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Genetic Diversity and Principal Component Analysis for Yield and its component Trait in Rice

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ABSTRACT: Breeding for aerobic rice is a vital step towards sustainable rice production, water conservation, climate change resilience, and ensuring food security for a growing global population. In this view the present investigation evaluated 30 diverse rice genotypes at the agriculture research farm of Lovely Professional University, Punjab. The study aimed to estimate genetic divergence for yield and its attributing traits during the *kharif* season of 2022. Randomized Block Design (RBD) with three replications was used and thirteen yield and its component traits were analysed. Among the eleven Clusters, Cluster 1 was largest comprising of thirteen genotypes followed by Cluster 2 with five genotypes. Maximum inter cluster distance was observed between cluster 2 and 11 (26.99), followed by cluster 2 and 10 (26.38) indicating that genotypes from these clusters were highly divergent and holds great promise as parents for hybridization. Maximum intra cluster distance was observed in cluster 8 (9.47) followed by cluster 2 (9.27). It reveals that genotypes present in the same cluster have low level of diversity and selection of parents with in the cluster for hybridization programme may not be considered promising. The first principal component (PC1) contributed 45.65 per cent towards variability. The characters namely, amylose content (0.23), Days to 50% flowering (0.320), plant height (0.15) and Days to maturity (0.14) explained maximum variance in this component. Among all the principal components, PC1 showed maximum variability of 45.65% with high Eigen value 1540, which decreased gradually indicating that maximum variation, was observed in PC1 as compared to the other PC's.

Keywords: Genetic divergence, Clusters, Principal component analysis, inter cluster, intra cluster and variability.

INTRODUCTION

Rice (*Oryza sativa* L.) is a short-day, C3 plant that is cultivated primarily in Asian agroecosystems. It belongs to the family *Poaceae* (Gramineae) and is an annual, semi-aquatic, and self-pollinating crop. There are two main domesticated species of rice: Asian rice (*Oryza sativa* L.) and African rice (*Oryza glaberrima* Steud.), both possessing the genome AA (2n = 24). According to data from the Punjab Agriculture department, paddy cultivation covers approximately 87% of the total area dedicated to kharif crops (grown from June to October) in Punjab. The data for the current 2022-23 kharif season reveals that out of the total 3.59 million hectares under kharif crops, paddy was cultivated on 3.13 million hectares (Anonymous, 2023). In India, it accounts for 20-25% of agricultural production and ensures food security for over half of the population. Rice production in India constitutes 55% of the total cereal production, with 116.48 million tons of rice being produced in the year 2018-19 from approximately 44.16 million hectares of planted rice land (AGRISTAT, 2019).

To develop varieties and hybrids based on specific needs, it is important to exploit the unique characters and existing variability present in the germplasm through crop improvement programs. An effective breeding program requires a comprehensive understanding of the nature and extent of genetic variability and the association among different traits within a species. Quantifying the level of divergence in experimental materials is highly valuable for identifying divergent genotypes that can be utilized in hybridization to generate new variations. The Mahalanobis D^2 statistic has been widely recognized as a powerful tool for plant breeders to select suitable parental genotypes with a broader range of variability for various traits (Sinha *et al*., 2020). The utilization of multivariate analysis tools like principal component analysis (PCA) has been found to be effective in assessing phenotypic diversity, identifying genetically distant Cluster s of genotypes, and selecting key traits that contribute to overall variation in genotypes. PCA enables the natural grouping of genotypes and provides a reliable indicator of differences among them. Consequently, the primary objective of any plant breeding program is to develop improved genotypes that surpass the existing ones in terms of economic yield. This calls for genetic enhancement through optimal utilization of allelic resources to create an ideal genotype (Pratap *et al*., 2012).

