Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

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Selection criteria for agronomic traits suitable for cold season through cluster and principal component analysis in rice (*Oryza Sativa* L.)

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Abstract
Thirty eight cold tolerant rice genotypes were evaluated for genetic variability, correlation and path analysis for yield and yield attributing traits. Genetic analysis revealed high GCV and PCV for tillers per plant, panicle exertion and seed yield per plant. High heritability coupled with high genetic advance as per cent of the mean was observed for plant height, tillers per plant and seed yield per plant. Seed yield per plant having positive significant correlation with plant height, tillers per plant, panicle exertion, panicle length, spikelet fertility and filled grains per panicle at the phenotypic level. Path coefficient analysis determined plant height, panicle exertion, panicle length and filled grains per panicle exhibiting direct positive effect on yield, selection for these traits helps in improving seed yield. Principal component analysis (PCA) revealed five most informative principal components which together accounted 87.56% of the total variance. The traits panicle length, plant height, panicle exertion, seed yield, filled grains per panicle, days to 50% flowering and test weight were determined to be having highest contributions towards total divergence as per Principal component analysis and D^2 analysis. Diversity analysis revealed that inter cluster distance was maximum between cluster III and V indicates that more diversity between the genotypes which provide ample opportunity to select donors to carry out hybridization programmes for the improvement of desirable traits in cold tolerant rice.

Keywords: Genetic variability, D^2, Correlation, Path analysis and PCA

Introduction

Rice (*Oryza sativa* L.) is one of the important staple food crop and dietary source of carbohydrates in Asian countries. It belongs to the family *Poaceae* and comprises about 20% of calories consumed worldwide. In India rice is a paramount cereal and staple food crop which occupies an area of 43.97 million ha which is the largest in the world, with an annual production of around 106.3 million tones as second largest in the world after China. Rice plays a pivotal role in the food security of India because it contributes 46% of the total cereal production and 43% of total food grain production and feeds more than 70% population of the country.

Rice is mainly grown during *kharif* and *rabi*. *Rabi* paddy is usually sown in November to January that coincides with the winter season. The prevailing cold temperatures adversely affect the *rabi* rice germination, growth at seedling stages, tillers formation and fertility and ultimately affecting the yield. The study of cold tolerant rice genotypes during *rabi* provides more information regarding genetic variability, genetic diversity and yield performance. Genetic variability is prerequisite for improving plant species in terms of important economic characters. The structure of a population can be estimated by heritability by determining the transmission of selected traits to the next generation (Ali *et al*., 2000) [1]. The genotypic and phenotypic coefficients of variations help to understand the clear picture of existing variability in the populations. Thus, in a given crop species the knowledge of genetic variability for the characters under improvement is important to design plant breeding programmes. Heritability with genetic advance is more helpful in anticipating the gain under effective selection (Idris *et al*., 2012) [8]. Correlation and path analysis determine the association between yield and its components and also bring out the relative importance of their direct and indirect effects, thus proving an understanding of their association with seed yield. Essentially, this kind of analysis could benefit the breeder to choose his selection strategies to improve seed yield. Principal component analysis is a multivariate analysis that attempt to simplify and analyze the inter-relationship among a large set of variables in term of a relatively a small set of variables or components without losing any essential information of original data set and each component explained per cent (%) variation to the total variability.

Thus, the present investigation is carried out with the objective of studying the genetic variability, trait relationship associations and genetic diversity in thirty eight cold tolerant rice genotypes for yield improvement.

Materials and methods
The experiment was carried out during *Rabi* 2018 at Institute of Biotechnology, Prof. Jayashankar Telangana State Agricultural University, Hyderabad. Thirty eight cold tolerant rice genotypes were evaluated in Randomized Complete Block Design with 3 replications. The observations were recorded on randomly selected five plants on days to 50% flowering, plant height (cm), tillers per plant, panicle length (cm), panicle exertion, spikelet fertility (%), test weight (g) and seed yield per plant (g). Genotypic (GCV) and phenotypic correlation coefficients (PCV) were estimated as per Falconer (1981), Heritability (h^2) in the broad sense was calculated from the formula given by Allard (1960) [2] and the Genetic advance as percent of mean was calculated as per Burton (1952) [5]. Correlation and Path coefficients as given by Dewey and Lu (1959) [6], Mahalanobis D^2 distances through INDOSTAT software and Principal component analysis (PCA) analysis using XLSTAT software were estimated.

