

RESEARCH ARTICLE

Unveiling Genetic Variation in Rice Hybrids Through Hierarchical Clustering and

Principal Component Analysis

Vijay Kumar Reddy C, Amarnath K and Ravi Kumar BNVSR* Unveiling Genetic Variation in Rice Hybrids Through Hierarchical Clustering and Principal Component Analysis

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Abstract

The present investigation was carried out with 67 rice hybrids along with eight checks (four varietal and four hybrid checks) to ascertain the extent of genetic diversity for yield and yield associated traits through multivariate techniques like hierarchical clustering and principal component analysis (PCA). By using Wards method of clustering, 67 rice hybrids along with eight checks were aggregated into eight clusters based on different traits in which cluster VI comprised of 15 hybrids is the largest one followed by cluster III and IV with 12 hybrids. The hybrids in cluster I and II had highest values for test weight and effective bearing tillers /m² respectively. Similarly, the hybrids in cluster III recorded maximum values for plant height and Grain yield. In PCA, the total variation was bisected into 10 major principal components (PCs) in which PC1, PC2, PC3 and PC4 with eigen values more than one describing 24.76%, 23.26%, 14.54 and 13.22%, respectively attributing for overall variation of 75.80%. From the present study, the hybrids viz., NRH 24, NRH 46, NRH 40, NRH 38, NRH 53, NRH 2; Hybrid checks HC2 (US 314), HC4 (HRI 174) and varietal check VC1 (BPT 5204) were identified to be genetically potential for commercial exploitation to enhance yield and its attributing traits in rice.

Keywords: Rice, Genetic diversity, Cluster analysis, Principal component analysis

Introduction

Rice (Oryza sativa L.), a global food grain and an important staple food crop for half of the global population. Globally, India holds second place in rice production next to China. The major rice producing states in India include West Bengal, UP, Andhra Pradesh, Punjab, Telangana and Tamil Nadu. Besides, West Bengal and Uttar Pradesh produce 30% of total quantity of rice produced in the country. In India, the rice crop reported a production of 203.6 million tonnes from 47.8 million ha with average productivity of 4259 Kg/ha (https://ipad.fas.usda.gov/ countrysummary/Default.aspx?id=IN&crop=Rice). In Andhra Pradesh, the crop is cultivated in total area of 2.13 million ha with production of 12.63 million tonnes and productivity of 5932 million tonnes (Agricultural statistics at a glance, 2022-23, Directorate of Economics and Statistics, Government of Andhra Pradesh, 2022-23). In the present national scenario, the population growth rate is reached to 1.58% and the requirement of rice was estimated to be around 140.7 million tonnes by 2025 (http:// worldfood.apionet.or.jp). In order to make India selfsufficient in rice, enhancement of rice productivity to larger extent is a prime requisite (Hossain, 1996; Mishra, 2002). Despite this, task is quite challenging for breeders as the options available are very limited. Hence, breeders need to identify genetically diverse and potential hybrids for their inclusion in crop

improvement programme by divergence studies for yield and its attributing traits.

Although yield is a complex and challenging trait which depends mainly on environment and other different variables. For an efficient selection, breeder can reduce the number of characteristics and this can be made possible by using a method known as PCA. PCA is one of the multivariate techniques utilized in data analysis that converts data into a series of new orthogonal variables called principal components by linearly combining the variables that account for majority of variance in the original variables (Abdi and Williams, 2010) and it comprehends non-parametric strategy from a complicated set of data (Tiwari et al., 2022). The main advantage of using PCA over cluster analysis is that each genotype can be assigned to one group only (Mohammadi, 2002). The cluster analysis is a pertinent method for concluding relationship among hybrids and amount of genetic distance from each other (Mellingers, 1972). Divergence analysis using PCA and hierarchical cluster analysis has been shown to be effective in determining potential hybrids useful for hybridization (Chaudhary et al., 2015). The prime objective of this investigation was to unveil genetic diversity and identify the best divergent hybrids for their inclusion in commercial rice improvement programme.

Materials and Methods

The experimental material consisted of 67 rice hybrids along with eight checks (four varietal and four hybrid checks) which were evaluated at Regional Agricultural Research Station (RARS), Nandyal, Andhra Pradesh, India during kharif, 2023. All the hybrids were evaluated in Augmented Block Design with plot size of 10 $m²$ per hybrid with a spacing of 20 cm x 15 cm. All the agronomic practices recommended by Acharya N.G. Ranga Agricultural University were followed to raise a healthy crop. Data was collected 5) among the studied hybrids. Vasudeva Reddy et al., from five competitive and randomly selected plants for recording yield and yield attributes viz., Days to 50% flowering (DFF), Days to maturity (DM), Plant

height (cm) (PH), Effective bearing tillers/m² (EBT/ m2), Panicle length (cm) (PL), Filled Grains per panicle (FGP), Unfilled grains per panicle (UFGP), Total grains per panicle (TGP), Spikelet fertility % (SF %), Test weight (g) (TW) and grain yield (Kg/m2)(GY) whereas DFF and DM data was recorded on plot basis. PCA (Hotelling, 1936) and cluster analysis were used to identify the most contributing traits for variation and diversity among hybrids, respectively (Peeters and Martinelli, 1989). The data generated by evaluation of hybrids s forwarded to statistical analysis to estimate genetic diversity through PCA and hierarchical clustering using JMP 17.0 statistical software (SAS Institute Inc., Cary, NC, USA).