MATERIALS AND METHOD

The current study was conducted at the agriculture research farm of the Department of Genetics and Plant Breeding, School of Agriculture, Lovely Professional

University, Punjab during the 2022 season. A Randomized Block Design (RBD) with three replications was employed for thirteen observations recorded *viz.* days to 50 percent flowering (DFF), days to maturity (DM), panicle length (PL), plant height (PH), flag leaf area (FLA), effective tillers per plant (ETPP), number of grains per panicle (GPP), chlorophyll content (CC), grain yield per plant (GYPP), 1000 grain weight (TW), grain L/B ratio (LBR), alkali spreading values (ASV), and amylose content (AC%). List of genotypes used in present investigation tabulated in Table 1. The recorded observations were analyzed statistically using the procedure for analysis of variance suggested by Panse and Sukhatme (1967) to estimate the variability. Standard statistical procedures were applied to the recorded data. Genetic divergence analysis was conducted using the D^2 statistics introduced by Mahalanobis (2008); and described by Rao (1952) along with Principal Component Analysis (PCA). The software utilized for the analysis were INDOSTAT 9.2 and R Studio.

RESULT AND DISCUSSION

Analysis of variance showed significant differences for yield and nutritional traits in the present investigation indicating existence of sufficient variation among the genotypes and therefore an ample scope for effective selection.

A. Grouping of genotypes into various Clusters

A perusal of the results on grouping of genotypes (Fig. 1 and Table 2) revealed that the 30 genotypes were grouped into eleven Clusters based on the relative magnitude of D^2 values using agglomerative hierarchical Clustering complete linkage based on Mahalanobis distance (Berkhin *et al*., 2006). The genotypes belonging to same Cluster had an average smaller D^2 value than those belonging to different Clusters. Among the eleven Clusters, Cluster 1 was largest comprising of thirteen genotypes followed by Cluster 2 with five genotypes, Cluster 8 with three genotypes and Cluster 5 with two genotypes whereas remaining Clusters *viz.*, Cluster 3, 4, 6, 7, 9, 10 and 11with only one genotype.

The genotypes present in different Clusters showed high degree of diversity than the genotypes present in the same Cluster Genotypes from same geographic location fell into different Cluster s indicating that Clustering of genotypes did not follow their geographic or location distribution. These findings are in conformity with the reports of Ashok *et al.* (2017); Prasad *et al*. (2018); Devi *et al*. (2019); Sudeepthi *et al*. (2020).

B. Average intra and inter-Cluster D^2 value

An analysis of inter and intra- Cluster distances (Table 3) revealed that the inter-Cluster D^2 values ranged from 7.88 (Cluster 3 and 4) to 26.99 (Cluster 2 and 11) indicating presence of broad spectrum of genetic diversity among the genotypes present in the Clusters. Maximum inter Cluster distance was observed between Cluster 2 and 11 (26.99), followed by Cluster 2 and 10 (26.38) indicating that genotypes from these Clusters were highly divergent and holds great promise as parents for hybridization. The greater the distance between two Clusters, the wider would be the genetic diversity between the genotypes. Therefore, hybridization between the genotypes of the above clusters is expected to result in greater variability and transgressive segregants. Minimum inter-cluster distance was observed between cluster 3 and 4 (7.88), indicating their close relationship and similarity concerning the characters studied for the genotypes in these two clusters. The inter-cluster distances were higher than the intra-cluster distances which indicate the existence of substantial diversity among the genotypes. Similar results of inter and intra cluster distances in rice were reported earlier by Behera *et al*. (2018).

Intra cluster D^2 values ranged from 0 (cluster 3) to 9.47 (cluster 8). Maximum intra cluster distance was observed in cluster 8 (9.47) followed by cluster 2 (9.27). It reveals that genotypes present in the same cluster have low level of diversity and selection of parents with in the cluster for hybridization programme may not be considered promising.

Table 1: List of genotypes studied in the present investigation.

Genotype No.	Genotype Name	Genotype No.	Genotype Name
1	Phule Samruddhi	**16**	Swarna sub-1
2	PR 126	**17**	PB-1509
3	Indrayani	**18**	RTN -6 (Ratnagiri- 6)
4	HUR-105	**19**	PB-7
5	HKR-127	**20**	Pusa-44
6	Samba Mahsuri (BPT-5204)	**21**	PB-1692
7	Karjat-7	**22**	PR-124
8	PR-128	**23**	PR-122
9	Sarjoo-52	**24**	HKR-126
10	HUR-2-1	**25**	IR-64-Sub1
11	Karjat-184	**26**	CR Dhan 800
12	DRR Dhan -50	**27**	HUR-36
13	HKR-47	**28**	Kalajeera
14	IR-82635-B-B-72-2 (BRR Dhan 66)	**29**	Ambemohar
15	PR 130	**30**	Phule Maval

Table 2: Clustering pattern of 30 rice genotypes for yield and its attributing traits.

