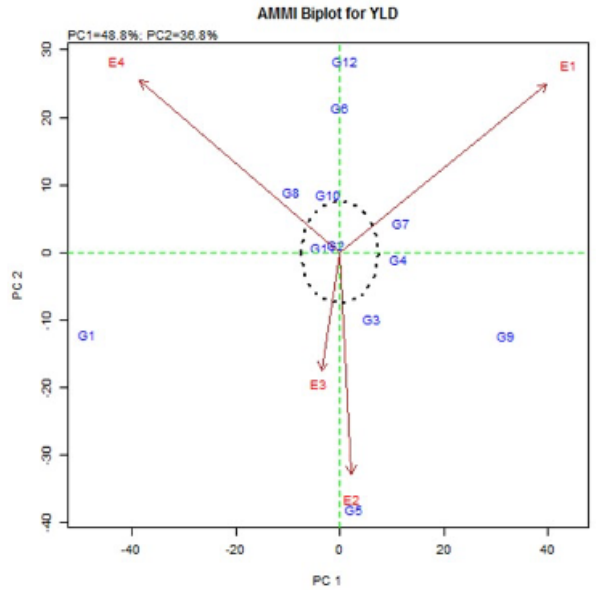

Figure 5. AMMI 2 biplot showing PC1 and PC2 scores of both genotypes and environments. The model accounted for 48.8% PC1 and 36.8% PC2 with a model fit of 85.6%.

Legend:

G1 - N22	G5 - NSIC Rc 218	G9 - NSIC Rc 298	E1 - Iguig
G2 - NSIC Rc 152	G6 - NSIC Rc 222	G10 - PR40330	E2 - Peñablanca
G3 - NSIC Rc 160	G7 - NSIC Rc 238	G11 - PR42026	E3 - Solana
G4 - NSIC Rc 216	G8 - NSIC Rc 240	G12 - PSB Rc10	E4 - Tuguegarao

The most adapted and stable genotypes were NSIC Rc 152 (G2) and PR42026 (G11) followed by NSIC Rc 240 (G8) and PR40330 (G10). Solana (E3) and Peñablanca (E2) were the most stable environments and less discriminating to genotypes. Iguig (E1) and Tuguegarao (E4) were highly interactive and discriminated genotypes effectively. PSB Rc 10 (G12) and NSIC Rc 222 (G6) performed better in Iguig (E1) and Tuguegarao (E4). NSIC Rc 160 (G3), Rc 216 (G4), Rc 238 (G7) and Rc 298 (G9) had high yields in Peñablanca (E2) and Iguig (E1). NSIC Rc 218 (G5) was specific to Peñablanca (E2) while N22 (G1) was negatively adapted to Iguig (E1).

AMMI stability value (ASV) and yield stability index (YSI)

AMMI stability value (ASV) for grain yield was calculated for the 12 genotypes (Table 6). Ranking based on yield, ASV and yield stability index (YSI) was included. Based on mean yield across environment, the highest yielding genotype was PR40330 (G10), with 6,934.17 kg/ha yield whilst N22 (G1) had the lowest yield at 2,996.92 kg/ha.

Genotypes with lower ASV were considered more stable genotypes hence, NSIC Rc 152 (G2) was the highest followed by PR42026 (G10), and the lowest was N22 (G1). Result of ASV validated the result and interpretation of AMMI biplot, confirming that the most stable and adapted genotypes were NSIC Rc 152 (G2) and PR42026 (G11).

The stability per se, should not be the only basis for choosing the best genotype since the most stable genotypes would not necessarily give the best yield performance (Mohammadi et al. 2007). Yield stability index (YSI) incorporated both mean grain yield, and based on this criteria, NSIC Rc 152 (G2) was the most desirable genotype followed by PR40330 (G10), and the lowest was N22 (G1) (Table 6). These results compliment the AMMI1 biplot interpretation (Fig. 4).

Recommended environment for screening high temperature tolerance

To further characterize the discriminating ability and relationship of environments, GGE biplots were used.

According to Yan and Tinker (2006), the most attractive feature of GGE biplot is its ability to show the which-won-where pattern of a genotype by environment dataset (Fig. 6). The interpretation of

Table 6. Ranking of genotypes based on mean yield, ASV and YSI.
PC1 – principal component 1, PC2 – principal component 2, ASV – AMMI stability value, YSI – yield stability index.

Genotypes	Code	Mean Yield	Mean Yield Rank	IPCA1	IPCA2	ASV	ASV Rank	YSI	YSI Rank
NSIC Rc 152	G2	6636.92	2	-0.83	1.14	1.48	1	3	**1**
PR40330	G10	6934.17	1	-2.33	8.55	8.96	3	4	2
NSIC Rc 216	G4	6536.50	4	11.28	-1.05	13.04	5	9	3
PR42026	G11	6316.33	8	-3.23	0.65	3.77	2	10	4
NSIC Rc 160	G3	6402.00	7	6.14	-9.77	12.06	4	11	5
NSIC Rc 240	G8	6508.08	5	-9.41	8.95	14.06	6	11	5
NSIC Rc 218	G5	6600.67	3	2.57	-38.07	38.19	10	13	7
NSIC Rc 222	G6	6486.75	6	0.01	21.38	21.38	8	14	8
NSIC Rc 238	G7	6196.17	9	11.71	4.34	14.17	7	16	9
PSB Rc 10	G12	5542.00	10	0.84	28.44	28.45	9	19	10
NSIC Rc 298	G9	5528.92	11	31.82	-12.33	38.68	11	22	11
N22	G1	2996.92	12	-48.58	-12.23	57.28	12	24	12

