International Journal of Chemical Studies

S Vanisri
Institute of Biotechnology, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad, Telangana, India

Charles Wesly K
Institute of Biotechnology, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad, Telangana, India

Priyanka B
Institute of Biotechnology, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad, Telangana, India

Sreedhar M
MFPI-Quality Control Lab, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad, Telangana, India

Ishwrya Laxmi
Department of Genetics and Plant Breeding, College of Agriculture, PJTSAU, Rajendranagar, Hyderabad, Telangana, India

V Srikant Rahul
Department of Genetics and Plant Breeding, College of Agriculture, PJTSAU, Rajendranagar, Hyderabad, Telangana, India

Corresponding Author:
S Vanisri
Institute of Biotechnology, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad, Telangana, India

Mahalanobis D² and principle component based genetic divergence, selection criteria and genetic variability studies in cold tolerant rice (*Oryza sativa*) genotypes

S Vanisri, Charles Wesly K, Priyanka B, Sreedhar M, Ishwrya Laxmi, and V Srikant Rahul

DOI: https://doi.org/10.22271/chemi.2020.v8.i4r.9862

Abstract
The present investigation was carried to assess the genetic variability, trait relationship, path analysis, D^2 analysis and principal component analysis in thirty eight genotypes. All the traits under this study shown the significant difference for ANOVA and high GCV and PCV is observed for Panicle exertion and seed yield per plant. High heritability coupled with high genetic advance as per cent mean was observed for seed yield per plant and tillers per plant. All the characters except test weight showed the positive and significant correlation with seed yield at phenotypic level. Path analysis revealed that filled grains per panicle and spikelet fertility showed positive direct effect on seed yield. Diversity studies indicated that inter cluster distance was maximum between cluster II and VII followed by cluster II and VI & cluster I and VII. Principle component analysis indicated that first five principal components contributed to 85.81% of the total diversity is contributed by plant height, seed yield per plant, spikelet fertility, panicle exertion, panicle length, filled grains per panicle and tillers per plant. Selection based on the results of the present study could be exploited in designing future plant breeding programmes for development of high yielding cold tolerant rice varieties.

Keywords: PCA, heatmap, genetic diversity, eigen values, rice, variability, correlation

Introduction

Rice (*Oryza sativa* L.) is one of the most important staple foods that consumed around the world and in India it is staple food for more than 60% of population. It accounts for about 43% of total food grain production and 46% of total cereal production in the country. Among rice growing countries, India has largest area under rice cultivation in the world *i.e.,* 43.86 million hectares and ranks second in the production with 99.24 million tonnes and the productivity of 2.49 t/ha next to wheat. (Ministry of Agriculture, Government of India, 2018-19). Shifts in growing season are a major concern in the recent past due to global climate change. Changes in yield performance and the associated traits under different growing seasons are to be studied in the present scenario and breeding programmes needs to be initiated in that direction to develop high yielding, adaptable varieties. Assessment of genetic variability is essential for the successful yield improvement through breeding. Further seed yield depends on various component characters and knowledge of correlation with yield and among yield component traits in addition to identification of the direct and indirect effects of the traits on yield would help in effective yield improvement. Genetic diversity among genotypes which offers better scope for selection in cross combinations involving divergent parents provide an opportunity for bringing together gene constellations of diverse nature and also promising segregant derivatives which result due to complementary interaction of divergent genes in parents are expected. (Murthy and Arunachalam, 1996 and Rahman, *et al.,* 1997) [17, 18]. Principal component analysis is an adaptive data analysis technique which is effectively used to visualize the similarity and difference between the genotypes and helps in identifying the quantitative characters contributing maximum towards genetic divergence (Jindal *et al.*, 2018). The present investigation was undertaken in the above context to obtain promising rice genotypes for further breeding programme.

Materials and Methods

Thirty eight rice genotypes were evaluated in RBD during mid *Kharif*, 2018 (Nursery sowings were done on 5th July, 2018) at College Farm, Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad. Observations were recorded on five randomly selected plants in each replication for traits days to 50% flowering, plant height (cm), tillers per plant, panicle length (cm), panicle exertion, spikelet fertility(%), test weight (gm) and seed yield per plant (gm). As stated to the formula given by Allard (1960) [1], broad sense Heritability (h^2) was calculated. The genetic advance as per cent mean was calculated according to Burton (1952) [5]. Genotypic and phenotypic correlation coefficients and path coefficient analysis as per Dewey and Lu (1959) [8], GCV, PCV as per Falconer (1981), D² analysis as per Mahalanobis (1936) through INDOSTAT software and Principal component analysis using XLSTAT software to reveal best relationship among traits.