Sr. No.	Clusters	No.	Genotype number
1.	Cluster 1	13	14, 24, 6, 18, 11, 22, 4, 29, 1, 30, 21, 7, 9
2.	Cluster 2	5	3, 26, 2, 13, 25
3.	Cluster 3	1	20
4.	Cluster 4	1	15
5.	Cluster 5	2	8,10
6.	Cluster 6	1	12
7.	Cluster 7	1	17
8.	Cluster 8	3	5,27,28
9.	Cluster 9	1	16
10.	Cluster 10	1	19
11.	Cluster 11	1	23

C. Cluster means

Cluster means represent average performance of all genotypes present in that particular cluster. Cluster means provides information on suitable parents for improvement of particular traits. In the present study cluster means revealed considerable variation between the clusters for all the characters. The cluster mean for grain yield and nutritional characters are presented in Table 4. A perusal of these results revealed considerable differences between the clusters for all characters under study.

Cluster mean values for days to 50 % flowering were highest in cluster 8 (100.35 days) and lowest in cluster 4 (74.95 days). Cluster mean for days to maturity was highest in cluster 6 (140.50) and lowest in cluster 7 (114.17), while for panicle length it was highest in cluster 3 (30.93) and lowest in cluster 8 (23.73). Plant height was highest in cluster 2 (121.25) and lowest in cluster 4 (92.64). Similarly, flag leaf area was highest in cluster 9 (101.27) and lowest in cluster 10 (47.47), while effective tillers per plant was highest in cluster 3 (13.85) and lowest in in cluster 9 (7.38). Further, number of grains per panicle was highest in cluster 11 (183.51) and lowest in cluster 6 (109.18). 1000 grain weight was highest in cluster 3 (30.77) and lowest cluster 9 (18.21). Chlorophyll content was highest in cluster 4 (47.04) and lowest in cluster 7 (30.03). Similarly, grain L/B ratio was highest in cluster 7 (4.33) and lowest in cluster 8 (2.16). Alkali spreading values was highest in cluster 5 and 11 (5.00) and lowest in cluster 2 (2.20). Amylose content was highest in cluster 5 (26.19) and lowest in cluster 11 (20.19). While Grain yield per plant was highest in cluster 3 (38.04) and lowest in cluster 9 (26.49).

Selection of genotypes from clusters with high mean for the respective traits is suggested for utilization in hybridization programme aimed at improvement of the respective traits. A perusal of these results also revealed that there was no single cluster with all the desirable traits, which ruled out the possibility of direct selection of genotypes for immediate use. Therefore, judicious combination of selected genotypes from the above divergent clusters may be carried out to obtain desirable segregants with respect to nutritional properties coupled with high yield potential. The results are in broad agreement with the reports of Sudeepthi *et al*. (2020); Singh *et al*. (2020).

D. Principal Component Analysis (PCA)

In the present study, the first five principal components with Eigen value more than one contributed 75.56% towards the total variability. The Eigen values, proportion of total variance represented by principal components of importance and the component loading of different characters for the principal components are presented in Table 5 and Fig. 2.

The first principal component (PC1) contributed 45.65 per cent towards variability. The characters namely, amylose content (0.23), days to 50% flowering (0.320), plant height (0.15) and Days to maturity (0.14) explained maximum variance in this component. The second principal component (PC2) contributed 24.51% of total variation. The characters Alkali spreading values (0.47) explained maximum loadings in this component. Likewise, the third principal component (PC3) contributed 7.87% of total variability. The characters amylose content (0.78), Alkali spreading values (0.25), L/B ratio 90.21) and days to 50 % flowering (0.12) explained maximum variance in this component. Similarly, the fourth principal component (PC4) contributed 6.08 % of total variation and the characters namely, Plant Height (0.63), Flag Leaf Area (0.32), amylose content (0.28), effective tillers per plant (0.27), grain yield per plant and 1000 Grain Weight (0.26), Alkali spreading values (0.25), L/B ratio 90.21) and days to 50 % flowering (0.12) showed maximum variance in this component.

