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Research Article

Analyzing genetic variations for head rice recovery under heat stress in rice (*Oryza sativa* L.)

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Abstract

High temperature induces yield losses in rice, by affecting pollination, fertilization and also by affecting grain quality. High temperature stress coinciding with the grain filling period affects starch granule compaction thereby leading to reduced head rice recovery (HRR). The broken grains significantly reduce the price of rice by 50% in the market. Hence, the development of rice varieties that exhibit a lower reduction in grain quality coupled with higher head rice recovery under high temperature stress has become a major mandate in rice breeding programs. The present study was undertaken to survey the genetic variation for head rice recovery in rice under both normal (wet season) and high temperature (dry season) conditions. Evaluation of 50 diverse rice germplasm lines across different temperature regimes during grain filling viz. mean temperature of 30.8°C (wet season; May to Sep-Oct) and 35.8°C (Dry season 2017; January to May) and 36.2°C (Dry season 2018; Nov-Dec to May) identified huge genetic variation for HRR in rice. During the wet season, HRR ranged between 20.6 per cent and 90.9 per cent. During summer, rice genotypes exhibited a significant reduction in HRR from 3.6 to 82.7 per cent. Stability analysis revealed that the rice genotypes viz. CO 39, ChiemChanh, CO 18, Guan-Yin-Tsan, IR36, Teqing, ARC 10818 and Cimarron exhibited stable head rice recovery across all seasons.

Keywords: Rice, heat stress, stability, head rice recovery

INTRODUCTION

Rice (*Oryza sativa* L.) serves as a staple food for more than 50 per cent of the Asian population (Bishwajit *et al.*, 2013). Rice is also a major source of income for many small and marginal farmers in Asia and Africa. Even though rice production has increased by several folds after the Green Revolution (1960s) and the introduction of hybrids (1980s), it has to be doubled by 2050 to meet the requirements of the growing population. But, yield plateau, diminishing natural resources, changing climate and increasing pests

and diseases are posing serious threats to increasing rice productivity and thereby reaching the goal of doubling rice production by 2050 (Ray *et al.*, 2013)

Rice is particularly vulnerable to heat stress (>35 °C), especially during the gametogenesis (Jagadish *et al.*, 2013) and flowering (Prasad *et al.*, 2006; Jagadish *et al.*, 2015) stages. Climate change is likely to have a negative impact on the world's rice output in the coming years. Global climatic

estimates show an increased frequency of heat spikes and warmer nights (IPCC, 2013), posing additional threats. According to climate simulations, by 2030, 16% of rice-growing land will be exposed to temperatures at least 5°C above the crucial threshold during the reproductive phase (Gourdji *et al.*, 2013).

Rice is sensitive to high temperature stress at almost all the stages of its growth and development. Heat stress will have negative impact on spikelet fertility and also grain quality if it coincides with flowering and grain-filling stages. High temperature (HT) stress at the ripening phase affects the grain quality and head rice recovery. Head rice recovery (HRR) is defined as the proportion of paddy rice that retains 75% of its length after milling. For a new rice variety to be accepted and adopted by farmers, it should satisfy the consumer requirements of minimum 55 per cent HRR or above. Hence, HRR is a crucial attribute by which new varieties are selected for release (Lapis *et al.*, 2019). Previously, Dalvi *et al.* (2007), Panwar *et al.* (2008), Waghmode and Mehta (2011), Padmavathi *et al.* (2013), Radhamani *et al.* (2017), Parimala *et al.* (2019) and Chandrashekhar *et al.* (2020) reported the existence of G x E interaction for quality traits in rice and yield related traits. Development of rice varieties exhibiting a lesser reduction in HRR during HT stress necessitates measuring the genetic variation of HRR in rice under both normal and HT conditions.

In the present study, HRR was estimated in a set of 50 diverse rice genotypes grown under contrasting temperature regimes during the grain filling stage. Genetic parameters namely, heritability, genetic advance and stability were estimated to identify rice genotypes exhibiting stable HRR across varying temperatures.

