

Genetic divergence and PCA for salinity tolerance in rice at reproductive stage

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ABSTRACT

Rice (*Oryza sativa* L.) is one of the most significant cereal crops, about 3 billion people, nearly half the world's population; depend on rice for survival and it offers up to 80% of daily energy intake in most of the Asian countries. Salinity is the second most constraints after drought and limits the rice production worldwide. In this context, study on genetic divergence for salinity tolerance is most important and accordingly an assessment had been done on the genetic diversity among 40 rice genotypes grown at CSSRI, RRS, Canning town, South 24-Pargana, W.B. in the soil salinity of 6 dS/m in two replications following randomized block design in plots of size 1m × 1m with spacing 20 cm × 15 cm during rabi, 2013. The data were recorded on four reproductive growth stage characters viz., pollen viability, number of unfilled grains per panicle, number of filled grains per panicle and grain yield per plant (g). All the forty genotypes showed differential performance with respect to the characters studied and had been grouped into 6 clusters. Cluster I comprised of highest i.e., 11 whereas Cluster II contained lowest i.e., 3 number of genotypes. The value of intra-cluster distance ranged between 6.28 and 58.96 whereas the inter-cluster distance varied from 34.39 to 2238.47. Considering the cluster mean, cluster V possessed the least value and cluster VI possessed the highest value for all the four characters. The relative increase of unfilled grain per panicle contributed the highest contribution (92.18%) followed by relative reduction in grain yield per plant (4.10%) and relative reduction in pollen viability (2.05%), respectively. Principal component analysis indicated that the three components, PC1, PC2 and PC3 could explain for a total of 98.110% of total variation. Among these, PC1, PC2 and PC3 explain for 90.077%, 5.584% and 2.449% of the total variation respectively. The component score coefficients of PC1, PC2 having positive values for majority of the variables under study suggested major component among the 3PCs. The bi-plot of PC1 against PC2 revealed considerable variation among genotypes in their response to salinity treatments where the genotype vectors ranged from 12 to 58 considering vector 1 and from -18 to 2 considering vector 2.

Key words: Rice, reproductive stage, genetic divergence, PCA, salinity tolerance

Rice is an important food crop for the entire world population. While active efforts are being made to increase rice productivity; a considerable amount of rice biomass for which genetic potential exists in the present-day cultivars is not harvested under field conditions, primarily because of the sensitivity of this crop to various stresses (Widawsky and O'Toole, 1990). Rice is a salt-sensitive crop, increasing its salt tolerance has enormous implications. The strategy to overcome

this problem is genetic improvement of salinity tolerance in present day varieties (Epstein et al., 1980). Knowledge on salt tolerance ability delivers useful information in plant breeding programs, in order to select best parents with diverse genetic background prior to intercrossing (Kanawapee et al., 2011). Considering the extent of sensitivity, rice is more sensitive during reproductive stages (Pearson and Bernstein, 1959; Zheng et al., 2001) and seed formation stage (Singh et

al., 2004; Babu, 1985). Reproductive stage, the most important as far as grain yield is concerned because the successful fertilization at this stage is ultimately translated to grain yield. Therefore, the important characters at reproductive growth stage had been chosen for investigation under salinity and control.

The materials in present experiment comprised 40 rice genotypes collected from ICAR-Central Soil Salinity Research Institute, Regional Research Station, Canning Town, South 24 Parganas district of West Bengal. All the 40 genotypes were grown in saline soil of $E_{c} = 6$ dS/m along with control at ICAR-Central Soil Salinity Research Institute, Regional Research Station, Canning Town, South 24 Pargana district of West Bengal in a randomized block design with two replications in plots of size $1\text{m} \times 1\text{m}$ with spacing $20\text{cm} \times 15\text{cm}$ during *rabi*, 2013. Thirty-one days old seedlings were transplanted @ 2-3 seedlings per hill at the farm. The usual recommended doses of N, P_2O_5 and K_2O fertilizers were applied @ 60:30:30 kg/ha. Half the amount of nitrogen and the entire amount of potash and phosphorus were applied as the basal dose at the time of final land preparation. One fourth of nitrogen was applied as first top dressing at 21 days after transplanting and the remaining one fourth nitrogen was applied as second top dressing at 21 days after first topdressing. The data were recorded on four reproductive growth stage characters *viz.*, pollen viability, number of unfilled grains per panicle, number of filled grains per panicle and grain yield per plant (g). The imposition of salinity caused reduction in pollen viability, decrease in number of filled grain, increase in the number of unfilled grains and decrease in grain yield per plant as compared to control. Therefore, for getting the true picture, the relative reduction percentage (RR%) for pollen viability, number of filled grain per panicle, grain yield per plant and relative increase percentage (RI%) for number of unfilled grain per panicle had been determined. The data were analysed in appropriate software (endostat).