Among all the principal components, PC1 showed maximum variability of 45.65% with high Eigen value 1540, which decreased gradually indicating that maximum variation, was observed in PC1 as compared to the other PC's. The yield and its component characters mainly contribute variation in PC1, PC2 and PC3. The PCA analysis identified that maximum contributing traits towards the existing variability. Hence, these characters may be considered as principal discriminatory traits for this germplasm indicating that selection is effective in favour of these traits. Similar results were reported by Beevi *et al*. (2015); Archana *et al.* (2018) for number of filled spikelets per panicle Prasad *et al*. (2018), for spikelet fertility and 1000 seed weight, for grain yield per plant and 1000 grain weight by Sowmiya and Venkatesan (2017), for plant height, spikelet per panicle and grain yield /plant by Raghavendra *et al*. (2018).

The PCA scores for 30 rice genotypes in the first three principal components were computed and were considered as three axes as X, Y and Z and squared distance of each genotype from these 3 axes were calculated and are presented in Table 6. These PCA scores for 30 genotypes were plotted in a graph to get the three-dimensional scatter diagram (Fig. 2).

Table 3: Average intra and inter–cluster D^2 values among eleven clusters of 30 rice genotypes.

Cluster Distances											
	Cluster 1	**Cluster 2**	**Cluster 3**	**Cluster 4**	**Cluster 5**	**Cluster 6**	**Cluster 7**	**Cluster 8**	**Cluster 9**	**Cluster 10**	**Cluster 11**
Cluster 1	**9.07**	16.89	10.57	12.38	14.43	12.41	12.06	14.71	13.32	16.57	13.78
Cluster 2	16.89	**9.27**	21.69	20.39	24.04	19.76	19.02	16.46	21.16	26.38	26.99
Cluster 3	10.57	21.69	**0**	7.88	16.17	9.06	9.09	20.08	16.02	9.71	10.06
Cluster 4	12.38	20.39	7.88	**0**	19.98	11.16	8.49	22.69	19.29	10.87	15.8
Cluster 5	14.43	24.04	16.17	19.98	**8.08**	16.82	17.48	14.86	13.73	18.5	12.76
Cluster 6	12.41	19.76	9.06	11.16	16.82	**0**	9.85	20.01	17.25	11.12	14.66
Cluster 7	12.06	19.02	9.09	8.49	17.48	9.85	**0**	19.88	17.67	9.43	14.76
Cluster 8	14.71	16.46	20.08	22.69	14.86	20.01	19.88	**9.47**	15.75	25.04	20.74
Cluster 9	13.32	21.16	16.02	19.29	13.73	17.25	17.67	15.75	**0**	22.27	13.28
Cluster 10	16.57	26.38	9.71	10.87	18.5	11.12	9.43	25.04	22.27	**0**	14.09
Cluster 11	13.78	26.99	10.06	15.8	12.76	14.66	14.76	20.74	13.28	14.09	**0**

Table 4: Mean values of eleven clusters by Tocher's method for 30 rice genotypes (*Oryza sativa* L.).

