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UNRAVELLING THE GENETIC DIVERSITY AMONG ADVANCED BREEDING LINES OF RICE (*ORYZA SATIVA* L.)

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ABSTRACT

Sixty advanced breeding lines with check varieties MTU-1121, TN-1 and RP Bio-226 were evaluated to examine genetic divergence and relationship among them by using Principal Component Analysis (PCA) and Cluster analysis for nine yield and its component traits during *kharif*, 2023 at RARS, Maruteru. The cumulative variance of 79.9 % was recorded by the first four principal components with eigen values greater than one. PC_1 contributed maximum towards divergence with a variation of 34.65 % followed by PC_2, PC_3 and PC_4 contributed 18.47 %, 14.46 % and 12.32 % variability respectively towards total divergence. PCA results revealed that the characters *viz.*, grains per panicle, ear bearing tillers per m^2 and grain yield per plant contributed high variation for genetic divergence. PCA biplot revealed that the genotypes *viz.*, NLR-1722, NLR-308, NLR-601, NLR-945, NLR-104, NLR-163, NLR-164, NLR-23, CM-453, CM-466, CM-470, BM-590, CM-464, CM-469 and VP-R-157 were divergent for most of the yield traits. Therefore, by utilizing these genotypes in a hybridization program, it may be possible to produce and select genotypes with high grain production that combine desired features, such as grains per panicle and ear bearing tillers per m^2. Cluster analysis results categorized 60 advanced breeding lines into 7 clusters, with cluster I having the lowest number of individuals and cluster IV having a greater number of individuals. The cluster analysis revealed the desired mean values for panicle length, ear bearing tillers per m^2, grains per panicle, spikelet fertility, test weight and grain yield per plant were found in clusters II, V, VI and VII. The genotypes within these clusters hold significance in rice improvement programmes.

***Keywords* :** Advanced breeding lines; Genetic divergence; Principal Component Analysis; Cluster analysis; Rice improvement programmes.

Introduction

Rice [*Oryza sativa* L.] is referred as "Global Grain" as it is the staple food crop for more than 50% population across the world contributing about 20% of the global calorie supply. Across the world, rice production accounts for 513.54 million metric tons and in India it is 137 million metric tons during the year 2023-2024 (United States Department of Agriculture, 2024). Developing high-yielding rice varieties that surpass current cultivated varieties primarily depends on the level of genetic variability in the population. Studies on genetic diversity to know the nature and degree of divergence for yield traits will help in designing efficient breeding programme by facilitating the selection of elite parental lines which can be further used in hybridization programme to develop superior and high yielding varieties (Talekar *et al.*, 2022). Principal Component Analysis (PCA) is an effective approach for quantifying genetic divergence among germplasm lines with respect to characters (Beena *et al.*, 2021). Cluster analysis offers a best method for selecting parents to realize high heterosis to strengthen hybrid breeding programme in rice.

Material and Methods

The present research investigation was conducted in the Godavari Zone of Acharya N. G. Ranga Agricultural University in *kharif*, 2023, at the Regional Agricultural Research Station, Maruteru, in the West Godavari district of Andhra Pradesh. The station is situated 5 meters above mean sea level, at 81.44^0 longitude and 26.38^0 N latitude. Experimental material for the present investigation comprised of sixty advanced breeding lines with three checks MTU-1121, RP Bio-226 and TN-1, that were evaluated in alpha lattice design with two replications. Nine yield and it's attributing traits *viz.,* days to 50% flowering, days to maturity, plant height, panicle length, ear bearing tillers per m^2, grains per panicle, spikelet fertility, test weight and grain yield per plant were considered for this study.

Statistical Analysis

Observations were recorded for the nine yield and it's attributing traits. The data was recorded on a plot basis for days to 50% flowering and days to maturity. Mean performance were recorded for the remaining traits by five randomly selected plants. PCA analysis was done as per the methodology suggested by Massay 1965 and Jolliffie 1986. Cluster analysis was done using mean data following Ward's method of diversity analysis. Principal component analysis and cluster analysis was performed in R software version 4.1.3.

Results and Discussion

Principal Component Analysis

In the current investigation, PCA was performed for nine yield and yield attributing traits for 60 advanced breeding lines of rice. Principal components that explain at least 5% of the variation in the data and have eigen values greater than 1 are to be taken into consideration, according to Brejda *et al.* 2000. Among the nine principal components, first four principal components had eigen value greater than one and they cumulatively explained 79.9 per cent of total variation present in the original data set. The details of eigen values, variance (%) and cumulative variance (%) by the principal components (PCs) in sixty advanced breeding lines of rice evaluated were furnished in table 1.

