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Stability analysis of backcross population for salinity tolerance at reproductive stage in rice

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Abstract

Significant yield reduction was recorded at flowering stage in rice varieties under salinity stress. But due to high genotype x environment interaction (GEI) the adaptability of the salt tolerant elite lines is generally poor. The identified source of tolerance, AC41585, was used in developing a backcross population. One hundred eighty BC_3F_4 lines were evaluated under stress (EC 8dSm^{-1}) and non-stress conditions in net-house during the years 2012 and 2013. Under salinity stress plant yield was observed to be associated positively with the number of panicles/plant, panicle length, harvest index and negatively with the percentage of spikelet sterility and degeneration. Genotypes such as, L-41, L-45, L-112, L-171, L-192 with low IPCA and higher mean were identified with general adaptability through AMMI analysis. In addition, 'which-won-where' pattern of GGE Biplot detected L-192 and L-41 as highest performing genotypes in saline and non-saline environments, respectively. Both the analyses identified stable introgression line L-171 with high yield stability index having phenotypic similarity with recurrent parent, IR 64. The elite lines selected through the present study could be used in rice breeding and also to investigate the molecular basis of salt tolerance at reproductive stage.

Key words: AMMI, GGE biplot, mapping population, genotype x environment interaction

Introduction

Both inland and coastal salinity are now becoming wide spread problems for rice cultivation in India and other rice growing countries in the world as well (Hossain et al. 2015). Sensitivity of rice crop to salinity stress varies with their growth stages. Rice is mainly susceptible to salt stress at early vegetative and from the panicle initiation to the grain filling stage (Munns and Tester 2008). A genotype with seedling stage salinity tolerance may not be tolerant at reproductive stage as well (Ahmadizadeh et al. 2016). Differential expression of genotypes to salt stress was found in both seedling and reproductive stages (Lekshmy et al. 2016). From Pokkali, one QTL (quantitative trait loci), named *Saltol* was detected (Bonnila et al. 2002) and incorporated to high yielding backgrounds (Islam et al. 2012) for imposing tolerance at seedling stage. For tolerance at reproductive stage, a few parameters such as panicle number/plant (PN), percent spikelet fertility (SF), etc. have been found responsible bringing about low yield reduction under stress (Zeng and Shannon 2000). To assess genotypic response to salt stress at flowering stage researchers used tolerance and susceptibility indexes, estimated from data of plant yield (PY) and important yield limiting factors under stress (Hosseini et al. 2012). But the main drawback in assessing the tolerance of a genotype was high genotype x environment interaction (GEI) for PY and most of the important yield contributing parameters which reduced the stability index of that genotype (Hossain et al. 2015). General adaptability of genotypes under both saline and non-saline environments is required as salinity stress at flowering stage is found occasional especially in coastal saline areas. Several developments have been observed in the front of stability assessment in the varietal performance in diverse environment and identifying lines with low GEI. The regression approach (Eberhart and Russell 1966), additive main effects and multiplicative interaction (AMMI) analysis (Gauch 1992), yield stability statistic approach (Kang 1993) and biplot analysis (Yan et al. 2007) are widely practiced methods in analyzing multi environmental trails. The AMMI model coupled with GGE-biplot analysis have been used in identifying salt tolerant

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rice genotypes with wide adaptability (Islam et al. 2016; Krishnamurthy et al. 2016). We developed a backcross derived mapping population using an accession of Pokkali (AC41585) as donor for salt tolerance at flowering stage (Chattopadhyay et al. 2013) and evaluated under both salinized and non-salinized condition in 2012 and 2013. The present investigation aimed at the identification of salt tolerant introgression lines and assessed their stability for PY and important yield attributing traits in saline and non-saline condition using AMMI and GGE Biplot analysis.

Materials and methods

Plant materials

For the present study an advanced backcross population was taken. This population was developed using Pokkali accession (AC41585) as tolerant to salt stress (EC = 8 dSm^{-1}) at flowering stage as donor to cross with salt susceptible recurrent parent IR 64 for three consecutive generations which was followed by selfing and single seed descent method (SSD) method to generate 200 BC_3F_3 lines.