MATERIALS AND METHODS

The field trials were conducted at Paddy Breeding Station, Tamil Nadu Agricultural University, Coimbatore, India (11°N, 77°E and 426.7m above MSL) across three different seasons *viz.* *kharif* (WS 2016) and *summer* (DS 2017 and DS 2018) (Table 1) involving 50 diverse rice accessions (Table 2).

Twenty-one day old seedlings were transplanted in a plot size of 2.4 m x 1.4 m at a spacing of 20 x 20 cm with a total of 104 plants per plot with each of the 50

lines replicated thrice. The field management practices were followed as per the TNAU crop production guide (http://agritech.tnau.ac.in/agriculture/agricrop_production_cereals_rice_tranpudlow.html). The seed materials were harvested 30 days after flowering and the grains were dried to 13 - 14% moisture content before measuring the head rice recovery.

The head rice recovery analysis was adapted from Singh *et al.* (2000). About 100 to 150 g of rough rice was taken and milled rice was obtained by dehulling or dehusking with a rice sheller (Rice Polishing Machine LTJM-2099, Garg Instrumentation, Haryana). Milled rice kernels were separated into head rice and broken kernel fractions with different sized separator/ sieves. Full kernel and ¾ size kernels were considered as head rice and weighed for calculating HRR percentage. Head rice recovery percentage was calculated as (DRR, 2014).

$$\text{Head rice recovery (\%)} = \frac{\text{Weight of full kernel}}{\text{Weight of rough rice}} \times 100$$

To assess the genetic diversity of HRR, descriptive statistics and frequency distribution were calculated using TNAU-STAT software (Manivannan, 2014). Stability analysis (AMMI model) was performed using the HRR data from all three seasons to identify stable genotypes.

RESULTS AND DISCUSSION

During the wet season of 2016, the maximum day temperature during the grain filling period ranged between 27.5°C and 31.9°C with a mean of 30.58°C. HRR ranged between 20.6 and 90.9 per cent with an average of 62.8 per cent (Table 3). In contrary, the maximum day temperature during the dry season was ranged between 31.7 and 35.8°C with an average of 34.97°C (Summer 2017) and 31.6 to 36.3°C with an average of 34.7°C (Summer 2018). This increased maximum day temperature had a significant effect on the HRR. During Summer, 2017, head rice recovery ranged from 4.3 to 88.1 per cent with an average of 37.4 per cent and during Summer, 2018, the HRR ranged from 2.8 to 88.4 per cent with an average of 33.56 per cent. From these results, it is clearly evident that a rise in temperature of >4°C during summer seasons over the wet season drastically reduced the average HRR.

Table 1. Details of season, harvesting time and mean temperature during grain filling stage

Season	Growing season	Mean temperature during grain filling stage (°C)	Temperature range (°C)
Wet season (WS 2016)	May - October 2016	30.58	27.5 – 31.9
Dry season 2017 (DS 2017)	January - May 2017	34.97	31.7 -35.8
Dry season 2018 (DS 2018)	November - May 2018	34.70	31.6 – 36.3

Table 2. Head rice recovery (%) and Interaction principal component analysis (IPCA) parameters of AMMI model for HRR across different seasons