Results and discussion
The analysis of variance (ANOVA) revealed that there is a significant difference among all the genotypes for all the traits studied (Table1). Plant height had a high variability as it ranged from 70 cm (HIMALAYA-741) to 126.60 cm (HIMALAYA-2216) with general mean of 102.89 g (Table 1) and days to 50% flowering varied from 98 days (K-116) to 126.5 days (V L DHAN-207) with general mean of 111.72. The genotype CHINA-1007 had highest numbers of tillers (17.60), while SHALIMAR-1 recorded higher panicle exertion (3.5). Panicle length varied from 15.0 cm (K-332) to 25.40 cm (HIMALAYA-2216), Spikelet fertility was high in SKAU-389 and filled grains per panicle for HPR-2373. Test weight was high in VIVEK DHAN-82 (26.70 g) with general mean of 21.12 g and seed yield ranged from 6.53g (K-475) to 34.87 g (HIMALAYA-2216) with general mean of 16.58 g.

The frequency distribution for the nine yield and attributing traits was depicted in the form of box plots (Fig. 2a) representing a vast genetic variability, while grain yield, panicle exertion, panicle length and spikelet fertility showed outliers indicating the scope for selection of those individuals for future breeding programmes. The traits, plant height and filled grains had higher variability compared to other traits as supported by heatmap (Fig.2b), The yellow and blue colour represents high and low diversity for expressed traits, respectively, while green representing median levels of expression. With respect to Quantile-Quantile plots, (Fig.3) normal distribution for days to 50% flowering, ear bearing tillers, spikelet fertility, panicle exertion and test weight was depicted. While the remaining traits had had few genotypes slightly deviating determining the presence of variability in those genotypes.

Genotypic (GCV) and phenotypic (PCV) coefficient of variation were calculated for each character and presented in Table 2, Fig.1. The magnitude of phenotypic coefficient of variation (PCV) was higher than genotypic coefficient of variation (GCV) for all the characters under study. Among the characters studied highest estimates of PCV and GCV were reported in panicle exertion (41.56, 30.71), seed yield per plant (36.85, 35.68) and tillers per plant (21.35, 20.16). Moderate for plant height (14.87, 15.57) and panicle length

(10.10, 10.56), while traits days to 50% flowering (6.30, 6.35), spikelet fertility (7.21, 8.84) recorded lowest GCV and PCV values respectively. Filled grains per panicle (29.90, 18.75) and test weight (11.90, 9.53) recorded high PCV with moderate GCV values. Singh *et al.* (2011) [17] and Babu *et al.* (2012) [4] also reported high PCV and GCV for most of the yield attributing traits. The high magnitude of PCV composed of high GCV indicates the presence of high genetic variability with less environmental influence. Therefore, on the basis of phenotype alone selection can be effective for the improvement of these characters. Similar results for low to moderate values of PCV and GCV were also found by Ananadarao *et al.* (2011) [3]. The traits studied exhibited high to moderate estimates of genetic advance as percent of mean. The value of genetic advance as per cent of mean was highest for yield per plant (71.17) followed by panicle exertion (46.75), tillers per plant (39.21), plant height (29.26) and filled grains per panicle (24.23) and for rest of the characters it had moderate values. Genetic advance estimates are depicted in Table 2, Fig 1. Heritability estimates along with genetic advance is more useful, than heritability alone, in predicting the effectiveness of selection (Johnson *et al.* 1955) [10]. In the present study the characters which showed high heritability associated with high genetic advance was plant height and yield per plant, these traits are under the control of additive gene action (Panse and Sukhatme, 1957) [14] and can be improved through simple or progeny selection methods, while the characters which showed high heritability coupled with moderate or low genetic advance can be improved by intermating superior genotypes of segregating population developed from combination breeding (Samadhia, 2005) [16]. Similar results was reported by Sahu *et al.,* (2015) [15] for high GCV, PCV and high heritability coupled with the high genetic advance percent of the mean.

Correlation analysis among yield and its attributing traits (Table 3,Fig.4) revealed that seed yield per plant had significant positive correlation with panicle length, plant heigh, panicle exertion, tillers per plant, filled grains per panicle and spikelet fertility. These results are in accordance with Lakshmi *et al.*, (2019) [12]. Selection for these traits could be considered as the criteria for higher seed yield, as they were mutually and directly associated. Positive significant associations were also found for filled grains per panicle with plant height, panicle exertion, panicle length and spikelet fertility. Similarly, panicle length had positive correlation with plant height, days to 50% flowering, tillers per plant and panicle exertion. Spikelet fertility recorded negative associations with days to 50% flowering, tillers per plant and test weight and is similar to the reports by Minnie *et al.* (2013) [13] and Ganapati *et al.* (2014) [7].