Experimental details

One hundred eighty two BC_3F_4 lines along with their parents were taken for this study. Seedlings at the age of twenty one days were planted in perforated pots filled up with well ground soil. The N:P:K was applied in pots at the rate of 100:50:50 kg/ha. Perforated pots were kept in plastic water tank. Standard procedure (Gregorio et al. 1997) with requisite modifications was followed to salinize potted plants. We know that NaCl is the main constituents (>80%) of sea water. For salinization, NaCl was dissolved to tank water to make water EC 8 dSm^{-1} and salt-water was allowed to enter inside the perforated pots to saturate soil. One perforated PVC pipe (as piezometer) was kept inside the soil of each pot with its opening just above soil surface. Grouping of genotypes was done based on their maturity duration (5 days interval). Stress was imposed in group wise on plants before booting. Salt stress was imposed on one set of pots and the other set was allowed to grow in normal condition at the net-house till maturity. The regular monitoring of soil electrical conductivity (EC), extrapolated through saturated water inside piezometer, was done by using a hand-held EC meter (Chattopadhyay et al. 2013).

Data analysis

Plant yield (cm) (PY) and yield attributing traits such as plant height (cm) (PH), days to 50% flowering (DAF), panicle number/plant (PN), panicle length (cm) (PL), per cent spikelet sterility (STE), per cent spikelet degeneration (DEG), straw weight (g) (SW) and harvest index (HI) were recorded from each plant from two replications in CRD trial in net house during 2012 and 2013. Vestiges of rudimentary rachis-branches left on the panicle were counted as degenerated spikelets at maturity and expressed in percentage of total spikelets (DEG) (Saha et al. 1998). The recorded data of two years, under saline and non-saline environments were subjected to analysis of variances using SPSS v. 15. Salt tolerance and susceptibility indices for each genotype were calculated using the following formula.

a. Yield stability index (YSI) = Ys/Yp (Bouslama and Schapaugh 1984)

b. Stress susceptibility index (SSI) = (1 – Ysi/Ypi)/SI; SI = 1 – Y s/Y p (Fischer and Maurer 1978)

(here Ysi = PY or yield attributing traits under stress, Ypi = PY or yield attributing traits under non-stress, Ys and Yp are average yield/yield traits under stress and normal condition, respectively)

Correlation analysis was performed with Windostat (Version 8.5, Indostat Services) software using Pearson's correlation coefficient method. Significance levels are indicated as: $^*P < 0.05$, $^{**}P < 0.01$. Data of PY and other traits collected from four environments such as Non-saline in 2012 (E1), saline in 2012 (E2), non-saline in 2013 (E3) and saline in 2013 (E4) were subjected to stability analysis using Additive Main effect and Multiplicative Interaction (AMMI) model (Crossa et al. 1991) to investigate GEI and identify genotypes with general and specific adaptability for saline and non-saline environments. GGE biplot analysis was done further to explain the source of variation of G (genotype) and GE (genotype x environment) (Yan et al. 2007). GGE biplot symmetric view was used in this study to explain the 'which-won-where' patterns for genotypes and environments.

Results and discussion

Correlation analysis

As compared to non-saline condition the major difference in correlation coefficient matrix under saline condition was the presence of the positive contribution of PL (0.384) and significantly higher negative contribution of STE (r = –0.528) along with DEG (r = –

0.112). Synonymous to our observation, it was reported earlier (Zeng et al. 2002) that PL contributed most significantly and positively while STE contributed negatively for PY under salt stress. In agreement with our observation tillers per plant and HI also were found sensitive to salinity stress induced at flowering stage in rice by many researchers (Surekha et al. 2008; Zeng et al. 2004).

Identification of tolerant lines with high mean plant yield and high YSI and low SSI for plant yield

Analysis of variances showed that population was significantly differ for most of the traits under both the conditions. A wide range of variation for tolerance was observed among the lines. Among yield attributing traits, PH, PL and HI were significantly reduced in salinized condition. PY and important yield attributing traits such as PL, SW and HI were revealing normal distribution over the environments. Genotypes were identified as tolerant or moderately tolerant based on higher mean PY than both the parents over the environments, high YSI and low SSI for PY, PL and HI (Table 1). SSI was used previously as important index to isolate salt susceptible genotypes from tolerant genotypes (Hosseini et al. 2012). Our observation revealed that YSI also could be the simplest salt tolerance index in rice which expressed the ability of a tolerant genotype to retain its potential yield and estimates of positive yield contributing traits under salt stress at flowering stage. A few tolerant introgression lines such as L-41, L-171, L-174, L-176, etc. were detected with high yielding ability and phenotypic similarities with IR 64 on the basis of PH, DAF (Table 2) and grain type (data not shown).