Results and Discussion

Analysis of variance showed the significant difference for all the characters studied indicates that there is ample scope for selection. The trait tillers per plant varied from 7.20 (SKAU-341) to 18.05 (SKAU-382). With respect to panicle length, the longest panicle was 27.10 (VIVEK DHAN-82), while the shortest one is 14.75 (HPR-2373). The mean value for filled grains per panicle is 94.51 with a range of 40.3 (K-429) to 165.4 (VIVEK DHAN-62), while seed yield per plant ranged from 5.68 (HIMALAYA-1) to 29.08 (HIMALAYA-2216).

The genetic variability is depicted in the form of box plots (Table 1, Fig. 2) showed the frequency distribution for nine quantitative traits among thirty eight genotypes. High PCV and GCV was observed for characters panicle exertion (33.84, 25.56) followed by seed yield per plant (28.95, 28.18), filled grains per panicle (26.05, 22.60) and tillers per plant (23.87, 23.56). While moderate values recorded for plant height and low values for spikelet fertility. Similar results were obtained by Kahani *et al.* (2015) [12], Das (2015) [7], Gautama *et al.,* (2016) [9] and Shet *et al.,* (2017) [22]. Quantile-Quantile plots (Fig. 3) showed normal distribution for all the traits except tillers per plant, filled grains per panicle and seed yield per plant which skewed towards the positive side. Hence selecting the genotypes which emerged as outliners in box plots and which skewed towards the positive side in Q-Q plots would be beneficial. High heritability coupled with high genetic advance as per cent mean was observed for seed yield per plant (95,56.52) followed by tillers per plant (97,47.90) and filled grains per panicle (75, 40.41). The results were in concordance with Rajput *et al.,* (2013), Shrivastava *et al.,* (2014) [23], Arvind *et al.,* (2015) and Chandra *et al.,* (2017) [6]. Depicted in Histogram (Fig. 1).

Traits relationship for yield and yield component characters were studied in the present investigation and the results are presented in (Table 2) and depicted in Correlogram (Fig. 4) revealed that the seed yield correlated significantly positive with filled grains per panicle, spikelet fertility, plant height, days to 50 % flowering, tillers per plant, panicle exertion and panicle length indicating that these characters could be considered as a criteria of selection for higher yield, as there were mutually and directly associated with seed yield. Similar results were obtained by Guru *et al.* (2016) [10], Shakhwat *et al.* (2017) [20, 21], Sowmiya *et al.* (2017) and Kalyan *et al.* (2017) [13]. While, a negative significant association of seed yield was shown by 1000 seed weight (-0.0151).

Direct and Indirect effects revealed (Table 3, Fig. 6) that the trait filled grains per panicle exerting the highest direct positive effect on seed yield per plant followed by plant height, tillers per plant and days to 50% flowering at the phenotypic level. The results are in concordance with earlier reports of Guru *et al.,* (2012), Seyoum *et al.,* (2012), Manikyaminnie *et al.,* (2013) [15] and Biswajit *et al.,* (2017) [3]. The positive direct effects of the remaining traits were low to be considered of any consequence. On the other hand, the negative direct effect on seed yield was recorded by panicle length. The high positive indirect effect on seed yield per plant was panicle length *via* plant height and spikelet fertility *via* plant height. Hence, these traits should be considered as important for selection in yield improvement.

Heat map (Fig. 5) depicted that filled grains per panicle exhibiting greater variation among the genotypes followed by plant height and spikelet fertility. The yellow and blue colour corresponds high and low diversity for expressed traits, respectively, while green representing median levels of expression. Thirty eight genotypes were grouped into seven clusters based on euclidean distances using Tocher's method in D² analysis. The distribution of genotypes into various clusters is displayed (Table 4). Among all 7 clusters, cluster I was the largest comprising about 13 genotypes followed by the cluster III (9 genotypes), cluster IV (6 genotypes) and remaining clusters II, V, VI and VII are monoclusters. The average Intracluster values (Table 5, Fig. 7) ranged from 0.00 to 108.00. While intercluster distances were maximum between cluster II & VII (668.03), followed by cluster I & VII (529.37) and cluster II & VI (503.96). Minimum intercluster distances were observed between cluster VI & VII (136.50) and cluster I & II (141.56). The relative contribution (Table 6) of plant height to the total diversity was the highest (34.28%) followed by seed yield per plant (27.14%) and days to 50% flowering (15.07%), while lowest contribution is from test weight (4.21%) followed by panicle length (2.42%). The characters filled grains per panicle, plant height, seed yield per plant and days to flowering contributed 76.47% towards total divergence.

PCA is the eigenvector-based multivariate analyses that best explains the variance in the data.