Fig. 6 was based on the inner product property of the biplot and was not altered by different single value partitioning methods. However, environment focused partitioning (SVP=2) was preferred because it accurately showed the relationships among environments. The GGE biplot graphically addressed important concepts such as cross-over GE, mega-environment differentiation and specific adaptation. Environments were grouped and each was referred as "mega-environment" which was defined as a subset of environments having the same, or at least similar winning genotypes (Gauch 2013). Different genotypes were selected for each mega-environment. In Fig. 6, environments were grouped into three with Peñablanca (E2) and Solana (E3) forming one mega-environment, both had PC1 scores closer to zero and negative PC2 scores. Although they did not have similarity in terms of temperature variables, both sites experienced extreme levels of stress. Peñablanca had the highest air temperature, while Solana had the highest canopy and panicle temperatures. Despite the stress, NSIC Rc 218 (G5) was their common winning genotype, and N22 (G1) was their lowest yielding genotype. Iguig (E1) and Tuguegarao (E4) formed different groups and were considered as unique environments. PR40330 (G10) was specific for Iguig (E1), and NSIC Rc 222 (G6) in Tuguegarao (E4).

Figure 7 shows the summary of interrelationships among test sites. Environment vectors, which are lines that connect the test environments to the biplot origin, approximates the relation between the test sites (Yan and Tinker 2006), based on the cosine of the angle between the vectors of two sites. Based on the angles, Solana (E3) and Iguig (E1) had positive correlation with Peñablanca (E2), while Solana (E3) and Tuguegarao (E4) with Iguig (E1). A positive correlation indicates non-cross-over GE signifying that genotype perform differently but the ranks remain unchanged (Bondari 2008). Positive correlation indicates non-additive response of genotypes under different environments. The magnitude of inter-genotypic variance increases and the environmental modification of the genotypes are in the same direction. Peñablanca (E2) and Tuguegarao (E4) had formed obtuse angle with each other which indicates negative correlation, signifying presence of GE crossover between these two environments. Under this type of GE, cultivar ranks changed across two environments. Right angle was formed between Solana (E3) and Tuguegarao (E4) indicating that these environments were not correlated. Genotypes in a non-correlating environment consistently performed better than the other genotypes by approximately the same amount across both environments (Baye et al. 2011).

From the same biplot (Fig. 7), discriminating ability of test locations was also determined by measuring the length of environment vectors. Discriminating ability of environment pertains to the capability of environment to differentiate genotypes based on their performance. When different rice genotypes were planted in a discriminating environment, more information can be obtained from their different responses. In addition, stable and location-specific genotypes can be efficiently selected. Iguig (E1) was found to be the most discriminating followed by Peñablanca (E2) and Tuguegarao (E4), while the least discriminating was Solana (E3). Least discriminating environments provided little information on the genotypes, and therefore, should not be used as test environment (Yan and Tinker 2006). Test sites were also ranked relative to ideal test environment, where most representative environments were good test environments for selecting generally adapted genotypes. It represents a good environment for screening genotypes, and in this case, under high temperature condition. Iguig (E1) was the most representative environment as well as most discriminating. Therefore, it was a good test environment for selecting generally adapted genotypes under high temperature condition.

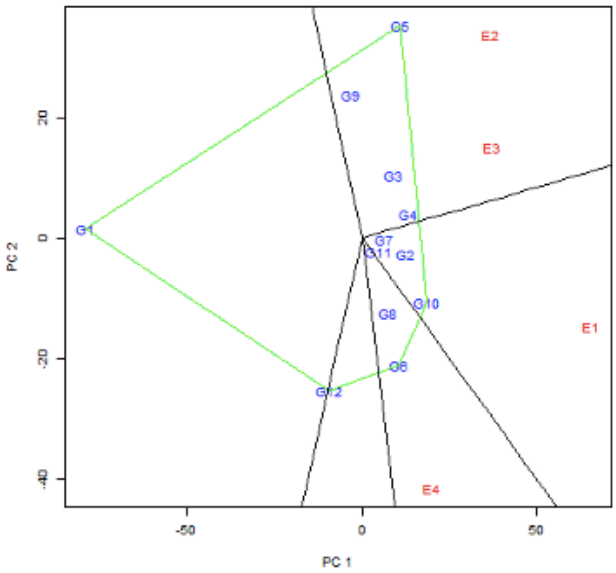

Figure 6. GGE biplot analysis showing mega-environments and their respective high-yielding genotypes.