Table 1. Correlation analysis of yield and yield attributing traits under saline and non-saline condition

Parameters	Condition	PH	DAF	PN	PL	STE	DEG	SW	HI
PH	Saline	1.000							
	Non-saline	1.000							
DAF	Saline	0.046	1.000						
	Non-saline	–0.014	1.000						
PN	Saline	0.121*	0.040	1.000					
	Non-saline	–0.186**	0.017	1.000					
PL	Saline	0.687**	0.100	0.244**	1.000				
	Non-saline	0.676**	–0.013	–0.258**	1.000				
STE	Saline	–0.037	0.119*	–0.180**	–0.025	1.000			
	Non-saline	–0.032	0.133*	0.130*	0.004	1.000			
DEG	Saline	0.112*	0.019	0.096	0.049	–0.022	1.000		
	Non-saline	-	-	-	-	-	-	-	
STWT	Saline	0.469**	0.270**	0.597**	0.451**	–0.022	0.110*	1.000	
	Non-saline	0.487**	0.252**	0.404**	0.354**	0.068	1.000		
HI	Saline	–0.024	–0.285	0.109*	0.059	–0.395**	–0.032	–0.222**	1.000
	Non-saline	–0.306**	–0.148**	0.161**	–0.264**	–0.274**	–0.487**	1.000	
PY	Saline	**0.267****	**–0.083**	**0.577****	**0.384****	**–0.528****	**–0.112***	**0.394****	**0.555****
	Non-saline	**0.146****	**0.088**	**0.572****	**0.069**	**–0.236****	**-**	**0.435****	**0.518****

At (n-2) df= 362 df, critical value of r at $p<0.05$ (*) = 0.09 and at $p<0.01$ (**) = 0.138

AMMI analysis and identification of tolerant genotypes with high adaptability

In AMMI analysis, GEI effect of each genotype was partitioned into effects due to individual environments and provided a graphical representation (biplot) to summarize information on main effects and interactions of both genotypes and environments simultaneously (Crossa et al. 1991). In our study, since the GEI was found to have significant effects on various important components of PY responsible for salt tolerance, AMMI stability model was applied for further partitioning of various variance components. Figure 1 represents the biplot of AMMI for 182 rice genotypes in four environments. The y-axis represents the IPCA1 scores, while the x-axis represents the seed per plant (mean effect) of the genotypes.

PH, PL, PY and HI were significantly ($p<0.05$) affected by genotypes (G), environments (E) and GEI

Table 2. Genotypes with more than 0.5 YSI for plant yield under salt stress at flowering stage and higher mean yield than IR64 and Pokkali (AC41585) based on pooled data over environment in two years (Year 2012 and 2013)