PC_1 contributed maximum towards divergence with a variation of 34.65 % and with an eigen value (3.118). PC_2, PC_3 and PC_4 contributed 18.47 %, 14.46 % and 12.32 % variability respectively towards total divergence. The first principal component accounts for maximum variability in the data as possible, and each succeeding component accounts for as much of the remaining variability as possible.

Table 1 : Eigen values, variance (%) and cumulative variance (%) by the principal components (PCs) in sixty advanced breeding lines of rice

Principal Components	Eigen value	% Var. Exp.	Cum. Var. Exp.
PC1	3.118	34.65	34.65
PC2	1.662	18.47	53.12
PC3	1.302	14.46	67.58
PC4	1.109	12.32	79.90
PC5	0.685	7.62	87.52
PC6	0.554	6.16	93.68
PC7	0.372	4.14	97.82
PC8	0.195	2.16	99.98
PC9	0.001	0.02	100.00

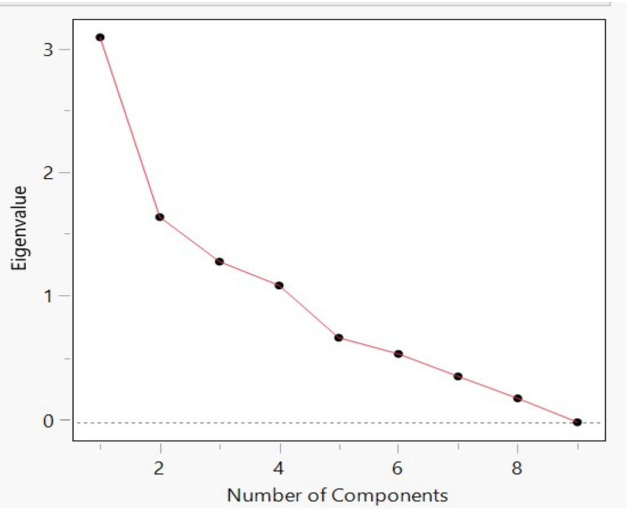


Fig. 1 : Scree plot depicting variation contributed by the principal components towards divergence

The scree plot, which is created by plotting the relationship between the principal component numbers (X-axis) and the eigen values (Y-axis), indicates the percentage of variance attributed to each principal component. The PC_1 showed 34.69 per cent variability with eigen value 3.118 which then declined gradually. The variation contributed by the principal components were depicted in Figure 1.

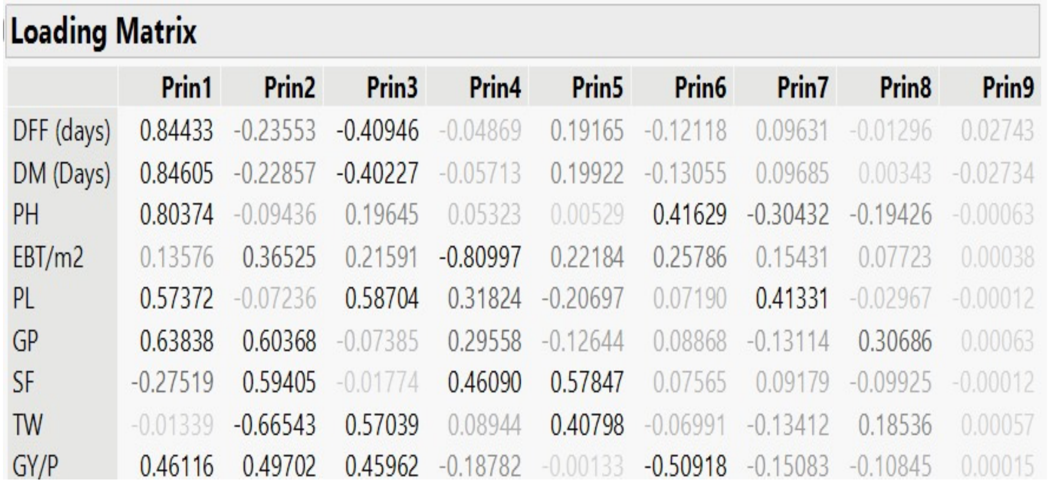
First principal component (PC_1) showed high positive loading for days to 50% flowering (0.84433), days to maturity (0.84605), plant height (0.80374), panicle length (0.57372), grains per panicle (0.63838) and grain yield per plant (0.46116), whereas it showed negative loadings for spikelet fertility (-0.27519). Similar findings accounted by Kumar *et al.* 2015 who assessed the principal component for yield, yield contributing and quality related traits in 30 rice genotypes and reported that the first principal component had high positive loadings for yield traits *i.e.,* grain yield per plant (0.278), panicle length (0.270) and days to 50% flowering (0.201) which contributed more to the diversity.