Path coefficient analysis (Table 4) revealed traits plant height, panicle exertion, panicle length and filled grains per panicle exhibited direct positive effect on seed yield indicating that selection for these characters is likely to bring about an overall improvement in seed yield directly as suggested by Madakemohekar *et al.* (2015) while days to 50% flowering, tillers per plant, spikelet fertility and test weight, exhibited an direct negative effect on seed yield, supported by the similar reports of Naseem *et al.* (2014) [9].

Thirty eight genotypes were grouped into seven clusters based on euclidean distances using Tocher's method. Among seven clusters (Table 5, Fig. 5) cluster I comprises maximum entries of 14 followed by cluster IV, III and II and V while, cluster VI and VII had single genotypes *viz,* HIMALAYA-2216 and RAJENDRA indicating a high degree of diversity among the

accessions, they can be directly used as parents for crossing programmes. Intra cluster D^2 values ranged from 0.00 to 73.93 (cluster IV), while inter cluster D^2 values were maximum between cluster III and V (563.34) followed by cluster V and VII (472.39). Highest inter-cluster distance between cluster III and cluster V revealed that these two clusters and members of these clusters are distinctly related to each other. More the inter-cluster distance more will be the diversity between the genotypes which provide ample opportunity to select a number of donors to carry out hybridization programmes (Joshi *et al*, 2018) [11]. The contribution of days to flowering (Table 7) was highest (54.77%) followed by, seed yield per plant (27.31%) and spikelet fertility (4.55%), while lowest contribution was from panicle exertion (0.57%) and No. of filled grains (1.14%). Traits days to flowering and seed yield per plant alone contributed 82.08% towards total divergence.

Principal component analysis (PCA) revealed five most informative principal components (Table 8, Fig 7) with Eigen values of 3.76, 1.68, 0.99, 0.97 and 0.46 respectively, which together accounted 87.56% of the total variance for all the characters (Fig. 6). According to principal component 1,

characters such as panicle length (0.445), plant height (0.426), panicle exertion (0.413), yield per plant (0.351), filled grains per panicle (0.339) and days to 50% flowering had relatively higher contributions (41.87%) to total morphological variability while second principal component accounted for 18.67% of the total variation with test weight (0.495) giving the highest contribution. In Scree plot (Fig. 6) shows the gradual decline of Eigen values upto 5 principle components. The first two principal components biplot including loadings of the various characters along with the genotypes spread over is given in Fig. 7. This Figure indicates that the PCA showed a clear differentiation between most of the cold tolerant genotypes from each others. Biplot interpret the near-real differentiation of the cold tolerant genotypes and morphological characters studied in this experiment. Selection of traits through panicle length, plant height, panicle exertion, seed yield, filled grains per panicle, days to 50% flowering and test weight lying in these two principal components would be beneficial in contributing to the total morphological diversity. Thus, the biplot can be used as a vital instrument to categorize, differentiate and address the genetic entities in breeding decisions.

Table 1: Analysis of Variance (ANOVA) table for seed yield and yield attributing traits

Character	Df	Plant height (cm)	Days to 50% Flowering	Tillers/ Plant	Panicle Exertion	Panicle Length	Spikelet Fertility	Filled grains/panicle	Test weight	Seed yield /plant
Genotype	37	0.3696	0.12	0.38	0.12	0.36	0.75	19.40	1.76	0.68
Replication	1	490.98***	99.77***	12.33***	1.14***	10.27***	89.35***	780.71**	10.36***	72.32***
Error	37	22.59	0.79	0.71	0.33	0.45	17.97	339.90	2.26	2.33
CV (%)		4.62	0.80	7.02	28.00	3.07	5.12	23.28	7.12	9.21
CD		9.63	1.81	1.70	1.17	1.36	8.59	37.36	3.05	3.10

CV: coefficient of variation; CD: critical difference.
Significant at the 0.01 probability level. * Significant at the 0.001 probability level.

Table 2: Estimates of mean, range, variability, heritability, genetic advance for seed yield and its components

| Characters | Mean | Range | | Coefficient of variation | | Heritability in broad sense h$^{2\,bs}$ (%) | Gen adv. As per cent of mean (at 5% level) |
		Min	Max	Phenotypic	Genotypic		
Plant height (cm)	102.89	70.0	126.60	15.57	14.87	91.00	29.26
Days to 50% flowering	111.72	97.50	126.50	6.35	6.30	98.00	12.87
Tillers/ plant	11.96	6.45	17.60	21.35	20.16	89.00	39.21
Panicle Exertion	2.07	0.00	3.50	41.56	30.71	55.00	46.75
Panicle length	21.93	15.00	25.40	10.56	10.10	92.00	19.92
Spikelet Fertility	82.86	63.42	94.29	8.84	7.21	67.00	12.11
Filled Grains/ Panicle	79.18	33.10	115.40	29.90	18.75	39.00	24.23
Test Weight	21.12	15.80	26.70	11.90	9.53	64.00	15.73
Seed Yield/ Plant	16.58	6.53	34.87	36.85	35.68	94.00	71.17