All the forty genotypes showed differential performance with respect to the characters observed (Table 1).

The range for relative reduction in pollen viability, RR-PV varied from 11.94 (IR11T138) to 33.57 (CSR 22). The relative reduction in number of filled

grain per panicle, RR-NFG/P varied from 17.01 (IR06M143) to 47.09 (BRRI Dhan 53). The relative increase percentage (RI%) for number of unfilled grain per panicle, RI-NUFG/P varied from 17.17 {Lal Minikit (WGL20471)} to 151.61 (BRRI Dhan 53). The relative reduction in grain yield per plant, RR-GY/P varied from 13.24 {Lal Minikit (WGL20471)} to 58.79 (Lalat). Eight genotypes revealed significant relative reduction for pollen viability. Again, eight genotypes revealed significant relative reduction for number of filled grain per panicle. Fifteen genotypes revealed significant relative increase for number of unfilled grain per panicle whereas ten genotypes revealed significant relative reduction for grain yield per plant. Moradi et al. (2003) hypothesized that slow growth of plants due to osmotic stress forced by a high concentration of salts in the root zone significantly decreased the grain yield.

The pollen viability was reduced under the saline condition. This resulted into poor fertilization and consequent poor seed setting. The poor seed setting leads to reduced grain yield per plant in saline environment. Almost all the varieties reduced their pollen viability under stress but those which reduce the pollen viability drastically in conjunction with very high reduction in grain yield were considered as the susceptible (sensitive) genotypes for reproductive stage (Kahtun and Flowers, 1995). In accordance with these studies, pollen viability, a very important trait that is greatly influenced by the ionic toxicity under salinity, was found to be a governing trait for the ultimate grain yield. In addition to that sodium distribution within the plant is not homogenous (Yamanouchi et al., 1987). Composition and development of the panicle and yield components such as tillering, spikelet number, sterility and grain weight may depend upon sodium concentration in the panicle (Khatun and Flowers, 1995). The changes in grain weight and sterility were significantly correlated with changes in the panicle sodium concentration at several panicle development stages (Folkard et al., 1999).

Analysis of genetic divergence

Grouping of 40 genotypes into different clusters

On the basis of Mahalanobis D^2 analysis, 40 genotypes used in the present experiment could be grouped into 6 clusters (Table 2). The clusters were formed on the basis of relative magnitude of D^2 values, following

Table 1. Relative reduction (RR) in pollen viability, number of filled grain per panicle, grain yield per plant and relative increase (RI) in number of unfilled grain per panicle of 40 genotypes of rice at 6 dS/m of soil salinity.