Second principal component (PC_2) enabled high positive loading for ear bearing tillers per m^2 (0.36525), grains per panicle (0.60368), spikelet fertility (0.59405) and grain yield per plant (0.49702), whereas it showed high negative loadings for test weight (-0.66543). These results were in accordance with Shoba *et al.* 2019 where PC_2 recorded positive loadings for ear bearing tillers per m^2 (0.16) and negative loadings with test weight (-0.73).

Principal component 3 (PC_3) exhibited high positive loading for panicle length (0.58704), test weight (0.57039) and grain yield per plant (0.45962), whereas high negative loading were recorded for days to 50 % flowering (-0.40946) and days to maturity (-0.40227). Talekar *et al.* 2022 reported that PC_3 showed positive loadings for panicle length (0.31) and negative loadings for days to 50% flowering (-0.70).

Principal component 4 (PC_4) exhibited high positive loading for spikelet fertility (0.46090) and negative loading for ear bearing tillers per m^2 (-0.80997). Details of loadings exhibited by principal components were depicted in loading matrix of table 2.

Table 2 : Details of loadings exhibited by principal components



Loading Matrix

	Prin1	Prin2	Prin3	Prin4	Prin5	Prin6	Prin7	Prin8	Prin9
DFF (days)	0.84433	-0.23553	-0.40946	-0.04869	0.19165	-0.12118	0.09631	-0.01296	0.02743
DM (Days)	0.84605	-0.22857	-0.40227	-0.05713	0.19922	-0.13055	0.09685	0.00343	-0.02734
PH	0.80374	-0.09436	0.19645	0.05323	0.00529	0.41629	-0.30432	-0.19426	-0.00063
EBT/m2	0.13576	0.36525	0.21591	-0.80997	0.22184	0.25786	0.15431	0.07723	0.00038
PL	0.57372	-0.07236	0.58704	0.31824	-0.20697	0.07190	0.41331	-0.02967	-0.00012
GP	0.63838	0.60368	-0.07385	0.29558	-0.12644	0.08868	-0.13114	0.30686	0.00063
SF	-0.27519	0.59405	-0.01774	0.46090	0.57847	0.07565	0.09179	-0.09925	-0.00012
TW	-0.01339	-0.66543	0.57039	0.08944	0.40798	-0.06991	-0.13412	0.18536	0.00057
GY/P	0.46116	0.49702	0.45962	-0.18782	-0.00133	-0.50918	-0.15083	-0.10845	0.00015

PCA results revealed that the characters *viz.,* grains per panicle, ear bearing tillers per m^2 and grain yield per plant contributed high variation for genetic divergence. PCA biplot revealed that the genotypes *viz.*, NLR-1722, NLR-308, NLR-601, NLR-945, NLR-104, NLR-163, NLR-164, NLR-23, CM-453, CM-466, CM-470, BM-590, CM-464, CM-469 and VP-R-157 were divergent for most of the yield traits. PCA biplot was depicted in Figure 2. Hence, using these genotypes in hybridization programme may result in the development and selection of genotypes with high grain yield combining desirable traits viz., grains per panicle and ear bearing tillers per m^2.