	PY			PL			HI			PH	DAF
Genotypes	Mean	SSI-PY	YSI-PY	Mean	SSI-PL	YSI-PL	Mean	SSI-HI	YSI-HI		
L-41	16.35	0.59	0.67	24.98	-0.64	1.07	0.41	0.44	0.85	98.75	98.25
L-112	13.08	0.60	0.67	26.98	-1.26	1.13	0.37	0.85	0.71	144.25	98.75
L-174	12.93	0.73	0.59	20.74	0.51	0.95	0.45	0.77	0.74	89.50	98.75
L-192	12.63	0.30	0.83	24.80	0.50	0.95	0.48	0.33	0.89	125.50	95.75
L-171	11.85	0.84	0.53	23.30	0.13	0.99	0.39	0.98	0.67	97.25	99.50
IR64	11.44	1.25	0.30	22.32	0.88	0.91	0.44	1.35	0.54	94.44	97.88
L-176	11.13	0.84	0.53	20.56	2.11	0.78	0.43	0.84	0.71	97.75	96.50
L-115	11.05	0.76	0.57	21.98	2.15	0.77	0.45	0.64	0.78	119.25	86.75
L-117	10.65	0.76	0.57	22.13	0.55	0.94	0.44	0.98	0.66	81.75	105.50
L-81	10.55	0.79	0.56	25.81	-0.37	1.04	0.29	1.58	0.46	136.25	100.50
L-79	10.40	0.80	0.55	25.23	0.56	0.94	0.38	0.43	0.85	119.50	95.75
L-136	10.40	0.89	0.50	28.23	0.59	0.94	0.36	0.87	0.70	130.75	97.00
L-4	10.38	0.12	0.93	23.48	-0.83	1.09	0.33	1.12	0.62	98.50	108.25
L-188	10.30	0.39	0.78	25.48	0.47	0.95	0.36	0.29	0.90	130.50	96.50
L-98	10.30	0.86	0.52	24.92	1.35	0.86	0.36	1.13	0.61	91.75	101.00
L-84	10.23	0.79	0.56	27.39	0.18	0.98	0.35	0.74	0.75	135.00	95.00
L-186	10.18	0.40	0.78	28.33	0.12	0.99	0.31	0.05	0.98	138.75	99.25
L-142	10.10	0.15	0.91	26.58	0.17	0.98	0.38	-1.05	1.36	136.25	93.00
L-20	9.88	0.75	0.58	22.74	0.35	0.96	0.40	0.98	0.67	103.00	96.00
L-31	9.70	0.66	0.63	23.25	1.09	0.89	0.41	0.97	0.67	113.75	86.50
Pokkali	9.70	0.38	0.79	24.96	1.03	0.89	0.44	0.25	0.91	132.88	89.88
Experimental mean	8.65	0.97	0.46	23.68	0.99	0.90	0.34	1.03	0.65	112.14	97.65
SD	2.08	0.31	0.17	2.25	0.87	0.09	0.07	0.70	0.24	21.06	4.77

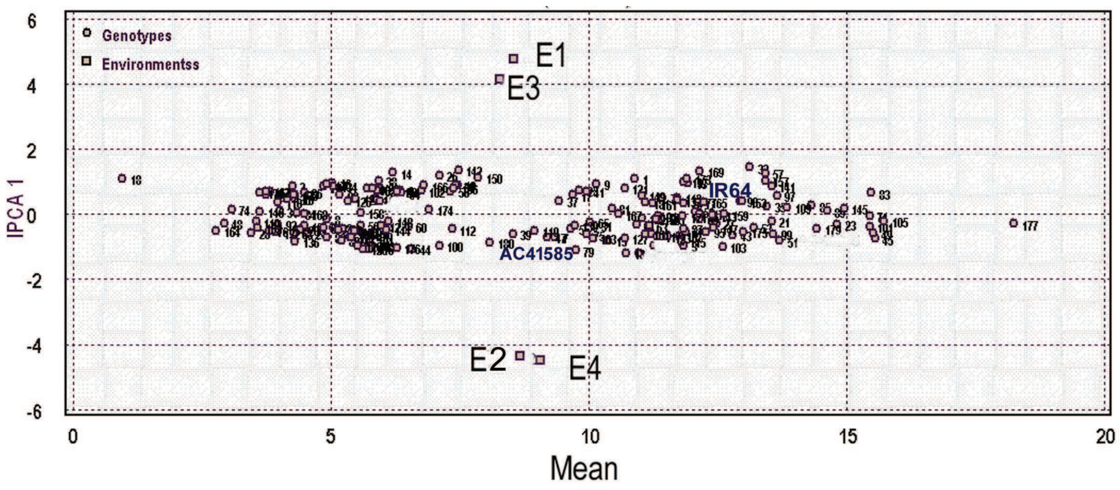

Fig. 1. Biplot derived from AMMI analysis of 182 rice genotypes evaluated in four environments based on PY and yield attributing traits