The five most informative principal components in the study explained 85.81% of the variation, with eigen-values of 3.42, 1.46, 1.19, 0.98 and 0.64 of variations among the traits respectively (Table 7). Similar results were reported by Mahendran *et al.* (2015) [14]. PC1 accounted for highest variability and highly contributed by plant height, seed yield per plant, spikelet fertility, panicle exertion, panicle length, filled grains per panicle and tillers per plant in positive direction. In second principal component has highest contribution of days to 50% flowering. PC3, PC4 were related to test weight and tillers per plant respectively. Scree plot (Fig. 8) explained the gradual decline of eigen values from PC1 to PC5. Selection of characters for seed yield per plant via days to 50% flowering, spikelet fertility and filled grains per panicle would be beneficial. The first two principal components biplot including loadings of the various characters along with the genotypes spread over is given in Fig. 9. The ordination of the rice germplasm revealed that accessions VL DHAN-206, VL DHAN-209, VL DHAN-208, VL DHAN-65, VL DHAN-207, VIVEK DHAN-62, VIVEK DHAN-82, RP-2421 and SUKARA DHAN-1 were distinct for the characters studied. Variables Days to 50 % flowering, filled grains per panicle and seed yield per plant showed highest variation can be seen on the right and left hand

quadrant of the plot. The variables that are on same quarter are very close to each other signify that there are positive correlations between them and results are similar with earlier statistical approaches in this paper. The genotypes and the traits lying in the same quarter representing the possibility of

selection of genotypes for the specific traits. Thus, the biplot diagrams can be used to select genotypes that might have favourable combinations of traits for use in a breeding projects.

Table 1: Estimates of range, variability, heritability, genetic advance for Seed yield and its components in rice germplasm lines

| Characters | mean | Range | | Coefficient of variation | | Heritability in broad sence $h^{2 \, bs}$ (%) | Gen adv. As per cent of mean (at 5% level) |
		Min	Max	phenotypic	genotypic		
Plant height (cm)	119.6	76.1	151.8	18.00	17.86	98.00	36.51
Days to 50% flowering	84	71	102	10.14	9.98	97.00	20.25
Tillers/ plant	11	7	18	23.87	23.56	97.00	47.90
Panicle Exertion	3.35	1.5	5.5	33.84	25.56	57.00	39.77
Panicle length	22.74	14.75	27.1	12.31	11.95	94.00	23.90
Spikelet Fertility	86	73	97	7.61	5.62	55.00	8.55
Filled Grains/ Panicle	94.51	40.3	165.4	26.05	22.60	75.00	40.41
Test Weight	23.12	19.35	27.19	10.08	7.89	61.00	12.73
Seed Yield/ Plant	18.80	5.68	29.08	28.95	28.18	95.00	56.52

Table 2: Phenotypic correlation coefficient analysis of yield and yield contributing characters in rice

Character	Plant height (cm)	Days to 50% flowering	Tillers/ Plant	Panicle Exertion	Panicle Length	Spikelet Fertility	Filled grains/panicle	Test weight	Seed yield /plant
Plant height (cm)	1.0000	0.2296*	0.1526	0.3657**	0.7308**	0.4065**	0.2990**	-0.1126	0.4681**
Days to 50% flowering		1.0000	0.0627	-0.1170	0.0717	0.0348	0.3353**	-0.3772**	0.3053**
Tillers/ Plant			1.0000	0.2601*	0.2115	0.2031	0.0713	-0.1740	0.3308**
Panicle Exertion				1.0000	0.3716**	0.3858**	0.3074**	0.0452	0.3478**
Panicle Length					1.0000	0.2919*	0.1364	-0.0973	0.3245**
Spikelet Fertility						1.0000	0.4166**	0.0609	0.4367**
Filled rains/panicle							1.0000	-0.0120	0.5020**
Test weight								1.0000	-0.0151
Seed yield /plant									1.0000

Table 3: Phenotypic Path coefficient analysis of yield and yield contributing characters in rice

Character	Plant height (cm)	Days to 50% flowering	Tillers/ Plant	Panicle Exertion	Panicle Length	Spikelet Fertility	Filled grains/ panicle	Test weight	Seed yield /plant
Plant height (cm)	0.2493	0.0572	0.0325	0.0912	0.1821	0.1046	0.0745	-0.0281	0.4681
Days to 50% flowering	0.0454	0.1980	0.0100	-0.0232	0.0142	0.0081	0.0664	-0.0747	0.3053
Tillers/ Plant	0.0320	0.0124	0.2458	0.0627	0.0453	0.0519	0.0179	-0.0452	0.3308
Panicle Exertion	0.0310	-0.0099	0.0216	0.0847	0.0315	0.0330	0.0261	0.0038	0.3478
Panicle Length	-0.0076	-0.0007	-0.0019	-0.0039	-0.0104	-0.0315	-0.0014	0.0010	0.3245
Spikelet Fertility	0.0516	0.0050	0.0260	0.0479	0.0371	0.1231	0.0513	0.0071	0.4367
Filled grains/panicle	0.0803	0.0901	0.0196	0.0826	0.0367	0.1120	0.2688	-0.0032	0.5020
Test weight	-0.0140	-0.0468	-0.0228	0.0056	-0.0121	0.0072	-0.0015	0.1241	-0.0151