Table 3: 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	0.5137**	0.1776	0.5170**	0.6587**	0.3585**	0.3716**	0.2598*	0.4648**
Days to 50% Flowering		1.0000	0.2559*	0.3984**	0.5215**	-0.0251	0.2017	-0.1264	0.0436
Tillers/ Plant			1.0000	0.3329**	0.3920**	-0.0793	0.1936	-0.2132	0.4131**
Panicle Exertion				1.0000	0.5427**	0.1084	0.3772**	0.1483	0.4616**
Panicle Length					1.0000	0.3169**	0.3838**	0.0879	0.5939**
Spikelet Fertility						1.0000	0.6096**	0.2112	0.2733*
Filled grains/panicle							1.0000	0.0731	0.3842**
Test weight								1.0000	0.0846

Table 4: 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)	0.2799	-0.2587	0.0364	0.0777	0.3170	-0.0240	0.0545	-0.0180	0.4648
Days to 50% Flowering	0.1438	-0.5036	0.0525	0.0599	0.2510	0.0017	0.0296	0.0088	0.0436
Tillers/ Plant	0.0497	-0.1289	0.2052	0.0500	0.1887	0.0053	0.0284	0.0148	0.4131
Panicle Exertion	0.1447	-0.2006	0.0683	0.1503	0.2612	-0.0073	0.0553	-0.0103	0.4616

Panicle Length	0.1844	-0.2627	0.0804	0.0815	0.4813	-0.0212	0.0563	-0.0061	0.5939
Spikelet Fertility	0.1003	0.0127	-0.0163	0.0163	0.1525	-0.0670	0.0894	-0.0146	0.2733
Filled grains/panicle	0.1040	-0.1016	0.0397	0.0567	0.1847	-0.0409	0.1466	-0.0051	0.3842
Test weight	0.0727	0.0637	-0.0438	0.0223	0.0423	-0.0142	0.0107	-0.0692	0.0846

Table 5: Distribution of 38 Genotypes into Clusters

Clusters	No. of Entries	Genotypes
I	14	CHINA-1039, SKAU-382, CHINA-988, JHELUM, SKAU-5, CHENAB, SKAU-339, SKAU-341, RP-2421, SUKARADHAN-1, HPR-2336, VIVEK DHAN-62, VIVEK DHAN-65, V L DHAN
II	5	SHALIMAR-1, VIVEK DHAN-85, SKAU-389, VIVEK DHAN-82, MTU 1010
III	5	K-475, K-429, HIMALAYA-741, K-116, K-332
IV	8	HPR-2143, HPR-1068, HPR-2373, HIMALAYA-1, V L DHAN-86, HPR-2513, TELLAHAMSA, CHINA-1007
V	4	V L DHAN-208, V L DHAN-209, V L DHAN-206, V L DHAN-207
VI	1	HIMALAYA-2216
VII	1	RAJENDRA

Table 6: 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	40.97	104.87	269.43	81.84	137.07	154.78	153.56
Cluster 2		44.30	119.67	155.52	333.72	173.43	94.89
Cluster 3			44.40	259.09	563.34	426.01	189.80
Cluster 4				73.93	159.99	294.05	218.66
Cluster 5					0.00	404.52	472.39
Cluster 6						0.00	93.52
Cluster 7							0.00

Table 7: Relative contribution of different characters to genetic diversity

S. No.	Characters	Contribution %
1	Plant Height	2.99%
2	Days to Flowering	54.77%
3	Tillers per plant	3.7%
4	Panicle Exertion	0.57%
5	Panicle Length	2.13%
6	Spikelet Fertility	4.55%
7	No. of Filled Grains/panicle	1.14%
8	Test Weight	2.84%
9	Seed yield per plant	27.31%