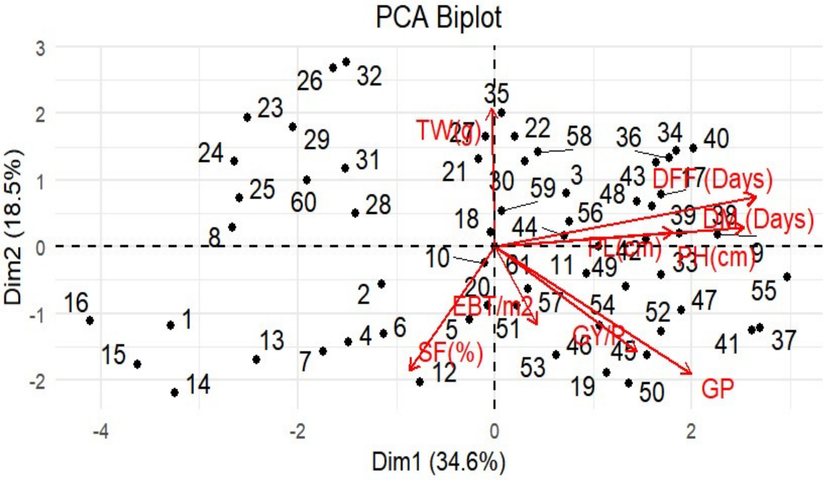


Fig. 2: PCA Biplot

1.	NLR-101	13.	NLR-27	25.	NLR-598	37.	CM-466	49.	CM-452
2.	NLR-213	14.	NLR-104	26.	NLR-945	38.	CM-467	50.	CM-453
3.	ISM Path-17	15.	NLR-163	27.	NLR-252	39.	CM-468	51.	CM-460
4.	160-2-1-2	16.	NLR-164	28.	NLR-296	40.	CM-469	52.	BM-587
5.	160-3-1-2	17.	RTCNP-170	29.	NLR-331	41.	CM-470	53.	BM-588
6.	441-1-3-2	18.	RTCNP-173	30.	NLR-497	42.	CM-471	54.	BM-589
7.	353-3-1-2	19.	NLR-79	31.	NLR-1699	43.	CM-472	55.	BM-590
8.	251-3-3-2	20.	NLR-328	32.	NLR-1722	44.	CM-459	56.	BM-591
9.	VP-R-157	21.	NLR-316	33.	CM-462	45.	CM-454	57.	BM-592
10.	MS-1	22.	NLR-271	34.	CM-463	46.	CM-458	58.	MTU-2077
11.	PNP-55	23.	NLR-308	35.	CM-464	47.	CM-446	59.	MTU-1121
12.	NLR-23	24.	NLR-601	36.	CM-465	48.	CM-449	60.	TN-1

Cluster analysis

Hierarchical cluster analysis was performed to generate dendrogram showing relationship among 60 advanced breeding lines of rice based on yield and its attributing traits. The clustering was done following Ward's method by employing Euclidean distance and a total of seven clusters were generated according to the distance between the genotypes. Classification of advanced breeding lines into different clusters were depicted in table 3. The dendrogram provides an explanation for the hierarchical order of genotypes by clustering comparable genotypes together. Within the clusters, there is little variance, but between the clusters, there is relatively more diversity as depicted by greater distance between them. Dendrogram for sixty advanced breeding lines was furnished in figure 3.

Cluster IV had the maximum number of advanced breeding lines (13), followed by cluster VI with eleven advanced breeding lines, clusters V and VII with ten advanced breeding lines in each cluster, cluster III with seven advanced breeding lines, cluster I with five advanced breeding lines and cluster II had the minimum number of advanced breeding lines (4).

Table 3 : Advanced breeding lines grouped in different clusters based on Ward's method

Cluster	Number of genotypes	Advanced breeding lines
1	**5**	NLR-101, NLR-104, NLR-27, NLR-163, NLR-164
2	**4**	160-2-1-2, NLR-23, 353-3-1-2, NLR-296
3	**7**	251-3-3-2, NLR-601, NLR-598, TN-1, NLR-308, NLR-331, NLR-1699
4	**13**	NLR-213, MS-1, NLR-328, 160-3-1-2, BM-592, 441-1-3-2, PNP-55, RTCNP-173, NLR-316, MTU-1121, MTU-2077, NLR-945, NLR-1722
5	**10**	ISM Path-17, VP-R-157, NLR-252, NLR-497, CM-462, BM-591, CM-463, CM-469, CM-465, CM-464
6	**11**	RTCNP-170, CM-472, CM-449, CM-467, CM-452, BM-589, NLR-271, CM-468, CM-471, CM-459, NLR-79
7	**10**	CM-466, CM-470, BM-590, CM-454, CM-446, CM-458, BM-587, CM-453,BM-588, CM-460