S.N.	Genotypes	RR-PV	RR-NFG/P	RI-NUFG/P	RR-GY/P
1.	IR10206-29-2-1-1	32.96	45.43	129.07	51.03
2.	IR52280-117-1-1-3	20.95	31.30	51.25	38.77
3.	Canning-7	21.78	35.37	66.41	36.50
4.	CSR-4	23.63	33.05	71.36	40.03
5.	CSRC (S) 32-B-B-B-3-B	24.34	36.04	82.51	43.22
6.	CSRC (S) 36-B-B-2-B	20.87	33.14	76.39	39.27
7.	CSRC (S) 50-2-1-2-B	22.73	32.32	67.61	35.54
8.	PUSA NR 580-6	30.80	41.52	145.98	55.73
9.	CSRC (S) 33-9-B-B-B	25.32	35.20	65.48	36.68
10.	CSRC (S) 50-2-1-1-4-B	24.01	37.50	82.75	34.73
11.	CSRC (S) 49-B-5-2-B-1	23.44	35.69	70.62	31.60
12.	CSRC (S) 53-1-B-1-B	21.22	34.84	81.92	42.49
13.	CSRC (S) 47-7-B-B-1-1	22.04	38.01	54.19	29.04
14.	RP2525-124-98-3	25.03	36.50	75.46	36.75
15.	IR77664-B-25-1-2-1-3-12-5-A5Y	24.97	34.60	87.78	43.14
16.	IR75395-2B-B-19-2-1-B	23.80	33.02	75.33	39.61
17.	CSR 12	23.52	35.22	59.52	44.87
18.	CSR 13	22.20	36.83	103.45	41.17
19.	CSR 22	33.57	42.62	115.48	52.49
20.	CSR 28	22.26	31.27	82.00	38.23
21.	CSR 29	24.75	33.09	84.77	30.34
22.	CSR 34	23.65	35.35	97.74	35.17
23.	CSR36	22.98	36.55	57.84	31.29
24.	BRR1 Dhan 47	12.11	22.43	37.50	19.46
25.	Bidhan-2	23.13	30.59	52.39	32.06
26.	IR06M143	14.75	17.01	21.24	18.32
27.	IR11T142	23.15	34.93	71.48	33.62
28.	IR11T138	11.94	20.18	28.29	17.23
29.	IR66946-3R-116-1-1	22.13	32.62	69.30	39.47
30.	IRRI 147	12.45	19.95	33.76	20.61
31.	Annada	31.91	43.89	133.73	56.58
32.	Lal Minikit (WGL20471)	13.73	21.48	17.17	13.24
33.	IR66946-3R-149-1-1	11.35	18.71	24.06	15.56
34.	Lalat	32.87	41.63	125.95	58.79
35.	Sada Minikit (IET4786)	23.14	31.71	75.80	31.98
36.	Boby	21.58	36.47	64.40	29.63
37.	BRR1 Dhan 53	31.59	47.09	151.61	54.65
38.	Rashi (IET1441)	24.25	37.38	55.40	40.41
39.	BRR1 Dhan 55	23.77	31.86	91.39	44.60
40.	Bina-8	22.97	33.32	101.21	34.52
	Mean	22.94	33.64	75.99	36.71
	C.V.	4.46	5.50	2.47	8.23
	S.E.M	0.72	1.31	1.33	2.14
	C.D at 5%	2.08	3.76	3.81	6.14
	Range lowest	11.35	17.01	17.17	13.24
	Range highest	32.96	47.09	145.98	58.79

Tocher's method (Rao, 1952). The principle followed was that the intra cluster D^2 values should be less than the inter cluster D^2 values.

Cluster I comprised of highest *i.e.*, 11 whereas Cluster II contained lowest *i.e.*, 3 number of genotypes.

Cluster III, V and VI each comprised of 6 genotypes and Cluster IV contained 8 genotypes. Earlier, Yadav et al. (2011) also grouped 9 genotypes of rice on the basis of salinity tolerance into 3 clusters. The genotypes belonging to the same cluster indicated their more

Table 2. Grouping of 40 genotypes of Rice into different clusters.

S. N.	Clusters	Number of genotypes	Genotypes
1.	I	11	CSRC (S) 49-B-5-2-B-1, IR11T142, CSR-4, IR66946-3R-116-1-1, CSRC (S) 50-2-1-2-B, Canning-7, CSRC (S) 33-9-B-B-B, IR75395-2B-B-19-2-1-B, Sada Minikit RP2525-124-98-3, Boby
2.	II	3	CSR 34, Bina-8, CSR 13
3.	III	6	CSRC (S) 47-7-B-B-1-1, CSR36, Rashi (IET1441), CSR 12, Bidhan-2, IR52280-117-1-1-3
4.	IV	8	IR77664-B-25-1-2-1-3-12-5-A5Y, BRRI Dhan 55, CSRC (S) 32-B-B-3-B, CSRC (S) 50-2-1-1-4-B, CSRC (S) 53-1-B-1-B, CSR 28, CSR 29, CSRC (S) 36-B-B-2-B
5.	V	6	IRRI 147, BRRI Dhan 47, IR11T138, IR66946-3R-149-1-1, IR06M143, Lal Minikit (WGL20471)
6.	VI	6	IR10206-29-2-1-1, Annada, Lalat, CSR 22, PUSA NR 580-6, BRRI Dhan 53

closeness than those belonging to different clusters. In respect of the character studied, grouping of most of the genotypes into more clusters indicated more divergence among them.