Cluster Means													
	DFF	**DM**	**PL**	**PH**	**FLA**	**ETPP**	**GPP**	**TW**	**CC**	**LBR**	**ASV**	**AC**	**GYPP**
Cluster 1	85.77	128.82	25.54	104.49	59.07	11.73	157.55	27.47	39.23	3.23	3.54	21.53	35.25
Cluster 2	96.97	139.32	25.04	121.25	60.57	10.02	147.33	22.36	36.09	2.94	2.20	23.99	29.63
Cluster 3	79.60	123.11	30.93	113.87	57.10	13.85	131.38	30.77	37.16	3.80	4.00	20.28	38.04
Cluster 4	74.95	114.58	30.50	92.64	52.19	13.32	139.78	29.04	47.04	4.25	3.00	21.52	36.23
Cluster 5	79.49	120.47	23.87	97.35	65.14	9.68	151.32	24.06	33.50	2.73	5.00	26.19	31.86
Cluster 6	91.75	140.50	26.64	116.62	81.87	13.32	109.18	26.21	36.25	3.89	4.00	23.13	35.55
Cluster 7	84.01	114.17	29.01	117.42	54.04	9.32	159.38	21.71	30.03	4.33	4.00	23.43	28.55
Cluster 8	100.35	139.97	23.73	113.39	52.43	10.38	158.91	22.36	34.41	2.16	4.00	24.46	27.67
Cluster 9	83.97	125.81	30.18	109.88	101.27	7.38	167.71	18.21	32.28	2.59	4.00	21.49	26.49
Cluster 10	76.61	116.44	30.06	107.21	47.47	12.65	141.05	29.11	33.05	4.64	4.67	24.25	30.63
Cluster 11	78.01	120.83	27.16	114.05	70.87	10.65	183.51	28.34	33.93	3.48	5.00	20.19	31.00

Table 5: Eigen values, proportion of total variance represented by the principal components, cumulative per cent variance and component loading of different characters.

	PC I	**PC II**	**PC III**	**PC IV**
Eigen Value (Root)	**1540.002**	**826.8153**	**265.5674**	**205.3281**
% Var. Exp.	**45.65916**	**24.51405**	**7.87375**	**6.08772**
Cum. Var. Exp.	**45.65916**	**70.17321**	**78.04697**	**84.13469**
Days to 50% flowering	0.2	0.07	0.12	0.23
Days to maturity	0.14	0.06	-0.06	0.3
Panicle Length (cm)	-0.07	-0.1	0	0
Plant Height	0.15	-0.03	-0.03	0.63
Flag Leaf Area (L × B)	-0.09	0.09	-0.23	0.32
ETPP	-0.08	-0.13	-0.19	0.27
No. of Grains /Panicle	-0.01	0.03	-0.17	-0.1
1000 Grain Weight	-0.18	-0.16	-0.27	0.26
Chlorophyll content	-0.03	-0.15	-0.22	0.14
L/B Ratio	-0.4	-0.81	0.21	-0.03
ASV	-0.81	0.47	0.25	0.19
AC %	0.23	-0.07	0.78	0.28
Grain Yield / Plant	-0.03	-0.15	-0.15	0.26

Table 6: PCA scores of divergence in 30 rice genotypes.

Genotype	X Vector	Y Vector	Z Vector
Variety 1	-29.864	-31.675	30.371
Variety 2	-17.575	-32.174	35.872
Variety 3	-13.19	-31.271	38.804
Variety 4	-33.186	-28.366	32.646
Variety 5	-22.766	-21.979	38.122
Variety 6	-31.708	-26.893	38.285
Variety 7	-28.32	-30.632	33.942
Variety 8	-34.181	-18.872	38.351
Variety 9	-23.728	-27.202	34.11
Variety 10	-33.707	-18.464	40.073
Variety 11	-32.662	-27.043	35.304
Variety 12	-32.505	-32.915	38.935
Variety 13	-17.395	-32.442	35.42
Variety 14	-30.104	-25.584	33.891
Variety 15	-33.166	-37.301	35.314
Variety 16	-30.136	-20.926	30.679
Variety 17	-31.948	-33.902	40.109
Variety 18	-27.832	-27.82	38.688
Variety 19	-39.748	-34.845	42.283
Variety 20	-35.51	-32.28	34.424
Variety 21	-25.213	-30.313	31.634
Variety 22	-29.89	-25.698	33.259
Variety 23	-40.236	-24.691	32.928
Variety 24	-28.778	-23.791	34.384
Variety 25	-13.449	-28.51	35.408
Variety 26	-13.865	-30.9	37.013
Variety 27	-21.799	-19.088	36.875
Variety 28	-21.448	-16.031	36.285
Variety 29	-27.97	-29.891	32.411
Variety 30	-28.017	-30.529	31.783