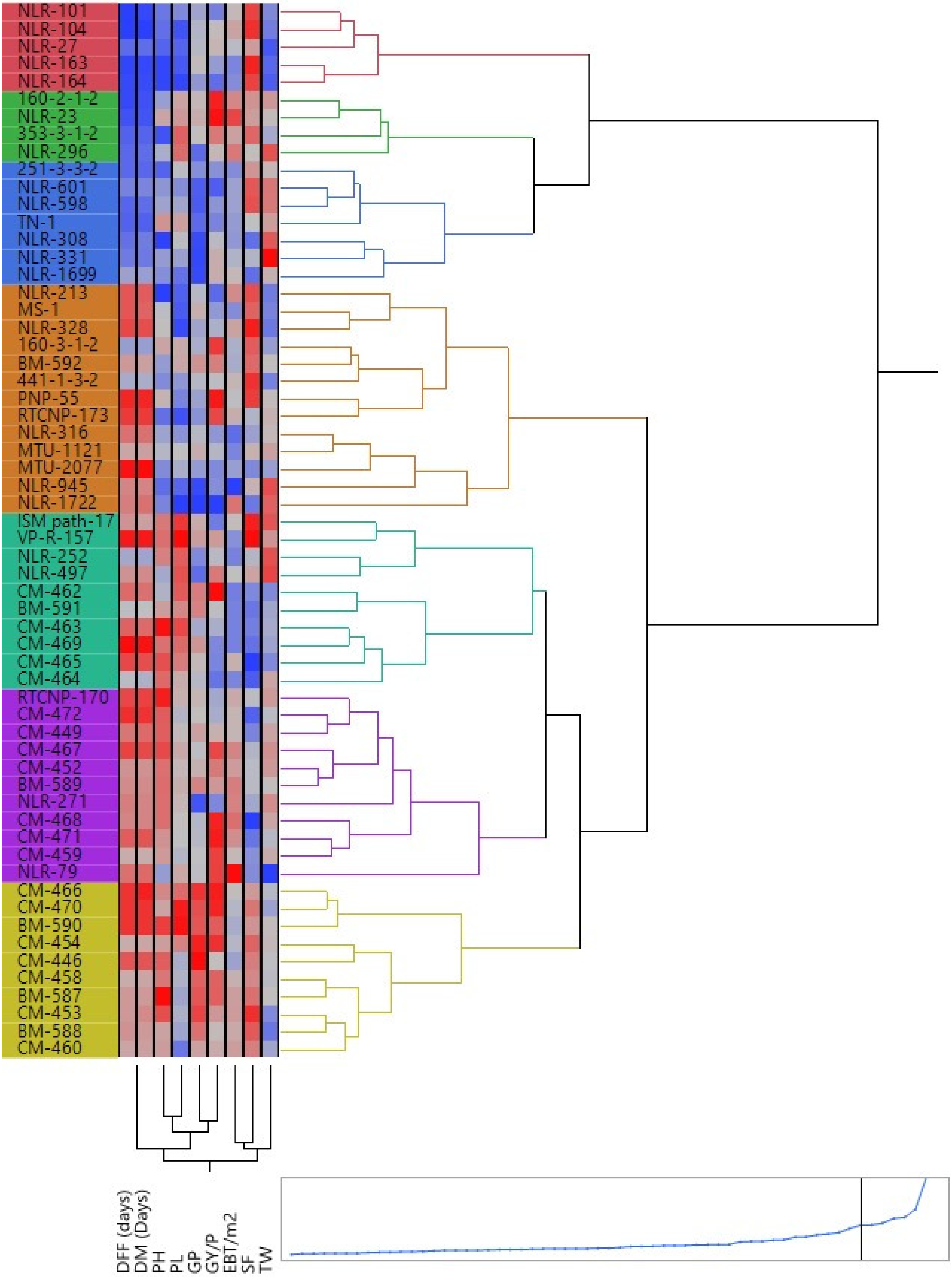
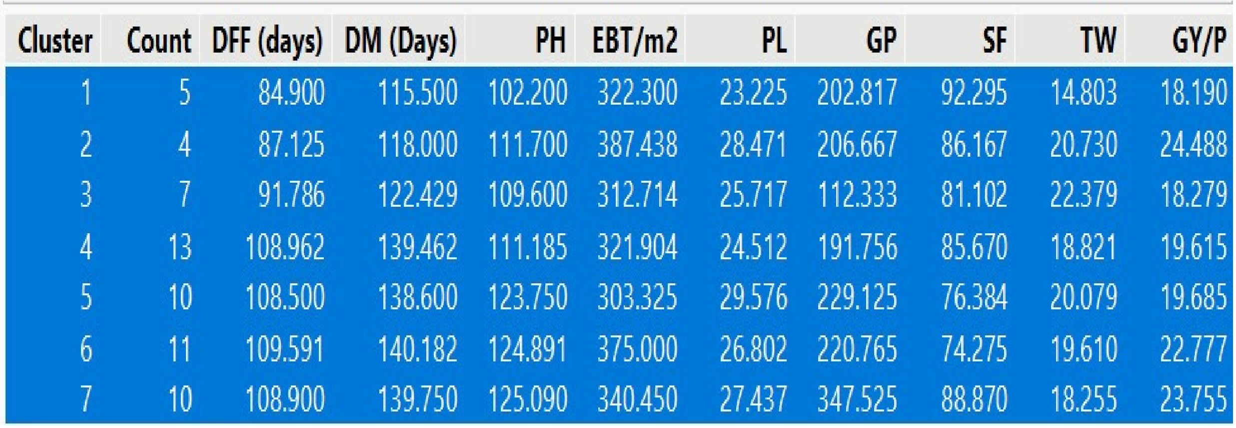