(Residual effect 0.7271)

Table 4: Distribution of 38 Genotypes into Clusters

Clusters	No. of Entries	Genotypes
I	19	SHALIMAR-1, SKAU-389, SKAU-339, CHINA-1007, V L DHAN-86, HPR-2143, SKAU-5, HPR-2513, SKAU-341, HPR-2336, CHINA-988, RP-2421, JHELUM, VIVEK DHAN-82, VIVEK DHAN-65, V L DHAN-221, SUKARADHAN-1, CHENAB, CHINA-1039
II	1	HIMALAYA-2216
III	9	K-475, K-332, K-429, K-116, HPR-1068, HIMALAYA-741, MTU 1010, HIMALAYA-1, TELLAHAMSA
IV	6	V L DHAN-206, V L DHAN-207, V L DHAN-208, V L DHAN-209, VIVEK DHAN-62, SKAU-382
V	1	RAJENDRA
VI	1	VIVEK DHAN-85
VII	1	HPR-2373

**Table 5:** Intra and Inter cluster average distances (D^2) for 9 quantitative traits in Rice

Clusters	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	Cluster 7
Cluster 1	108.00	141.56	264.54	206.92	197.07	333.74	529.37
Cluster 2		0.00	487.28	153.74	291.00	503.96	668.03
Cluster 3			100.76	354.44	186.68	173.77	293.83
Cluster 4				89.22	354.30	436.68	398.47
Cluster 5					0.00	150.97	385.33
Cluster 6						0.00	136.50
Cluster 7							0.00

Table 6: Relative contribution of different characters to genetic diversity

S. No.	Characters	Contribution %
1	Plant Height	34.28%
2	Days to Flowering	15.08%
3	Tillers per plant	11.66%
4	Panicle Exertion	4.11%
5	Panicle Length	2.42%
6	Spikelet Fertility	4.85%
7	No. of Filled Grains	7.97%
8	Test Weight	0.85%
9	Seed yield per plant	27.14%