indicating the presence of genetic variation and possible selection of stable entries (Table 3). GEI was further portioned by principal components (IPCA$_1$, IPCA$_2$, IPCA$_3$). For PH two significant IPCA could able to explain 77% of the total GEI. On the other hand for PY, IPCA$_1$ could alone explain 61% variance of total GEI. Therefore, IPCA$_1$ value might be reliable determinant for stability of yield over environments. Low GEI and IPCA of a genotype indicated stability of the genotype over the range of environments. Genotypes such as L-41, L-45, L-112, L-171, L-192 and Pokkali had low IPCA (near to 0) and same sign with genotypic variance. They had also significantly higher values than the mean (Table 4). Therefore, they were stable under both the environments (saline and non-saline). Stable salt tolerant genotypes also identified by researchers in field experimentation based on least interaction with environments (saline vs. non-saline) (Islam et al. 2016). On the other hand, lines such as L-63 and L-114 had high IPCA (>1) and different sign with genotypic variance showing specific adaptability to favourable (non-stress) environment. Similarly, due to different sign of genotypic variance and IPCA and higher yield than the mean, lines such as L-174 and L-175 and also IR 64 could be specifically adaptable for favourable environment. Genotypes identified based on similar criteria in earlier investigation for the favourable, non-saline environment. As per the AMMI analysis among the 5 most stable genotypes over the environments, four of them L-41, L-192, L-112 and L-171 were found tolerant to salt stress at flowering stage. Among these lines, L-41 and L-171 were identified phenotypically similar with IR 64 based on PH, DAF and grain type.

Table 3. Genotype, environment and GEI effect derived through AMMI analysis of lines belonged to backcross derived mapping population from IR64/Pokkali

Source of variations	MSS		GEI		Contribution to total GEI (%)	
	Genotypes (G)	Environments (E)	IPCA$_1$	IPCA$_2$	IPCA$_1$	IPCA$_2$
PH	1121.4**	3566.21**	957.28**	664.8*	45.87	31.51
DAF	41.69	437.69**	40.39	39.21	-	-
PN	5.49	26.08**	10.11**	5.45*	51.53	27.47
PL	17.71**	94.66**	11.81**	9.44	41.65	32.90
STE	368.67	1823.14**	357.23	351.29	38.97	37.90
DEG	575.68**	38.57	124.97**	111.95*	39.90	37.00
SW	66.71	402.66**	130.14**	50.97	58.89	-
PY	55.12**	23.15*	33.67**	11.77	61.09	-
HI	0.04**	0.06**	0.02**	0.01*	43.30	32.00

*P<0.05, **P<0.01

Table 4. Identification of high yielding stable genotypes using mean, genotypic and interaction PCA effect

Line	General mean yield (g)	Mean yield under saline (g)	Mean yield under non-saline (g)	Genotype variance Index	IPCA 1
L-41	16.35	13.10	19.60	-3.54	-0.08
L-45	12.90	6.15	19.65	-2.96	-0.65
L-63	12.75	7.30	18.20	4.76	1.26
L-78	11.88	6.65	17.10	-3.44	-0.46
L-97	12.23	6.65	17.80	-2.86	0.80
L-112	13.08	10.45	15.70	-2.91	-0.80
L-114	13.13	7.55	18.70	-1.29	0.81
L-162	12.25	5.70	18.80	-0.81	1.14
L-171	11.85	8.20	15.50	3.96	0.00
L-173	11.93	6.90	16.95	2.59	0.33
L-174	12.93	9.60	16.25	-4.91	0.70
L-175	12.10	7.05	17.15	1.41	-0.74
L-192	12.63	11.45	13.80	-0.58	-0.87
Pokkali	9.9	8.80	10.90	2.44	0.36
IR 64	11.16	5.25	17.64	-2.71	0.41
Mean	8.67	5.29	12.03	-	-
CD (p<0.05)	3.3	2.2	2.5		

GGE Biplot analysis

Figure 2 displays the ‘which-won-where’ pattern of rice genotypes with high mean grain yield in multi environment trial (MET). In this biplot, a polygon was formed by connecting the vertex genotypes with straight lines and the rest of the high mean genotypes placed within the polygon. The polygon view of a biplot is generally considered as the best way to visualize the interaction patterns between genotypes and environments (Yan et al. 2003). The vertex genotypes in this study were L-192, L-41, L-160, L-133, L-118, L-18 and L-132. These genotypes were the best or worse genotypes in any of the environment. Test environments fell in two sections. The first section contained non-saline environments (E1 and E3) and also one saline environment, E2. Adjacent to E3, the vertex genotype with highest grain yield was L-41. The second section contained the test environment, E4. This could be considered as the ideal salinity stress environment to discriminate genotypes for their performance in saline situation as compared to non-saline situation. The vertex genotype in this section was L-192. So this line had the highest PY in E4 environment. The other genotypes inside this section could be considered as high yielding under saline environment.