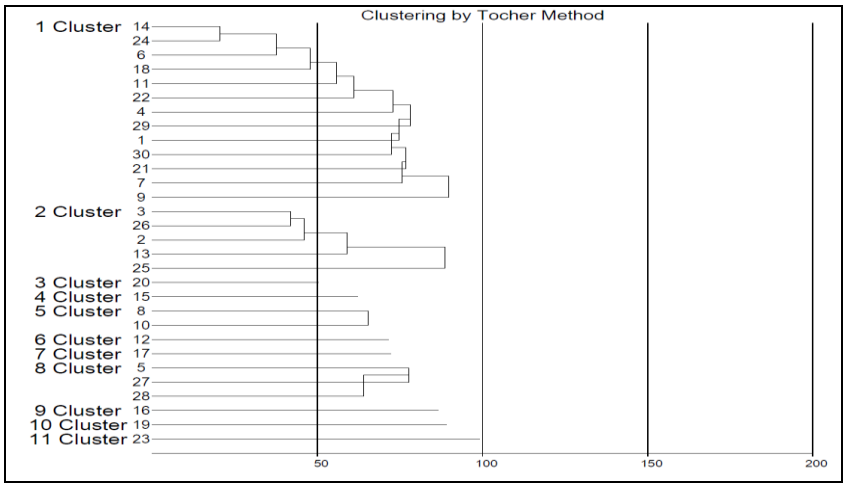

Fig. 1. Dendrogram showing relationship among 30 rice genotypes in 11 clusters based on Mahalanobis distance.

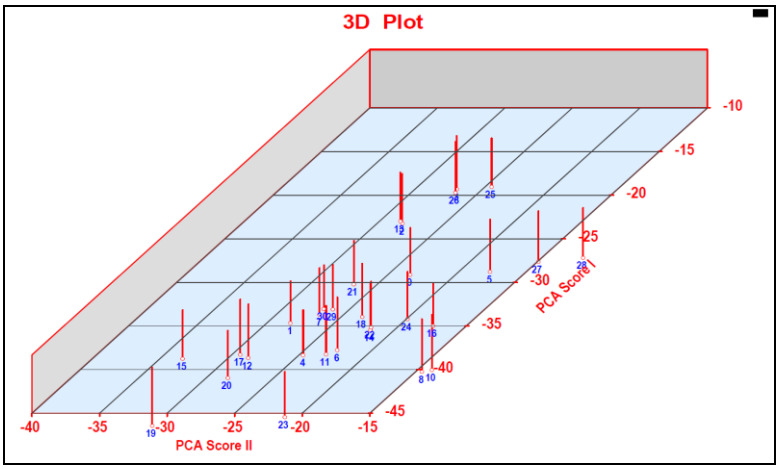

Fig. 2. Three-dimensional graph showing relative position of 30 rice genotypes based on PCA score.

CONCLUSIONS

Based on the findings of the present investigation, it can be concluded that there is sufficient genetic variability observed in the evaluated quantitative traits. This suggests that there is potential for selecting genotypes with high grain yield by targeting different clusters and conducting appropriate crosses to improve overall yield. The study has also identified a genotype that performed exceptionally well, which could be recommended for further evaluation and potential commercialization. For future breeding programs that involve hybridization, it is advisable to select parental material from different clusters rather than within clusters. This approach would maximize the potential for combining favorable traits and enhancing overall performance. Among the eleven Clusters, Cluster 1 was largest comprising of thirteen genotypes followed by Cluster 2 with five genotypes. Maximum inter cluster distance was observed between cluster 2 and 11, followed by cluster 2 and 10 indicating that genotypes from these clusters were highly divergent and holds great promise as parents for hybridization. Maximum intra cluster distance was observed in cluster 8 followed by cluster 2. It reveals that genotypes present in the same cluster have low level of diversity and selection of parents with in the cluster for hybridization programme may not be considered promising. Additionally, it is recommended to replicate the study across multiple seasons and locations, involving a larger number of genotypes. This would provide more robust and reliable predictions of genotypic performance across diverse environments, helping to validate the current results. Furthermore, to complement the findings of the current study, it is suggested to incorporate molecular characterization techniques in future rice research. Molecular characterization can provide additional insights and confirmation of the observed outcomes, further enhancing our understanding of the genetic basis of the traits under investigation.

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Conflict of Interest. None.

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