Table 7: Eigen values, contribution of variability and factor loading for PCA

	P1	P2	P3	P4	P5
Eigenvalue	3.425	1.465	1.199	0.988	0.646
Variability (%)	38.058	16.273	13.322	10.980	7.182
Cumulative %	38.058	54.332	67.654	78.634	85.816
Plant height (cm)	0.783	-0.023	-0.283	-0.428	-0.080
Days to 50% flowering	0.332	0.836	0.173	-0.083	-0.203
Tillers/ plant	0.443	-0.066	-0.281	0.759	-0.295
Panicle Exertion	0.683	-0.348	-0.124	0.196	0.424
Panicle length	0.667	-0.114	-0.536	-0.359	-0.134
Spikelet Fertility	0.740	-0.275	0.324	0.020	0.130
Filled Grains/ Panicle	0.649	0.218	0.581	-0.013	0.211
Test Weight	-0.176	-0.709	0.449	-0.200	-0.393
Seed Yield/ Plant	0.772	0.026	0.250	0.177	-0.313
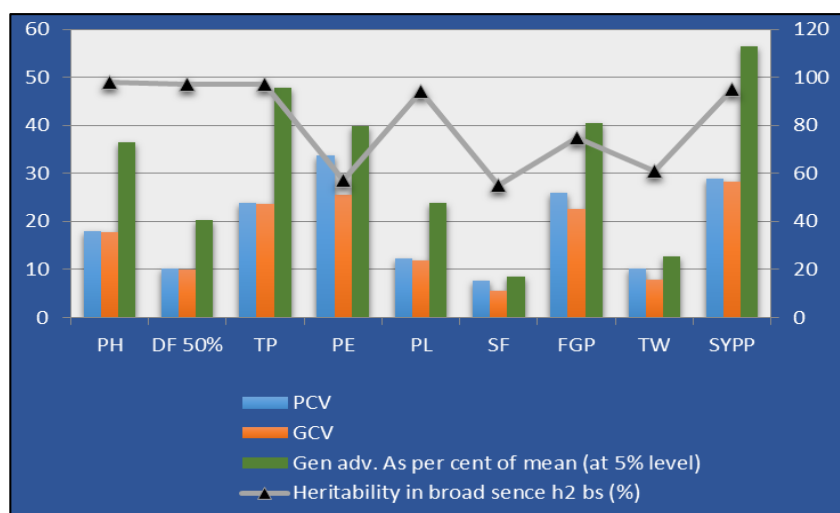
Fig 1: Histogram of heritability in broad sense (h^2), genetic advance, phenotypic and genotypic co-efficient of variation (GCV) for Seed yield and its component characters in rice.
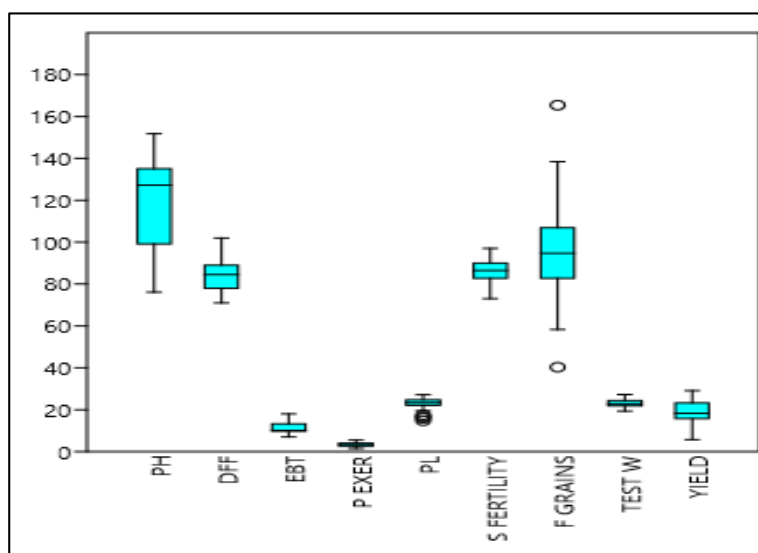
Fig 2: Box-plots showing variation of the data from the 9 quantitative traits evaluated in 38 accessions
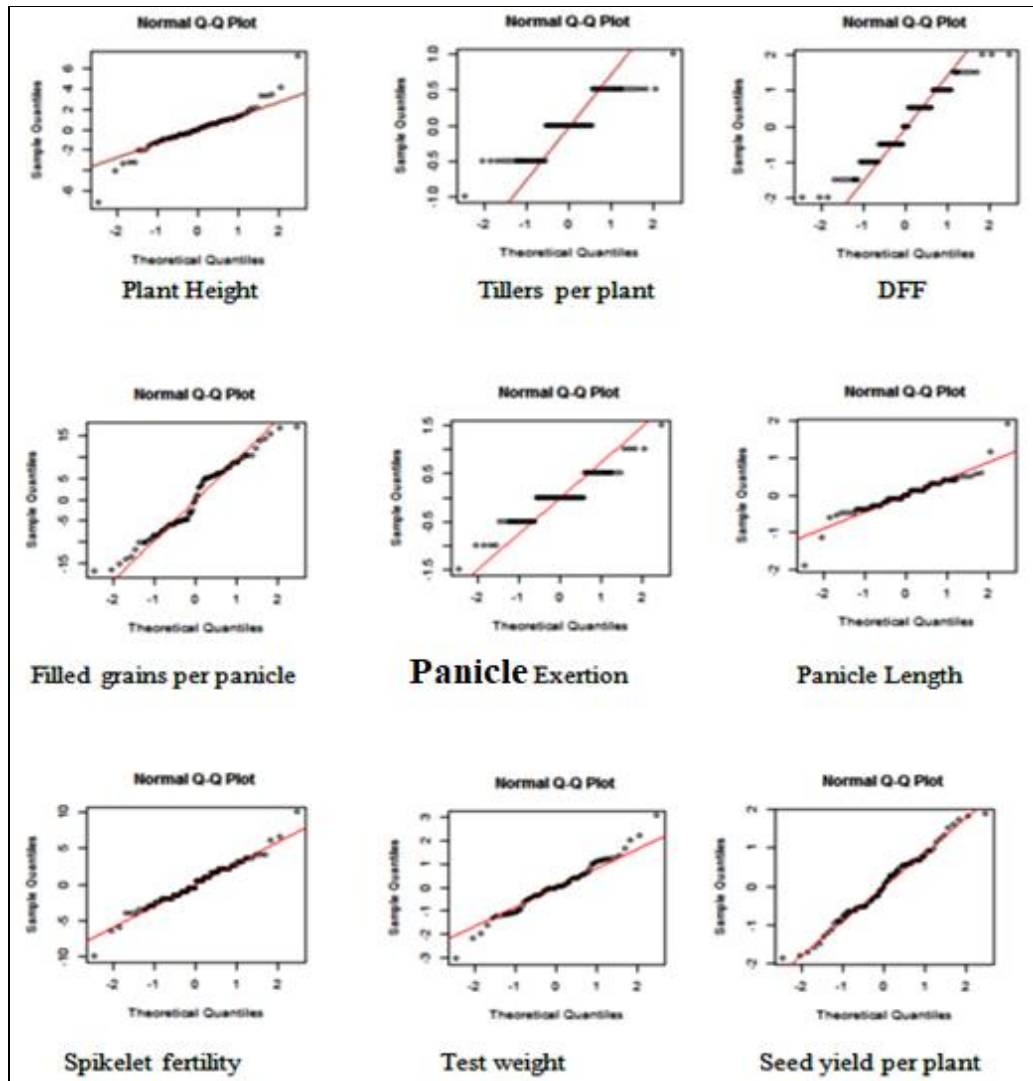
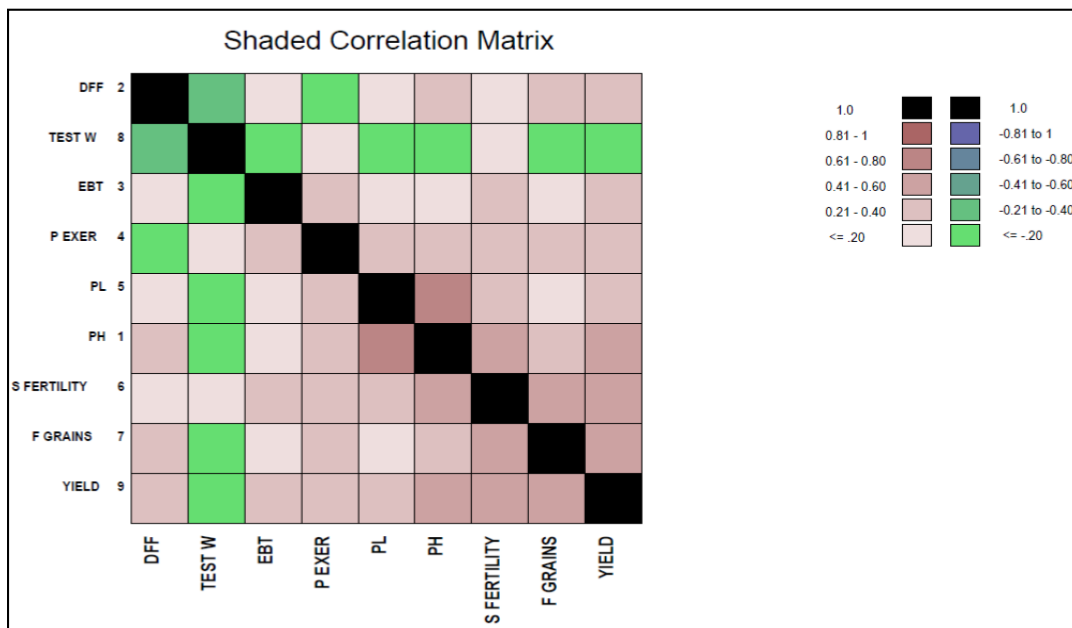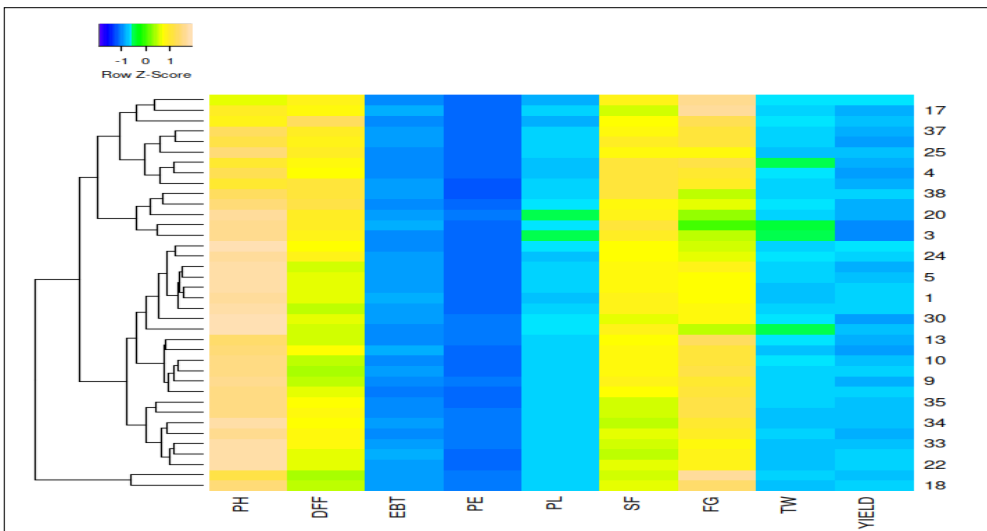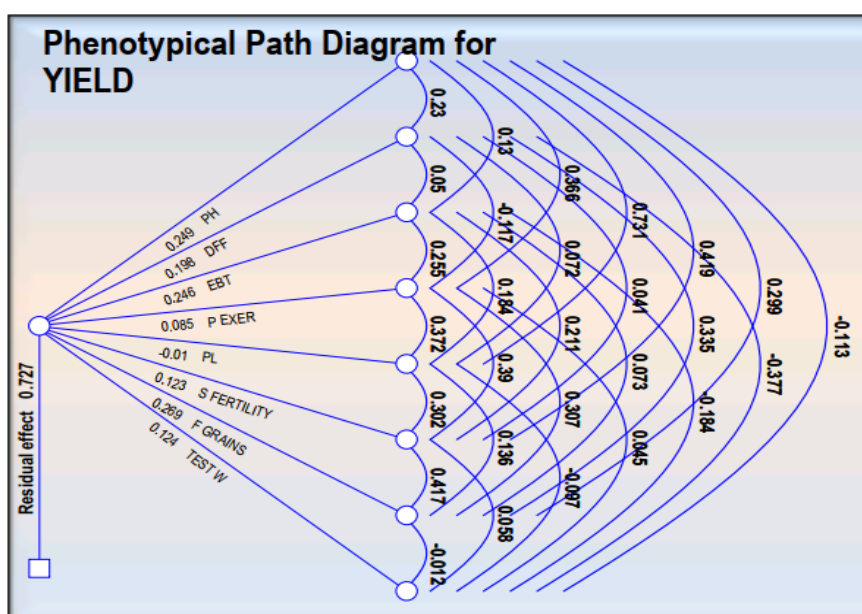**Fig 3:** Quantile-Quantile plots showing the distribution for yield and its attributing traits
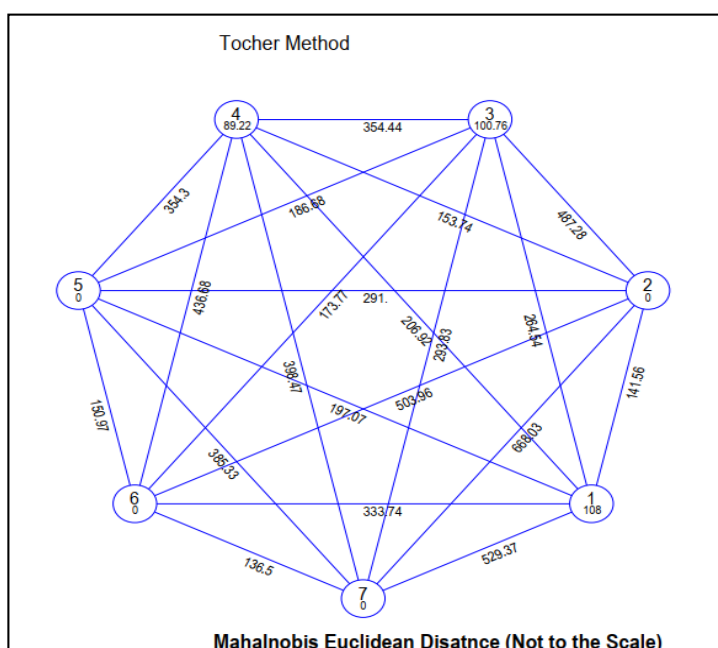