G. No.	Genotype	Country of origin	WS 2016	DS 2017	DS 2018	AMMI results		
			HRR (%)			HRR	IPCA1	IPCA2
G1	IR64-21	Philippines	58.7	46.5	44.2	49.8*	-1.2	0.1
G2	Minghui 63	China	80.9	10.5	7.6	33	3.7	0.3
G3	Shan-Huang-Zhan-2	China	83.3	11.4	15.9	36.8	3.5	1.0
G4	CHITRAJ (DA 23)::IRGC 6208-1	Bangladesh	48.8	10.4	30.0	29.7	0.0	2.2
G5	CO39::IRGC 51231-1	India	78.5	46.8	31.8	52.3*	1.0	-1.0
G6	DE ABRIL::IRGC 50463-1	Brazil	55.5	22.5	20.3	32.8	0.6	0.2
G7	FANDRAPOTS::IRGC 10984-1	Madagascar	38.8	32.2	30.9	34.0	-1.7	0.1
G8	GIE57::IRGC 8231-1	Vietnam	30.3	27.4	26	27.9	-2.0	0.1
G9	JC92::IRGC 9176-1	India	72.4	32.6	35.4	46.8	0.9	0.7
G10	LAL AMAN::IRGC 46202-1	India	54.1	21.5	20.4	32	0.5	0.3
G11	MADAEL::IRGC 7722-1	Sri Lanka	55.7	4.9	3.6	21.4	2.0	0.4
G12	MAKALIOKA 34::IRGC 6087-1	Madagascar	54.4	34.4	35.8	41.5	-0.7	0.5
G13	MILYANG 23::IRGC 34393-1	South Korea	35.0	17.7	17.1	23.2	-0.8	0.2
G14	MTU9::IRGC 7919-1	India	59.2	40.3	38.0	45.8	-0.6	0.1
G15	PATIK::IRGC 43530-1	Indonesia	71.7	58.4	22.6	50.9*	0.4	-3.0
G16	PIN KAE::IRGC 5803-1	Thailand	80.1	72.7	68.8	73.9*	-1.5	-0.1
G17	RTS4::IRGC 8177-1	Vietnam	33.5	26.6	25.3	28.5	-1.7	0.1
G18	VANDANA::IRGC 117398-1	India	50.5	41.0	36.5	42.6	-1.3	-0.1
G19	RTS14	Vietnam	55.2	36.8	20.7	37.6	0.0	-1.2
G20	AI-CHIAO-HONG	China	55.5	17.3	16.4	29.8	0.9	0.3
G21	BINULAWAN	Philippines	84.1	82.7	77.9	81.6*	-2	-0.2
G22	CHANG CH'SANG HSU TAO	China	54.3	25.0	20.6	33.3	0.4	0.0
G23	CHIEM CHANH	Vietnam	70.6	49.6	47.0	55.7*	-0.4	0.1
G24	CO18	India	78.5	42.8	40.8	54.1*	0.8	0.2
G25	GUAN-YIN-TSAN	China	85.4	64.6	51.6	67.2*	0.0	-0.9
G26	IR36	Philippines	82	49.4	44.7	58.7*	0.6	0.0
G27	KUN-MIN-TSIEH-HUNAN	China	54.1	19.3	46.1	39.8	-0.6	2.8
G28	ORYZICA LLANOS 5	Colombia	77.3	22.2	18.7	39.4	2.5	0.2
G29	PAO TON HUNG	China	53.1	23.1	19.6	31.9	0.4	0.1
G30	PAPPAKU	China	72.1	69.4	60.1	67.2*	-1.7	-0.6
G31	PEH-KUH-TSAO-TU	Taiwan	80.3	76.7	73.1	76.7*	-1.8	-0.1
G32	TEQING	China	67.2	41.9	43.6	50.9*	-0.3	0.5
G33	TKM6	India	87.7	24.9	23.6	45.4	3.0	0.4
G34	17/79/02-005::IRGC 51080-1	Sri Lanka	71.5	59.3	13.3	48.1	0.8	-3.9
G35	849::IRGC 5970-1	Madagascar	20.8	14.2	11.1	15.4	-1.6	0.0
G36	AGAMI M 1::IRGC 4158-1	Egypt	84.2	73.2	38.1	65.1*	0.2	-2.9
G37	AI LAN KE 1110::IRGC 67034-1	China	56.2	39.6	37.6	44.5	-0.8	0.1
G38	ARC 10818::IRGC 21079-1	India	73.8	53.3	50.4	59.2*	-0.5	0.1
G39	BADA DHAN::IRGC 26540-1	Bangladesh	48.8	22.2	40.4	37.2	-0.9	2.0
G40	BALGALA GURMATIA::IRGC 61074-1	India	80.4	35.7	34.3	50.1*	1.5	0.3
G41	BANDIOUROU::IRGC 15980-1	Senegal	57.8	38.6	36.4	44.3	-0.6	0.1
G42	BIRAIN 360::IRGC 6550-1	Bangladesh	70.1	59.9	56.8	62.3*	-1.3	0.0
G43	BYAT KYAR::IRGC 33004-1	Burma	61.6	24.9	23.5	36.7	0.8	0.3
G44	CHI TON HUANG 1::IRGC 51280-1	China	53.8	39.2	37.3	43.4	-1.0	0.1
G45	CHINA 98-45-1::IRGC 1598-1	China	71.3	62.4	59.4	64.4*	-1.4	0.0
G46	CIMARRON::IRGC 116967-1	Venezuela	67.2	42.4	38.0	49.2*	0.0	-0.1
G47	DA5::IRGC 5855-1	Bangladesh	70.3	26.8	25.6	40.9	1.4	0.3
G48	DA NUO (ZHAN)::IRGC 72024-1	China	31.4	11.4	10.8	17.8	-0.6	0.3
G49	DENG DENG QI::IRGC 72671-1	China	53.7	26.2	14.8	31.6	0.5	-0.7
G50	E 5168::IRGC 68021-1	China	69.5	36.9	34.9	47.1	0.5	0.2
		E1 (Wet season 2016)				62.8*	7.9	0.3
		E2 (Dry season 2017)				37.4	-3.6	-5.5
		E3 (Dry season 2018)				33.6	-4.3	5.2
		Grand mean				44.6		
		SE				1.4	CD (5%)	3.8