Cluster analysis of the 40 genotypes based on the characters under study has been illustrated by dendrogram. The results of grouping the genotypes in different clusters following D² analysis were also confirmed with the help of dendrogram where it clearly showed 6 clusters reflecting the number of genotypes belonging to each cluster (Fig. 1.). The results suggest that classification of germplasm using D² statistics would provide a set of groups from which parents may be selected for further breeding programme.

Average intra-cluster (Diagonal) and inter-cluster distance in 40 genotypes

Average intra-cluster and inter-cluster distances among 40 genotypes of rice comprising 6 clusters have been presented in table 3.

The value of intra-cluster distance ranged between 6.28 and 58.96. The intra-cluster distance values indicate the closeness of the genotypes falling in the same cluster. The low intra-cluster distance reveal less genetic difference and high intra-cluster values

Table 3. Intra (diagonal) and inter cluster distance in 40 genotypes of rice.

	1	2	3	4	5	6
	Cluster	Cluster	Cluster	Cluster	Cluster	Cluster
1 Cluster	7.11					
2 Cluster	143.21	6.28				
3 Cluster	40.78	306.31	15.11			
4 Cluster	34.39	53.23	124.27	12.42		
5 Cluster	437.42	1008.60	262.40	655.19	22.92	
6 Cluster	731.12	271.71	1051.62	499.55	2238.47	58.96

indicate more genetic difference between genotypes within the same cluster. Therefore, less heterogeneity is expected from the former and the highest from the later. Cluster VI possessed maximum intra-cluster distance *i.e.*, 58.96 followed by cluster V (22.92), cluster III (15.11), cluster IV (12.42) and cluster I (7.11) and cluster II had minimum intra-cluster distance *i.e.*, 6.28. Therefore, hybridization between genotypes belonging

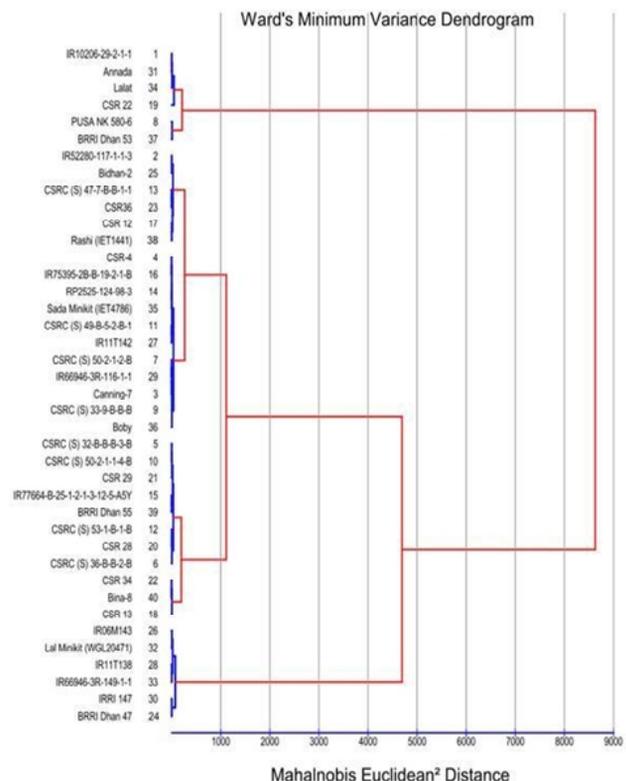


Fig. 1. Cluster analysis of 40 rice genotypes using data for various reproductive growth phase characters showing salt tolerant and susceptible genotypes.

to these different clusters would be facilitative for gene transfer and it bears more prospect between cluster VI and Cluster V.

The average inter-cluster distance varied from 34.39 to 2238.47. The highest inter-cluster distance was observed between cluster VI and V (2238.47) followed by VI and III (1051.62), V and II (1008.60) and others. It indicated that these cluster pairs were most divergent or in other words, these cluster pairs had genotypic constituents contributed by most distantly related parents, in respect of the characters studied. Minimum inter-cluster distances were observed between cluster IV and I (34.39) followed by III and I (40.78) and IV and II (53.23). Minimum inter-cluster distance indicates close relationship between constituent's genotypes of these clusters. The above results reveal that most of the genotypes of rice under study were highly variable considering individual character but considering constellation of characters also they belong to the different groups. Anandan et al. (2011) grouped the genotypes into 12 distinct clusters based on D² statistics. Consisting of 19 genotypes, Cluster I was the largest cluster, followed by Cluster II with nine genotypes. Meanwhile, Clusters III, IV, VI, VII, IX, and XII were mono-genotypic clusters. The maximum inter-cluster distance was exhibited between Cluster IX and X (144.91), followed by Clusters II and X (131.87) as well as clusters VII and X (126.27).