Fig 4: Correlogram visualizing the correlation in yield and its attributing traits

Fig 5: Heatmap depicting the genetic variability in for yield and its attributing traits

Fig 6: Phenotypical Path diagram for yield

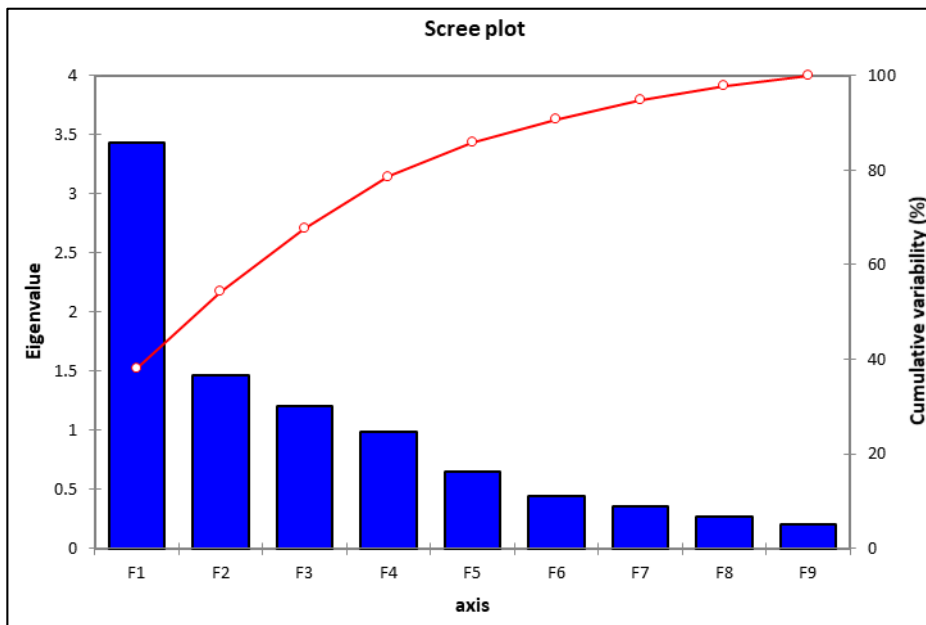**Fig 7:** Cluster diagram depicting intra and intercluster distances. (The figure is not exactly to the scale)