*Significant at 5% level

Bhaskaran and Sebastian (2017) had earlier reported that a 4.4°C increase in day temperature resulted in a 24.9 per cent reduction in HRR. Similarly, it was reported that high air temperature during grain filling significantly reduced the HRR (Truong *et al.*, 2012; Liu *et al.*, 2013; Abayawickrama *et al.*, 2017). Frequency distribution analysis clearly indicated a greater variability for HRR across three seasons (**Fig. 1**). During WS 2016, 10 genotypes recorded more than 80 per cent head rice recovery with a maximum HRR of 90.9 per cent. However, during DS 2017, only one genotype registered a HRR of more than 80 per cent and during DS 2018, none of the entries showed head rice recovery of more than 80 per cent. This indicates that high temperature during the grain filling stage had a significant negative effect on the percentage of head rice recovered.

Genetic variability parameters such as genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability (h^2) and genetic advance as percentage of the mean (GAM) were estimated for all the 50 rice accessions. High PCV and GCV values were observed during all three seasons (**Table 3**). PCV was higher than their corresponding GCV, which signifies the influence of environmental interaction. Higher GCV and PCV values indicated that the traits

are genetically controlled and amenable for selection. This also indicated that head rice recovery percentage could be improved through hybridization and selection (Bisne *et al.*, 2009). The results showed a higher heritability and high GAM for head rice recovery during all three seasons. Previous studies also indicated that HRR exhibited a higher heritability with high GAM (Singh *et al.*, 2021). Similarly, Devi *et al.* (2016), Nirmaladevi *et al.* (2015) and Subudhi *et al.* (2011) also observed high heritability for HRR.

Genotype x Environment (G x E) interaction is a major problem in the study of quantitative traits. Hence, the identification of stable genotypes over a wide range of environments is an important but challenging task for breeders. The AMMI analysis of variance revealed highly significant variance due to genotypes and environments for HRR percentage. Variance due to genotype x environment interactions was significant for HRR percentage (**Table 4**). The G x E interaction was again partitioned into two, IPCA 1 and IPCA 2 axes without any residual value. Both the IPCA scores representing the interaction pattern were significant for HRR percentage. The significance of two IPCA scores suggested the presence of a complex, multidimensional variation in genotypes by environment data.

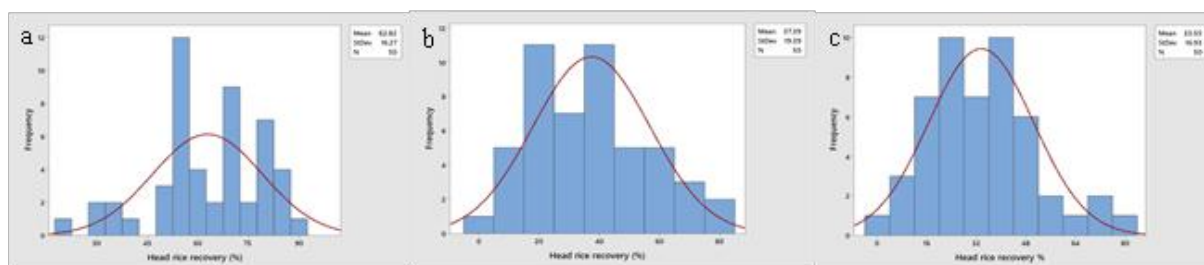


Fig. 1. Frequency distribution of head rice recovery (%) during three different seasons
a - Head rice recovery during the wet season 2016; b - Head rice recovery during the dry season 2017; c - Head rice recovery during the dry season 2018.