Table 8: Eigen values, contribution of variability and factor loading for PCA

	PC1	PC2	PC3	PC4	PC5
Eigen value	3.77	1.68	1.00	0.97	0.46
Variability (%)	41.87	18.67	11.06	10.83	5.13
Cumulative %	41.87	60.54	71.60	82.43	87.57
Plant height (cm)	0.426	0.075	0.350	-0.057	0.059
Days to 50% flowering	0.309	-0.344	0.452	-0.418	0.027
Tillers/ plant	0.207	-0.501	-0.401	0.156	0.648
Panicle Exertion	0.413	-0.108	0.120	0.226	-0.429
Panicle length	0.445	-0.101	0.052	0.000	0.169
Spikelet Fertility	0.241	0.532	-0.294	-0.320	0.212
Filled Grains/Panicle	0.339	0.270	-0.361	-0.380	-0.115
Test Weight	0.125	0.495	0.373	0.518	0.410
Seed Yield/ Plant	0.351	-0.042	-0.374	0.481	-0.369
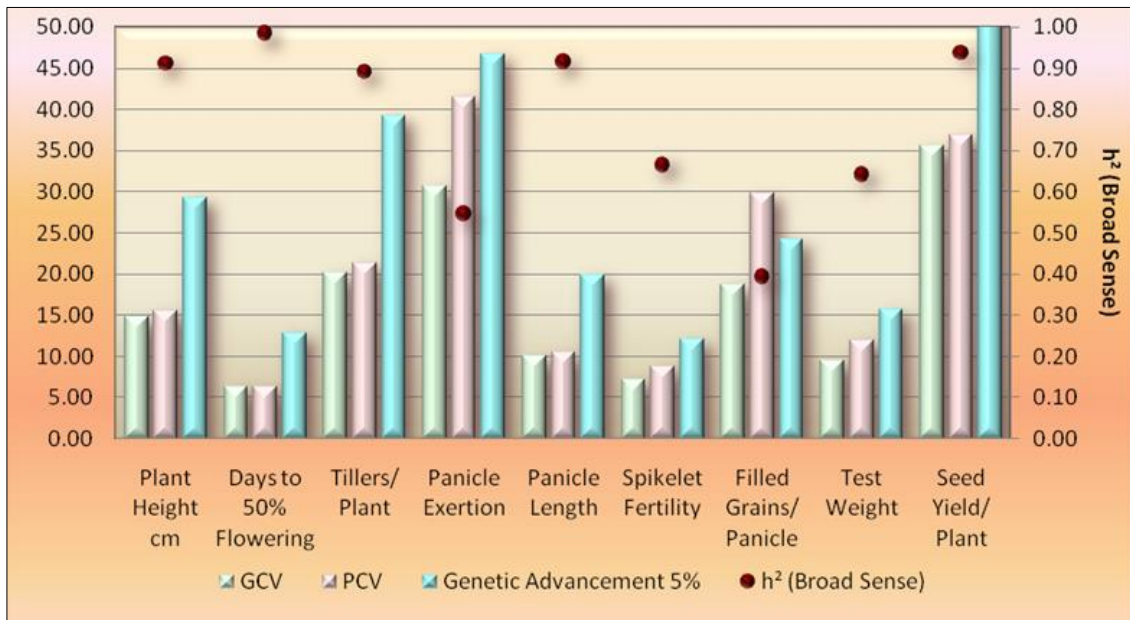
Fig 1: Histogram of GCV, PCV, GA at 5% of mean, h^2 (bs) for seed yield and its component traits
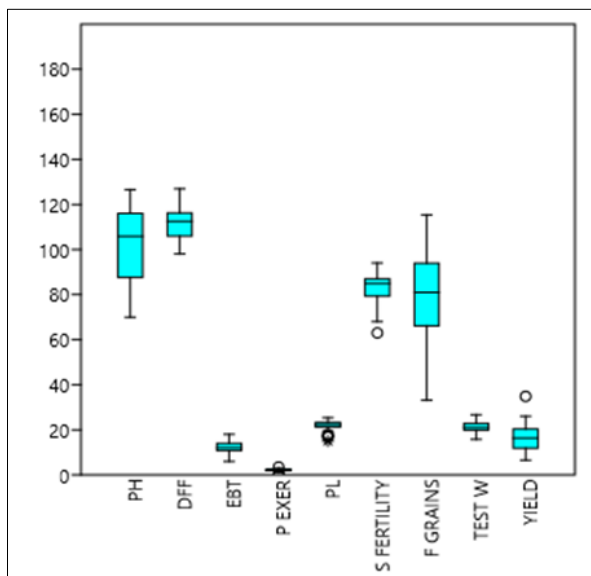
Fig 2a: Box-plots showing variation of the data from the 9 quantitative traits evaluated in 38 accessions.
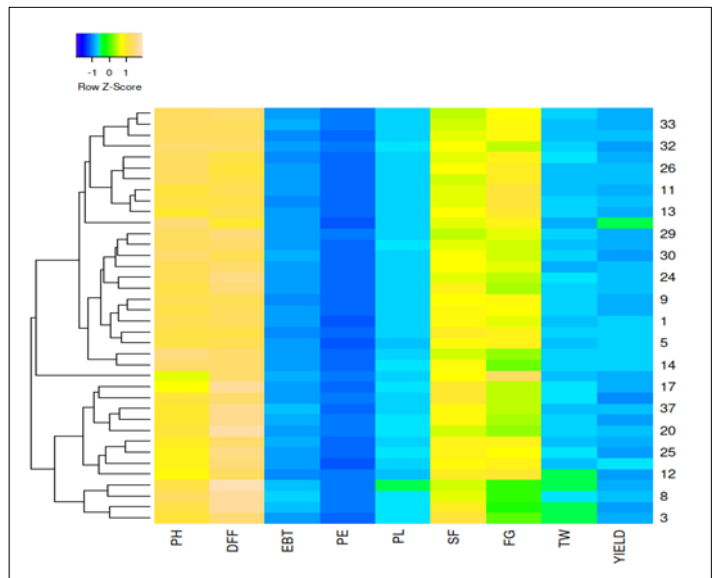
Fig 2b: Heatmap depicting the genetic variability in for yield and its attributing trait
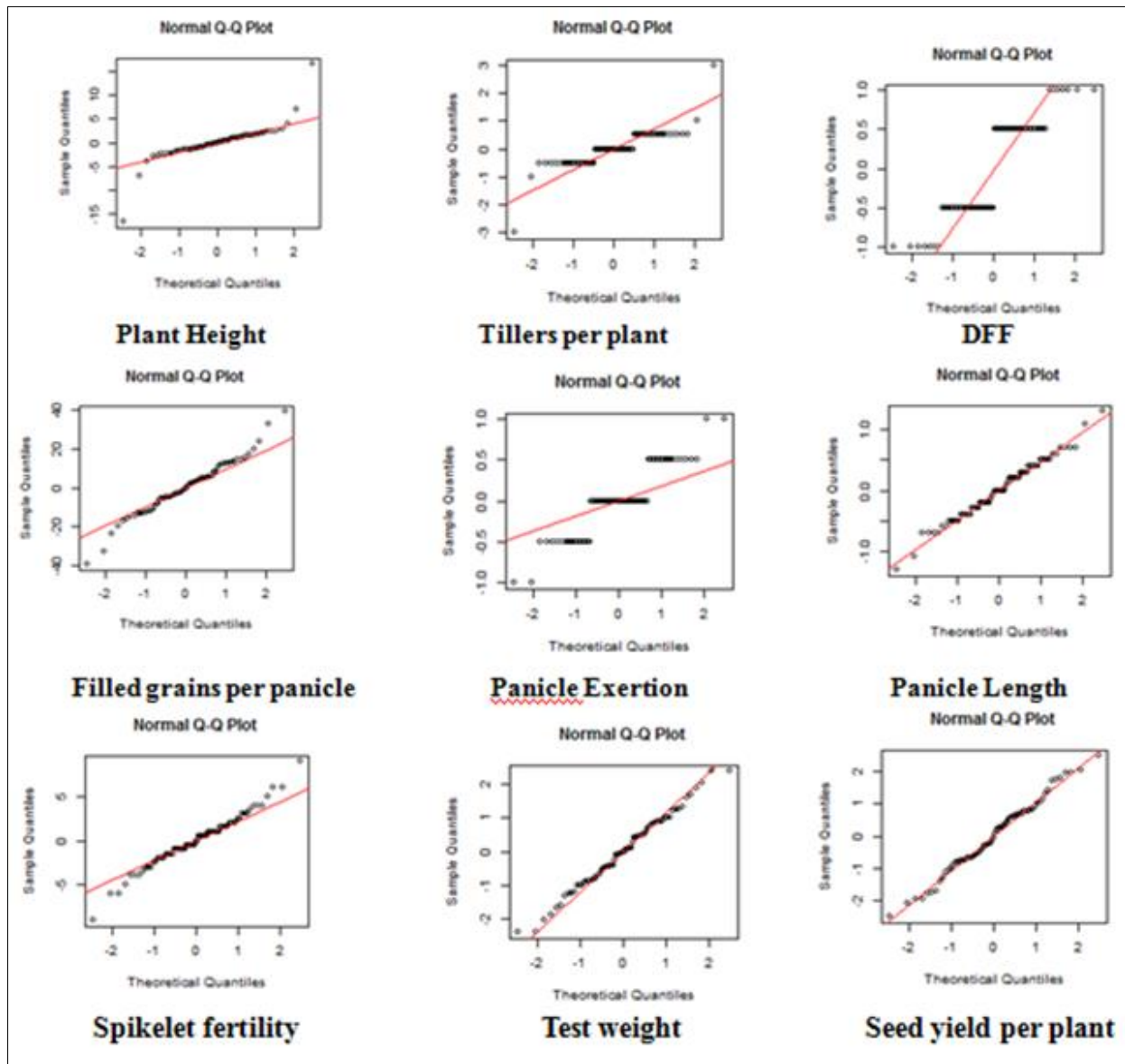
Fig 3: Quantile-Quantile plots showing the distribution for yield and its attributing traits