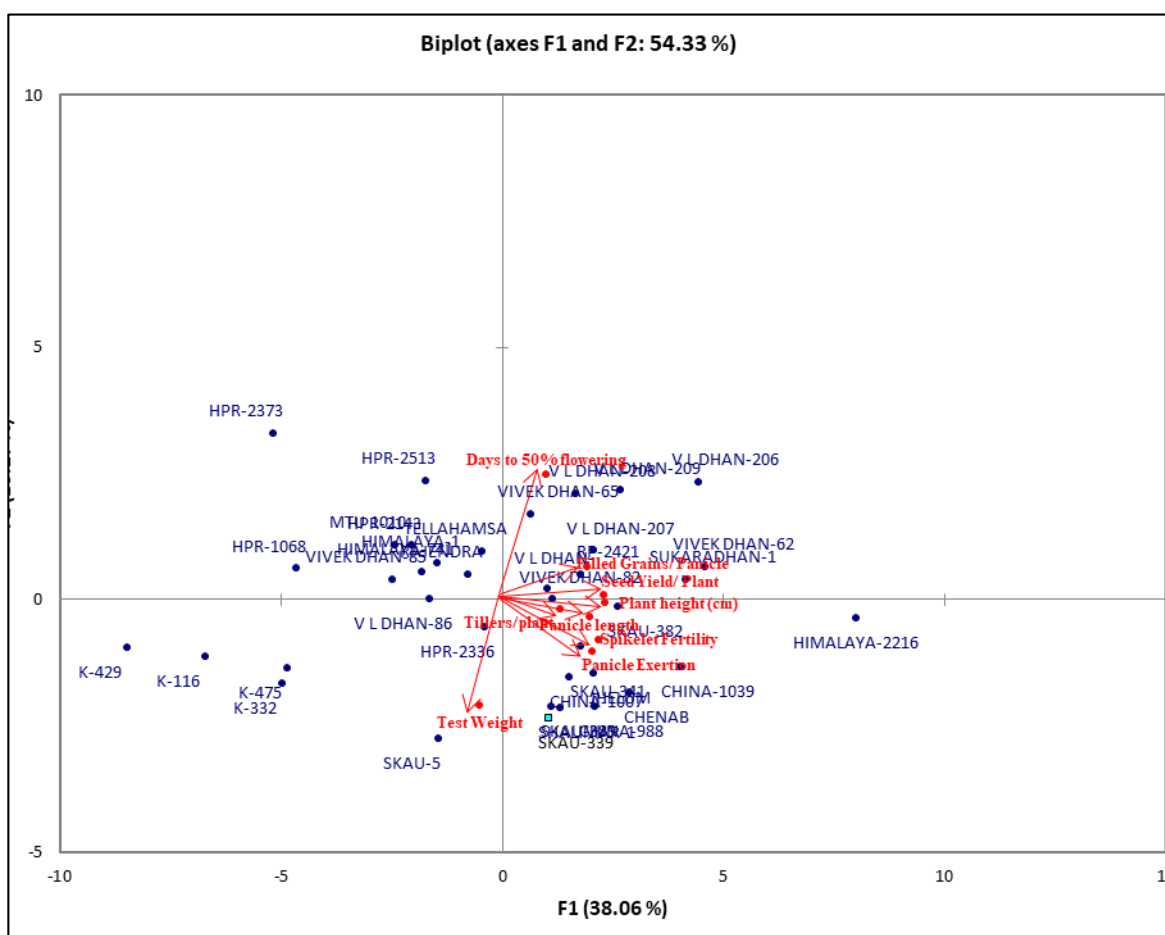**Fig 8:** Eigen values of principal components for yield and its attributing traits

Fig 9: Biplot of Principal component analysis

Conclusion

The results conclude that significant differences among genotypes for all the characters indicate the presence sufficient variation among the genotypes. High GCV and PCV recorded for the traits seed yield per plant, panicle exertion, filled grains per panicle and tillers per plant, while high heritability coupled with high genetic advance as per cent mean was shown for all characters except panicle exertion, spikelet fertility and test weight. There is positive associations between seed yield per plant and to all characters except test weight. Selection for these traits would be effective to enhance yield potential. The traits filled grains per panicle, tillers per plant showed highest positive direct effects on seed yield. Clusters II &VII, I & VII and II & VI has the highest inter cluster distance and genotypes present in these clusters are more diverse and can be used as parents for further breeding programme to improve yield. PCA studies identified that only few characters play a prominent role in classifying the variation existing in the germplasm. About 85.81% of the total variability was explained by first five

principal components. As there is a greater level of variability present among the genotypes and traits towards diversity, there is an ample scope for enhancement of the new varieties with the use of these germplasm in rice breeding programmes.

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