Legend:

G1 - N22	G5 - NSIC Rc 218	G9 - NSIC Rc 298	E1 - Iguig
G2 - NSIC Rc 152	G6 - NSIC Rc 222	G10 - PR40330	E2 - Peñablanca
G3 - NSIC Rc 160	G7 - NSIC Rc 238	G11 - PR42026	E3 - Solana
G4 - NSIC Rc 216	G8 - NSIC Rc 240	G12 - PSB Rc10	E4 - Tuguegarao

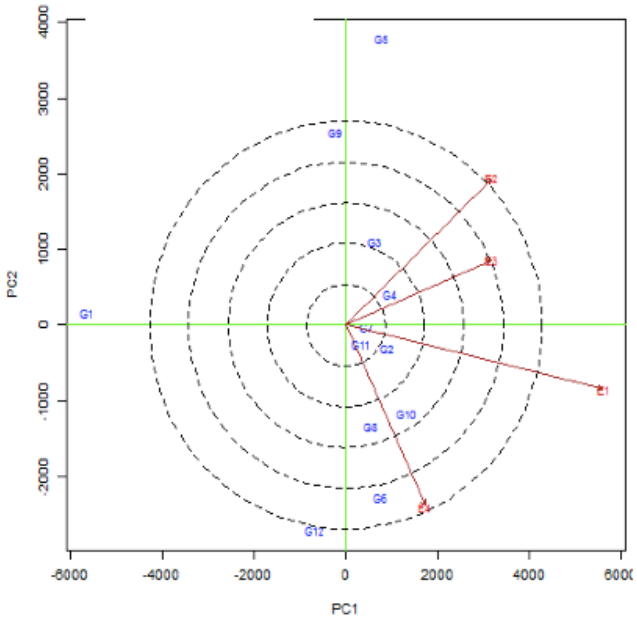

Figure 7. Environment vector view of GGE biplot showing the relationships among the test environments and their ability to discriminate genotypes based on yield performance.

Legend:

G1 - N22	G5 - NSIC Rc 218	G9 - NSIC Rc 298	E1 - Iguig
G2 - NSIC Rc 152	G6 - NSIC Rc 222	G10 - PR40330	E2 - Peñablanca
G3 - NSIC Rc 160	G7 - NSIC Rc 238	G11 - PR42026	E3 - Solana
G4 - NSIC Rc 216	G8 - NSIC Rc 240	G12 - PSB Rc10	E4 - Tuguegarao

CONCLUSION AND RECOMMENDATION

Due to changing climate, global temperature is expected to increase continuously and can affect rice production. The Philippines is a rice-growing region where most of the populations rely on rice for sustenance, justifying the country's goal to be rice-secure by increasing productivity, be safe, and available in the market. But with threat from climate change-induced stresses, possible decrease in rice production is expected. Cagayan Valley is one of the highest temperature stress-prone areas in the Philippines. This study validated that high temperature stress occurred in rice farms. All the test environments had high temperature of ≥35^{0}C with 46.1^{0}C air temperature. Air, canopy and panicle temperature were significantly correlated to yield and spikelet fertility. As a consequence of high panicle and canopy temperature in Solana, yield and spikelet fertility declined by 53.6 and 24.5%, respectively.

Combined analysis of variance revealed that GEI of spikelet fertility was not significant, which indicates low genetic diversity of test genotypes. But in the presence of stress, tolerant genotypes based on spikelet fertility can be selected. Consequently, only yield had significant GEI and was further analyzed using AMMI and GGE. Genotypes, environment and GEI were found significant *(p*≤0.05) where environment contributed the greatest proportion (43.1%) of the total variation, an indication of a diverse environment as evident of different temperature levels. Temperature level in each location had major effect in selecting rice for high grain yield and wide adaptation under high temperature conditions. Genotype contributed 35.5% of the total yield variation which is an indication that genotypes differed in their average performance across environments. GEI significant effect, on the other hand, demonstrated differences in genotypes' response to variation in a particular environmental condition. GEI was high at 21.5% but G was higher at 35.5% demonstrating that genotypes were adapted but the degree of their adaptation and interaction to environments was significantly different.

AMMI biplot, ASV, and YSI showed NSIC Rc 152, PR42026 and PR40330 as the most adapted, stable, and high-yielding genotypes while PSB Rc 10 and NSIC Rc 222 performed better in Iguig and Tuguegarao. NSIC Rc 160, Rc 216, Rc 238, and Rc 298 had high yields in Peñablanca and Iguig. NSIC Rc 218 was specific to Peñablanca. GGE biplot showed Iguig as the least predictable, most discriminating, and best representative environment. It means that Iguig was the best test location for screening and selecting adapted genotypes under high temperature condition.

NSIC Rc 152, NSIC Rc 218, NSIC Rc 222, PR40330, and PR42026 can be recommended for planting in other high-temperature prone areas. Consequently, PR40330 passed the National Cooperative Test (NCT) for high temperature in 2020 and registered as NSIC 2020 Rc 600. PR40330, PR42026, and NSIC Rc 152 due to their high yield, stability, and acceptable phenotype, can be used as parents for development of new heat-tolerant lines. Using the NCT protocol, a greater number of genotypes must be studied and evaluated under high temperature environments in the country to recognize their adaptation and tolerance.

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