Cluster mean

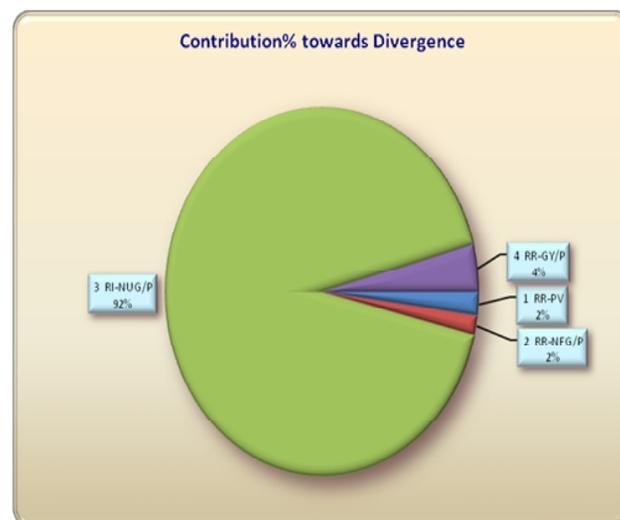
The results with respect to cluster mean have been presented in the table 4. Cluster V possessed the least value for all the four characters which is desirable for salinity tolerance therefore, it is assumed that this cluster contained the genotypes that will be tolerant to salinity. Similarly, Cluster VI possessed the highest value for all the four characters which is desirable for salinity susceptibility therefore, it is assumed that these clusters

Table 4. Cluster mean for different reproductive growth phase characters of 40 genotypes of rice.

Clusters	RR-PV	RR-NFG/P	R. I. NUFG/P	RR-GY/P
1 Cluster	23.42	34.04	70.88	36.18
2 Cluster	22.94	35.17	100.80	36.95
3 Cluster	22.64	35.07	56.43	35.15
4 Cluster	23.27	34.04	83.69	39.50
5 Cluster	12.72	19.96	27.00	17.40
6 Cluster	32.28	43.70	133.64	54.88

Table 5. Contribution of individual characters towards total genotypic divergence of 40 genotypes of rice.

Sl. No.	Characters	Percent contribution towards divergence D ² statistics
1.	RR-PV	2.05
2.	RR-NFG/P	1.67
3.	Increase in Chaffyness/P	92.18
4.	RR-GY/P	4.10



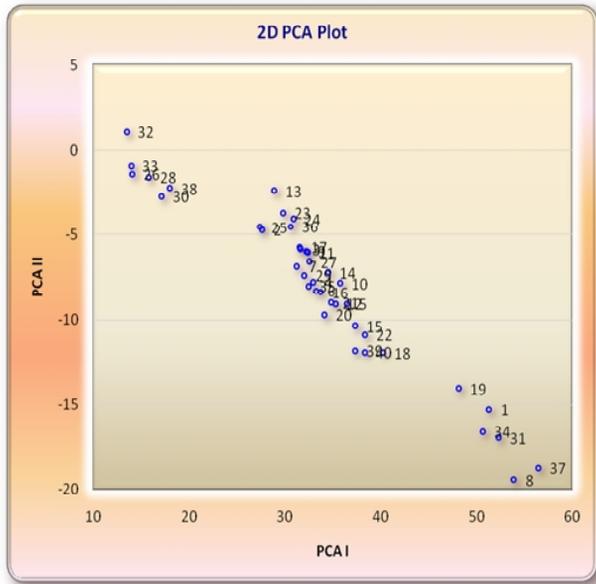
contained the genotypes that will be susceptible to salinity. Considering the mean value for above parameters for reproductive growth under study (Table 1) also confirms the same result. Therefore, for selecting susceptible and tolerant genotypes, genotypes from Cluster VI and V were considered.

Contribution of individual character towards total genetic divergence of 40 rice genotypes

Contribution of individual character toward total genotypic divergence in 40 genotypes under study presented in table 5 revealed that the highest contribution was of the character relative increase of unfilled grain per panicle (92.18%) followed by relative reduction in

Table 6. Principle component analysis for 4 characters and the variation accounted by each PC at 6 dS/m of soil salinity.