Table 3. Descriptive statistics for head rice recovery percentage across different seasons

Parameters	WS 2016		DS 2017		DS 2018	
	Temp (°C)	HRR (%)	Temp (°C)	HRR (%)	Temp (°C)	HRR (%)
Grand Mean	30.58	62.8	34.97	37.4	34.7	33.56
S.E.	0.15	3.2	0.13	3.1	0.19	3.02
Range	27.5 - 31.9	20.6 - 90.9	31.7 - 35.8	4.3 - 88.1	31.6 - 36.3	2.8 - 88.4
CD(5%)	-	8.9	-	8.7	-	8.46
CV(%)	-	8.8	-	14.4	-	15.60
PCV(%)	-	26.9	-	53.1	-	52.01
GCV(%)	-	25.4	-	51.1	-	49.62
h^2 (%)	-	89.4	-	92.6	-	91.01
GAM(%)	-	49.4	-	101.4	-	97.51

Table 4. Stability ANOVA from AMMI model for HRR percentage in rice germplasm lines across different seasons

Source	df	MSS	%TSS
Genotype	49	2040.74**	
Environment	2	37965.99**	
G x E	98	369.51**	
IPCA1	50	353.35**	73.2
IPCA2	48	134.43**	26.8

** Significant at 1% level of probability

Among the two AMMI components, the first IPCA (73.2%) explained most of the proportion of genotype \times environment interaction than the second IPCA (26.8%). AMMI with two interaction principal component axes together explained 100 per cent of G \times E interaction.

The AMMI 1 biplot for HRR clearly indicated that the three environments differed in both main and interaction effects. A total of eight genotypes viz. CO39 (G5), ChiemChanh (G23), CO18 (G24), Guan-Yin-Tsan (G25), IR36 (G26), Teqing (G32), ARC 10818 (G38) and Cimarron (G46) showed IPCA 1 score close to zero with high main effects (**Table 2**). This indicated that the above mentioned lines were stable and had general adaptability overall seasons for HRR.

Among the three seasons studied, environment 1 (WS 2016) with temperatures ranged from 27.5 - 31.9°C had increased head rice recovery (62.84%) as compared to the other two seasons (DS 2017, DS 2018) with temperatures ranged from 31.7 - 35.8°C (37.4% HRR) and 31.6 - 36.3°C (33.5% HRR), respectively (**Table 2**). This indicates high temperature stress plays a major role in grain quality, especially head rice recovery. Among the 50 lines studied, seven lines viz. CO39, (G5) Patik (G15), CO 18 (G24), Guan-Yin-Tsan (G25), IR 36 (G26), Agami M 1 (G36) and Cimarron (G46) exhibited high main effect with positive IPCA 1 score near to origin. Since the environment E1 had a positive IPCA 1 score, it had a positive interaction with these genotypes and environment E1 can be considered as the favorable environment for the selected genotypes (**Fig. 2**). On the other hand, three lines, Chiem Chanh (G23), Teqing (G32) and ARC 10818 (G38) had a high main effect with negative IPCA 1 score and significant mean performance for this trait. The environments E2 and E3 had negative IPCA 1 scores and therefore E2 and E3 can be considered as favorable environments for these three genotypes.