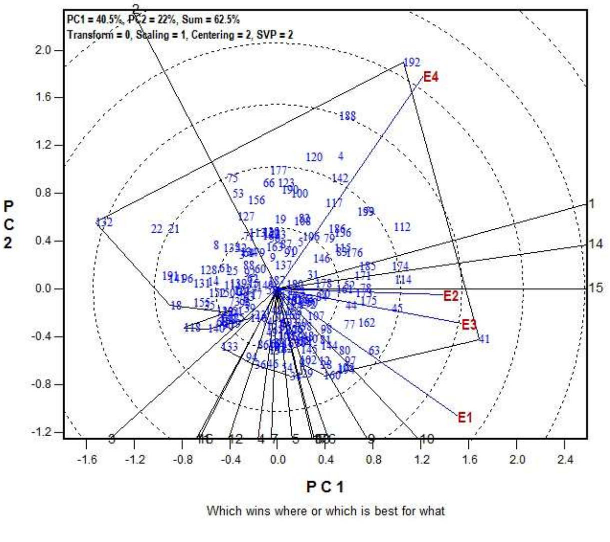

Fig. 2. GGE Biplot for best genotypes from the selected high mean genotypes in different environments for Plant yield

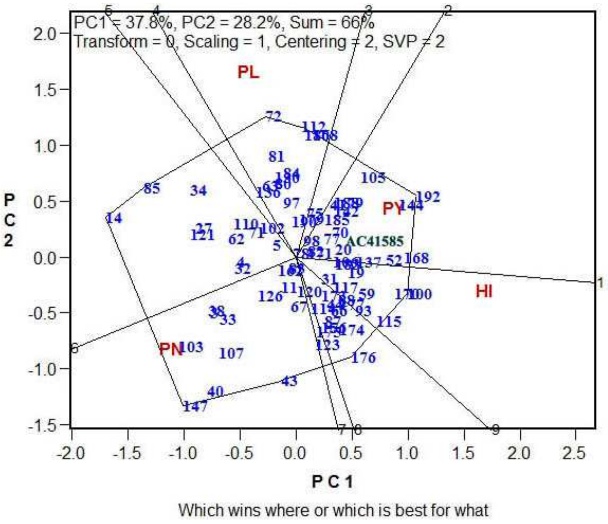

Fig. 3. GGE Biplot for best performing entries for PY, PN, PL and HI under salinity stress (8 dSm^{-1})

Under stress condition PC1 and PC2 jointly explained 66% of the variation (Fig. 3) which were mainly constituted by the positive effects of PY, PL, PN and HI. These traits revealed the genotypes with high performance located in the vertex of the polygon derived through G x GE interaction of high mean genotypes. The genotypes which were located at the middle of the polygon were the average performers for all these traits. These vertex genotypes for each sector could able to give highest response for salinity stress tolerance parameters located within the same sector (Krishnamurthy et al. 2016). Here in the sector where PY is located, as vertex genotype, L-192 was the best performer under salinity stress. Inside the PY sector, other high performing genotypes were L-20, L-144, L-105 and AC41585. Inside the PL sector the highest performer for this trait were the vertex genotypes, L-72 and L-112.

In non-saline environment PC1 and PC2 jointly explained 72% variation (Fig. 4). The vertex genotype, L-12 was located in the same sector with PY and HI. IR64 located nearer to the vertex with high performance under non-saline condition. The vertex genotype L-44 had the highest PN and L-23 had the highest PL as revealed from their sectorial position.