Characters	PC1	PC2	PC3
RR-PV	0.508	0.039	0.817
RR-NFG/P	0.483	0.814	-0.300
RI-NUFG/P	0.504	-0.394	-0.490
RR-GY/P	0.504	-0.426	-0.046
% variation	90.077	5.584	2.449
Cumulative (%)	90.077	95.661	98.110
Eigenvalue	3.603	0.223	0.098



grain yield per plant (4.10%) and relative reduction in pollen viability (2.05%), respectively. The values regarding contribution of each character has been presented in pie chart. The lowest contribution was in case of relative reduction in number of filled grain per panicle (1.67%). Anandan et al. (2011) revealed that the number of grains per panicle (42.71%), followed by the grain yield per plot (29.81%), was the major contributor to the total divergence.

Principal component analysis of 40 genotypes of rice

The principal component analysis (PCA) was performed with the objective to identify the main components of different reproductive growth parameters *viz.*, relative reduction in pollen viability, relative reduction in number of filled grain per panicle, relative increase in number of unfilled grain per panicle and relative reduction in grain yield per plant that would best reveal the response of different genotypes to salinity treatment. The results obtained are presented in the table 18. One principal component having Eigen values greater than 0.5 were extracted from the original data accounting 90.077% of total variation for treated population. The component score coefficients, Eigen values, individual and cumulative percentage of variance are presented in table 6. The sign of the score coefficient indicates the direction of the relationship between the component and the variable. Principle component analysis

performed by Anandan et al. (2011) revealed that PC1 and PC2 accounted for 82.88% and 11.14% of the variance, respectively. The highest contributing variable was the number of grains per panicle in PC1 and the plant height in PC2.

The table 6 indicated that the three components, PC1, PC2 and PC3 could explain for a total of 98.110% of total variation. Among these, PC1, PC2 and PC3 explain for 90.077%, 5.584% and 2.449% of the total variation respectively. PC1 explains for maximum percentage of variation and comprised of all the four characters with positive value. The PC2 and PC3 contributed 8.033% of total variation but it comprised of two and three characters with negative value and two and one characters with positive value respectively. The component score coefficients of PC1, PC2 having positive values for majority of the variables under study suggested major component among the 3PCs.

The bi-plot of PC1 against PC2 (Fig. 2) revealed considerable variation among genotypes in their response to salinity treatments where the genotype vectors ranged from 12 to 58 considering vector 1 and from -18 to 2 considering vector 2.

The lowest value for vector 1 was produced by V32 followed by V33, V26, V28, V30 and V38 and others. Interestingly, these genotypes had lowest mean value for all the four characters (table 13). On the contrary, the highest value for vector 1 was produced by V37, followed by V8, V31, V34, V1, V19 and others. These were the genotypes that produced highest mean values for all the four characters. Vector 2 had a lower range of variation with compared to that of vector 1. The highest value for this vector was produced by V32, V33, V26, V28, V38, V30. All these genotypes had lower relative reduction for all the four characters. The lowest value for this vector was exhibited by V8.

Considering the mean table, six genotypes, *viz.*, IR11T138, Lal Minikit (WGL20471), IR66946-3R-149-1-1, IR06M143, IRRI 147 and BRRI Dhan 47 showed less (11.35 - 14.75%) reduction in pollen viability coupled with less reduction in grain yield per plant (13.24 - 20.61%) were considered as tolerant whereas six genotypes, *viz.*, IR10206-29-2-1-1, PUSA NR 580-6, BRRI Dhan 53, CSR 22, Annada and Lalat showed more reduction (30.80 - 33.57%) in pollen viability coupled with more reduction (51.03 - 58.78%) in grain

yield per plant as susceptible. Therefore, the genotypes under these two groups were considered as tolerant and susceptible genotypes at reproductive growth stage. The susceptible genotypes belonged to cluster VI whereas tolerant genotypes belonged to cluster V which showed highest inter-cluster distance and indicated that these cluster pairs were most divergent, in respect of the characters studied. Therefore, can be potentially exploited by the rice breeders in conventional breeding programs or by breeders/researchers in identifying various desirable traits and selection of suitable parents for their use in rice improvement.

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