The IPCA 1 component accounted for 73.2 per cent of G \times E interaction, while IPCA 2 accounted for 26.8 per cent in AMMI 2 biplot indicated that this model fit 100 per cent. A total of 31 genotypes viz., Chitraj (G4) (DA 23), CO 39 (G5), De abril (G6), JC92 (G9), LalAman

(G10), Makalioka 34 (G12), Milyang 23 (G13), MTU9 (G14), Patik (G15), RTS14 (G17), Ai-Chiao-Hong (G20), ChangCh'sangHsu Tao (G22), ChiemChanh (G23), CO18 (G24), Guan-Yin-Tsan (G25), IR36 (G26), Kun-Min-Tsieh-Hunan (G27), PaoTou Hung (G29), Teqing (G32), 17/79/02-005 (G34), Agami M 1 (G36), AiLanKe1110 (G37), ARC 10818 (G38), BadaDhan (G39), Bandiourou (G41), ByatKyar (G43), ChiTou Huang 1 (G44), Cimarron (G46), DaNuo (ZHAN) (G48), DengDeng QI (G49) and E 5168 (G50) were positioned close to the origin for IPCA 1 and IPCA 2 scores (**Fig 3**). This showed minimal interaction of these genotypes with environments. The remaining lines were scattered away from the origin in the biplot revealing that the genotypes were more sensitive to environmental interactive forces. Among the three environments, E1 (wet season, 2016) is the less interacting environment for head rice recovery and would be adjudged the best season for improved HRR as compared to the other two dry seasons of 2017 and 2018. Among these 31 lines, ten lines viz. CO 39 (G5), Patik (G15), ChiemChanh (G23), CO18 (G24), Guan-Yin-Tsan (G25), IR 36 (G26), Teqing (G32), Agami M 1 (G36), ARC 10818 (G38) and Cimarron (G46) exhibited high significant mean values for HRR percentage.

The "which-won-where" biplot was been constructed to identify the best performing genotypes for HRR for each season. The polygon view of the GGE biplot is used for visualization of the best performing genotypes for a specific environments (Das *et al.*, 2018). Polygon is constructed by joining the genotypes far away from the biplot origin to contain all the genotypes inside the polygon. Genotypes positioned in the vertices of the polygon are the best performer or poor performer in one or more environments (Yan and Tinker, 2006). From the results (**Fig. 4**), it is evident that the rice genotypes viz. CO 39 (G5), Patik (G15), ChiemChanh (G23), CO 18 (G24), Guan-Yin-Tsan (G25), IR36 (G26), Teqing (G32), Agami M 1 (G36), ARC 10818 (G38) and Cimarron (G46) are better performing genotypes in terms of HRR for the environment E1 (wet season 2016). Furthermore, the genotypes viz. IR 64-21 (G1), PinKaeo (G16), Binulawan (G21), Pappaku (G30), Peh-Kuh-Tsao-Tu (G31), Birain 360 (G42) and China 98-45-1 (G45) are good performers for HRR in environments E2 (dry season 2017) and E3 (dry season 2018).

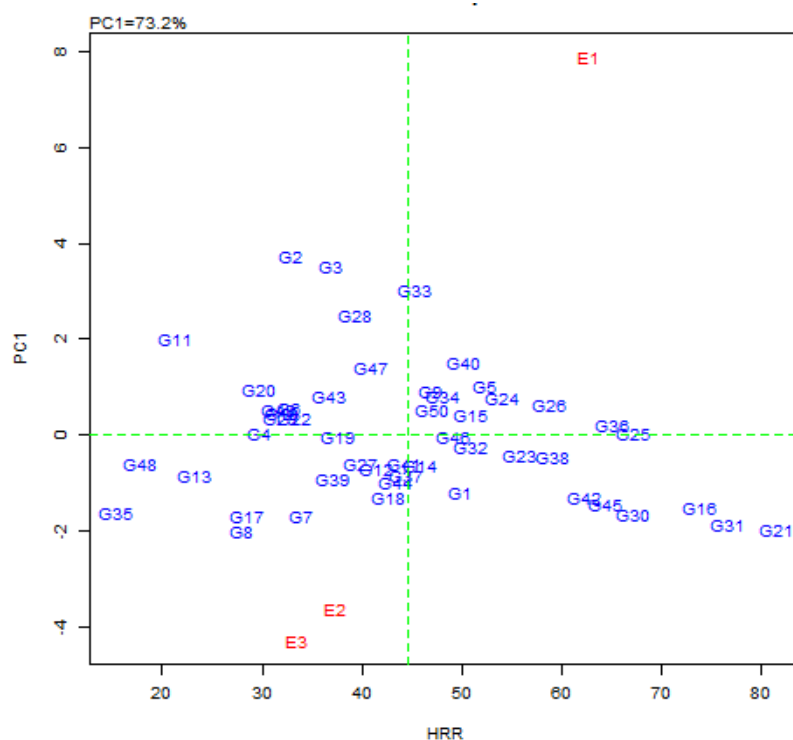


Fig. 2. AMMI 1 model for HRR showing mean of genotypes and seasons against their IPCA1 scores