Fig. 3 : Dendrogram and heatmap for 60 advanced breeding lines employing Euclidean distance and Ward method of clustering.

Cluster means for yield and its attributing traits included in the study were depicted in table 4. Early flowering lines were grouped under cluster I and cluster II with average duration of 85 days and 87 days respectively. Similary, lines with early maturity were grouped in clusters I and II with an average duration of 115 days and 118 days respectively. Lines with highest ear bearing tillers per m^2 were found in cluster II and cluster VI with an average number of 387 and 375 tillers per m^2 respectively. Lines with highest panicle lengths were found in clusters II and V with an average panicle length of 28.5 cm and 29.6 cm respectively.

More grains per panicle were recorded by lines present in clusters VII and V with an average of 247 and 229 grains per panicle respectively. Highest spikelet fertility was shown by the lines present in clusters I and VII with an average of 92.3 % and 88.9 % respectively. The lines present in clusters II, III and V recorded higher test weights with an average of 20.7 g, 22.4 g and 20.1 g respectively. Higher grain yields per plant were recorded by lines present in clusters II, VI and VII with an average of 24.5 g, 22.8 g and 23.7 g respectively.

Table 4 : Cluster means for yield and its attributing traits



Cluster	Count	DFF (days)	DM (Days)	PH	EBT/m2	PL	GP	SF	TW	GY/P
1	5	84.900	115.500	102.200	322.300	23.225	202.817	92.295	14.803	18.190
2	4	87.125	118.000	111.700	387.438	28.471	206.667	86.167	20.730	24.488
3	7	91.786	122.429	109.600	312.714	25.717	112.333	81.102	22.379	18.279
4	13	108.962	139.462	111.185	321.904	24.512	191.756	85.670	18.821	19.615
5	10	108.500	138.600	123.750	303.325	29.576	229.125	76.384	20.079	19.685
6	11	109.591	140.182	124.891	375.000	26.802	220.765	74.275	19.610	22.777
7	10	108.900	139.750	125.090	340.450	27.437	347.525	88.870	18.255	23.755

The cluster analysis revealed the desired mean values for panicle length, ear bearing tillers per m^2, grains per panicle, spikelet fertility, test weight and grain yield per plant were found in clusters II, V, VI and VII. The genotypes within these clusters *viz*., 160-2-1-2, NLR-23, 353-3-1-2, NLR-296, ISM Path-17, VP-R-157, NLR-252, NLR-497, CM-462, BM-591, CM-463, CM-469, CM-465, CM-464, RTCNP-170, CM-472, CM-449, CM-467, CM-452, BM-589, NLR-271, CM-468, CM-471, CM-459, NLR-79, CM-466, CM-470, BM-590, CM-454, CM-446, CM-458, BM-587, CM-453, BM-588 and CM-460 can be used in rice improvement programmes.

Conclusion

Principal Component Analysis revealed that the characters *viz.,* grains per panicle, ear bearing tillers per m^2 and grain yield per plant contributed high variation for genetic divergence. PCA biplot revealed that the genotypes *viz*., NLR-1722, NLR-308, NLR-601, NLR-945, NLR-104, NLR-163, NLR-164, NLR-23, CM-453, CM-466, CM-470, BM-590, CM-464, CM-469 and VP-R-157 were divergent for most of the yield traits. Therefore, by utilizing these genotypes in a hybridization program, it may be possible to produce and select genotypes with high grain production that combine desired features, such as grains per panicle and ear bearing tillers per m^2. Cluster analysis results categorized 60 advanced breeding lines into seven clusters, with cluster I having the lowest number of individuals and cluster IV having a greater number of individuals. The cluster analysis revealed the desired mean values for panicle length, ear bearing tillers per m^2, grains per panicle, spikelet fertility, test weight and grain yield per plant were found in clusters II, V, VI and VII. The genotypes within these clusters hold significance for rice improvement breeding.

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Conflicts of interest

The authors declare no competitive or financial conflicts of interest.

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