Figure 5 displays the ‘which-won-where’ pattern of rice genotypes with high mean in MET output obtained using the mean vs. stability option. The performance and stability of genotypes for PY, PL, PN and HI were evaluated here by an average environment coordination (AEC) method (Yan 2002). The average environment is defined by the average PC1 and PC2 scores of all four environments. A red line with one direction arrow passes through the origin (marked by a small circle) of the biplot, called AEC

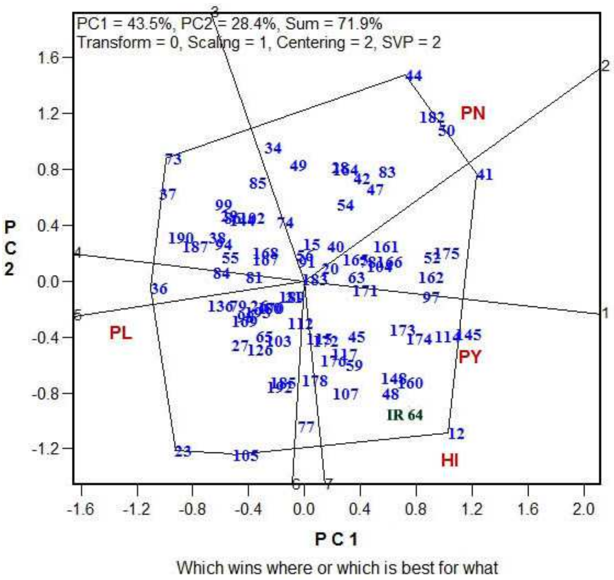

Fig. 4. **GGE Biplot for best performing entries for PY, PN, PL, HI under non-saline environment**

abscissa. Perpendicular to this line is AEC ordinate which is indicated by double arrow and greater GEI effect and reduced stability can be expected in points of this line which is far from the biplot origin. Genotypes with high mean for PY, HI, PL and PN were placed in six sectors (Fig. 5) of the GGE Biplot. The vertex genotype L-174 with low instability shared the same sector with PY. L-175 and L-114 with high PY were also with low instability. PN also belonged to same sector and vertex genotype L-41 with moderate stability was nearer to this trait. Vertex genotype, L-192 shared the same sector with HI but with relatively high instability. In the biplot, the ideal genotype were close to origin and had shortest vectors from the AEC. In the sector where PY was located, L-171 was close to origin and also very short vector from the AEC. This could be considered as the high yielding stable genotype for all environments.

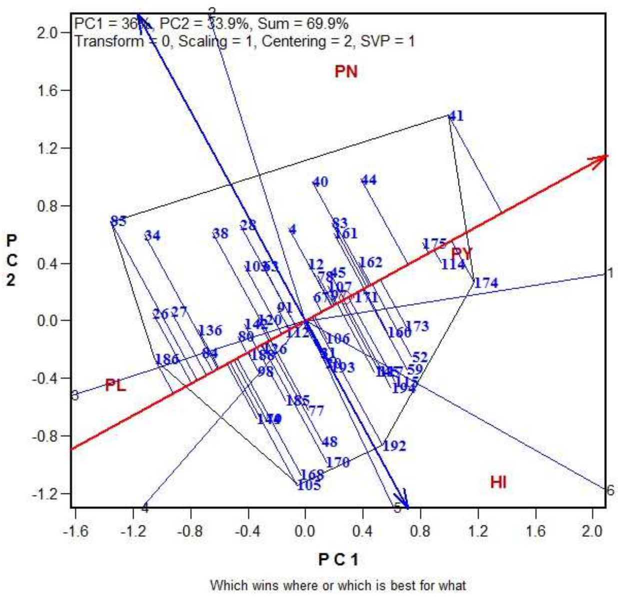

Fig. 5. **GGE Biplot displaying the ranking of genotypes selected based on higher than mean values of HI, PL, PN and PY for both high value and stability performance over the environments**

Stable high yielding genotypes such as L-171, L-41, etc. were phenotypically similar with the recurrent parent, IR 64. They had high yield stability index and yield had higher than the mean yield. These introgression elite lines could be evaluated further for their suitability in the coastal areas and also used in genetic analysis for detection of genes/QTLs responsible for salt tolerance at flowering stage in rice.

Authors' contribution

Conceptualization of research (KC, ONS, RKS); Designing of the experiments (KC, BIM, RKS); Contribution of experimental materials (BCM, KC); Execution of field/lab experiments and data collection (KC, BCM, RKS, ONS); Analysis of data and interpretation (KC, RKS); Preparation of manuscript (KC, RKS).

Declaration

The authors declare no conflict of interest.

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