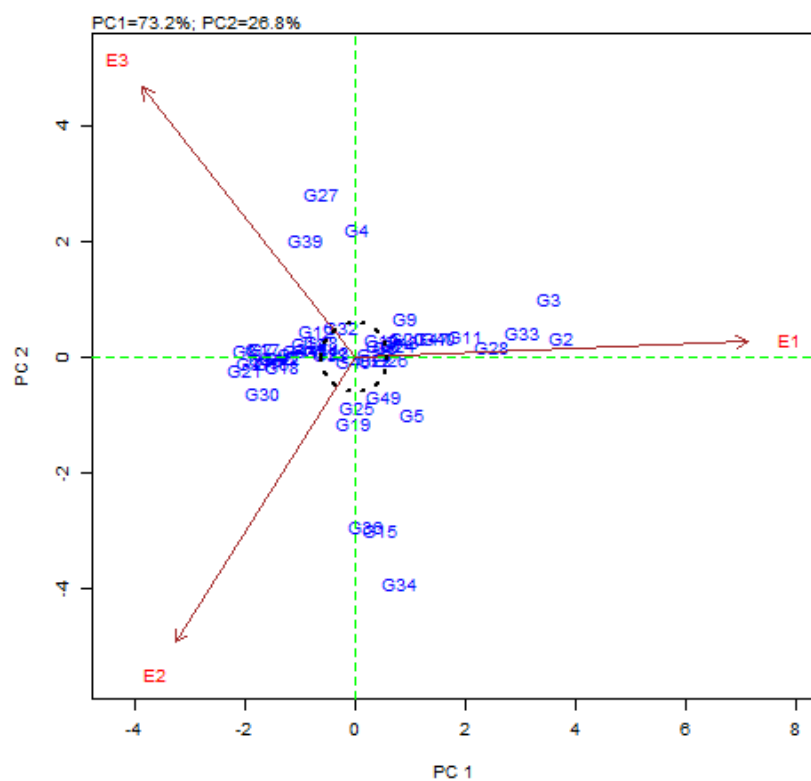


Fig. 3. AMMI 2 model for head rice recovery showing IPCA1 vs IPCA2 scores of rice genotypes across seasons



The interrelationship between the environments is depicted in **Fig. 5**. The cosine of the angle between two environmental vectors in a GGE biplot reflects the correlation between them (Yan and Tinker, 2006). The acute angle between two environmental vectors indicates a positive correlation between the concerned test environments while the obtuse angle indicates a negative correlation between them. In the environment-vector view of the GGE biplot, concentric circles help in the visualization of the magnitude of length of the environment vectors, which is proportional to the standard deviation within the respective environment (Das *et al.*, 2018).

In GGE biplot analysis, E2 (DS 2017) exhibited a positive correlation with E3 (DS 2018) as the E2 environment vector made an acute angle with the E3 environment vector while E2 and E3 environment vectors formed approximately a right angle with E1 (WS 2016) indicating independent nature of E1 (wet season) and E2, E3 (dry seasons) environments. This is consistent with the high degree of variability in the observed head rice recovery of genotypes which might be attributed to contrasting agroclimatic conditions of these seasons and the presence of a high degree of cross-over interactions due to higher G x E interactions.

By considering AMMI 1, AMMI 2, and GGE biplot results, eight genotypes viz. CO 39 (G4), ChiemChanh (G23), CO 18 (G24), Guan-Yin-Tsan (G25), IR36 (G26), Teqing (G32), ARC 10818 (G38) and Cimarron (G46) were found to exhibit stable HRR and general adaptability overall environments. These genotypes with superior mean values of HRR and less interaction with the environment identified by the AMMI model could be used in breeding programs to develop high temperature tolerant rice varieties with improved HRR.

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