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

Fig 5: Cluster diagram of 38 genotypes based on D2 values by Tocher method

Fig 6: Scree plot depicting Eigen value and percentage of cumulative variability in 38 rice genotype

Fig 7: Biplot of Principal component

Conclusion

The present study revealed that traits had medium to high variability levels with high genotypic and phenotypic coefficients for tillers per plant, panicle exertion and seed yield per plant. High heritability coupled with high genetic advance as per cent of the mean for plant height, tillers per plant and yield per plant. Yield per plant had positive relations with plant height, tillers per plant, panicle exertion, panicle length, spikelet fertility and filled grains. Path coefficient analysis suggests that plant height, panicle exertion, panicle length and filled grains per panicle exhibited direct positive effect on yield. Additionally, the PCA showed that first two components explained 60.54% of the existing variation in genotypes as depicted by biplot. Thus, the present study finds immense applications in rice improvement programmes.
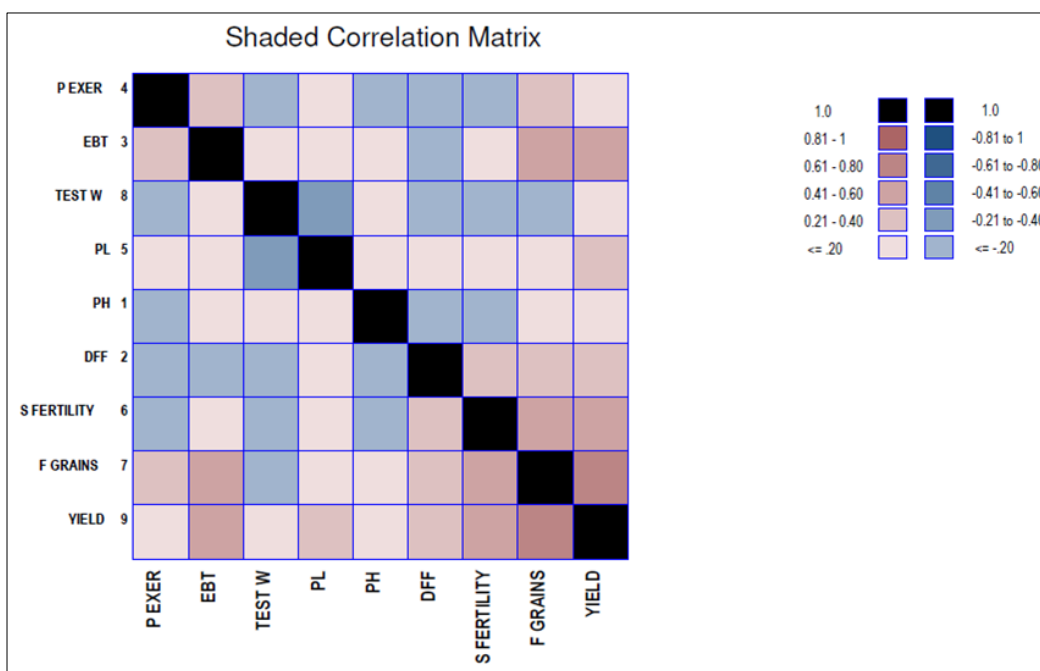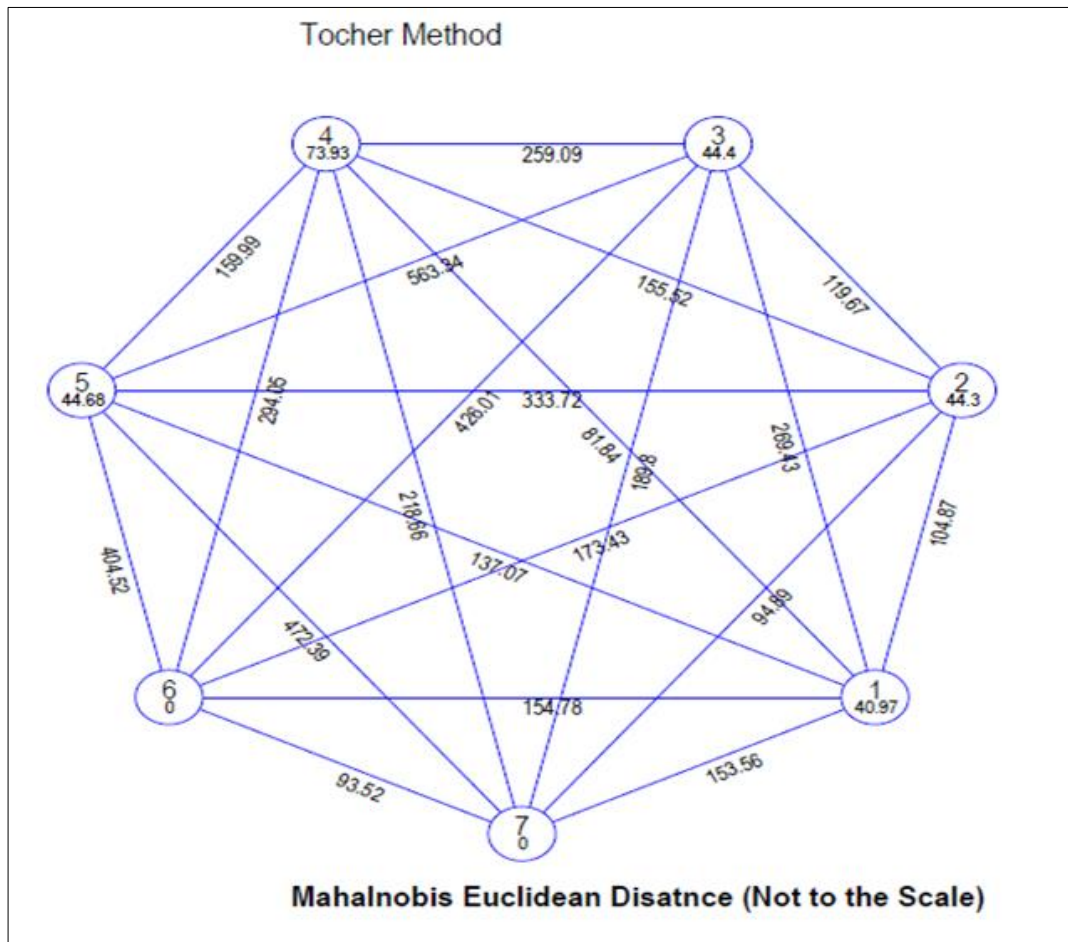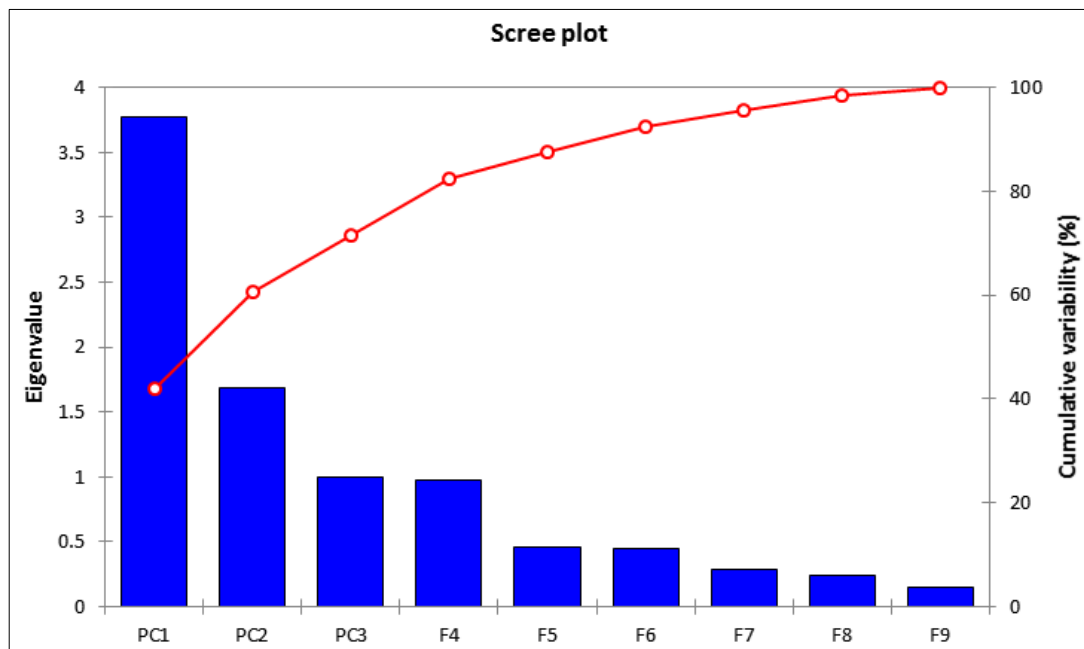
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