Results and Discussions

The performance of 67 rice hybrids together with eight checks evaluated for eleven yield and yield attributes is presented in Table 1. The hybrid NRH 3 was found to be early flowering (81 days), while VC2(MTU 1262) was identified as late flowering (114 days). DM ranged from 109 days (NRH2) to 144 days (NRH 51). NRH 36 was found as taller (123 cm) whereas VC1 (BPT 5204) noticed as shorter (87.70 cm) with an average of 106.98 cm. The hybrid NRH 43 was found to bear a greater number of effective tillers $(726 / m^2)$, while least number of effective tillers $(238 / m^2)$ was displayed by hybrid, NRH 8. The mean value of PL was found to be 25.19 cm with range of 21.22 cm (HC2) to 27.86 cm (NRH 55). The TGP ranges from 160 (HC 2) to 599 (NRH 40) with mean value of 337. The mean SF % was recorded as 84.55% in which NRH 34 topped the list with 93.64% and NRH 17 occupied the bottom position with 60.06%. The TW ranged from 11.27 g (NRH 40) to 23.27 g (NRH 11) with average TW value of 15.98 g. The mean GY was recorded as 1.27 Kg/m² with ranges from 0.612 Kg/m2 (NRH 26) to 2.152 Kg/m2 (NRH (2023) reported same kind of experimental results. while evaluating hybrids for yield and its component traits in rice.

Hierarchical cluster analysis was conducted with 67 rice hybrids along with eight checks using Wards method which provides the best result to get the finest possible classification. The cluster analysis revealed the aggregation of hybrids into eight clusters (Table 2 and Figure 1). The cluster means computed for eleven major yield attributing characters revealed the existence of ample amount of variation among the clusters (Table 3). The highest and lowest cluster means were recorded for the traits EBT/m^2 (631.4) and GY (1.00), respectively. Maximum cluster mean of overall traits was noticed in cluster VII (174.60) followed by cluster VIII (154.20). In contrast, the least cluster mean was displayed by cluster I et the finest possible classification. The cluster clusters, the highest numbers of hybrids words

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 (631.4) and Tejaswini et al., (2018), Muthuramu and Sakthivel (121.70). This clearly infers the existence of ample amount of genetic divergence in the hybrids of these clusters. Further, among the eight divergent clusters, the highest numbers of hybrids were grouped in cluster VI with 15 hybrids followed by 12 hybrids in cluster III and IV. The hybrids in cluster IV showed highest mean values for DFF, DM, PH, EBT/m² and SF %. The hybrids of cluster III and cluster VIII showed maximum value for GY. Ravikumar et al., (2015), Tejaswani et al., (2016), (2018), Dhakal et al., (2020), Kusuma Kumari et al., (2021) and Amudha and Ariharasutharsan (2021) also documented same kind of clustering of accessions into distinct clusters.

Wards method

DFF-days to 50% flowering, DM-days to maturity, PH-plant height, EBT/m²- Effective bearing tillers/ per panicle, S m², PL-panicle length, FGP-filled grains per panicle, weight, GY-g

UFGP-unfilled grains per panicle, TGP-total grains per panicle, SF %- spikelet fertility %, TW-test weight, GY- grain yield

Table 3: Cluster means of various characters of rice hybrids under study												
Cluster No.	DFF	DM	PH	EBT/m^2	PL	FGP	UFGP	TGP	SF%	TW	GY	Mean
Cluster I	96.9	125.7	109.1	381.5	25.2	199.5	50.0	249.4	80.3	19.8	1.2	
Cluster II												
												121.7
Cluster III	98.3 96.8	126.9	106.4 114.7	631.4	25.2 26.2	235.6	44.2	279.9	84.1	17.3	1.3	150.1 145.7
Cluster IV	87.1	125.5 115.5	101.2	484.3 373.5	25.7	287.6 322.4	37.1 34.6	324.7 357.1	88.7 90.4	15.8 16.2	1.5 1.2	138.6
Cluster V	94.8	124.5	101.2	417.9	23.8	227.5	28.6	256.1	88.5	15.3	1.3	125.4
Cluster VI	100.5	129.3	110.5	386.1	25.1	263.0	87.7	350.7	74.7	16.3	1.3	140.5
Cluster VII	94.8	123.8	104.8	408.1	25.3	448.2	84.6	532.8	84.0	13.6	1.0	174.6
Cluster VIII	103.7	132.2	106.1	386.8	24.8	365.7	54.6	420.3	87.0	13.2	1.4	154.2
	96.6	125.4	106.7	433.7	25.2	293.7	52.7	346.4	84.7	15.9	1.3	
Mean values Bold figures indicate maximum and minimum values							greater than one explaining 75.80% of total variation.					
in each character. DFF-days to 50% flowering, DM-							The eigen values and total cumulative per centage of					

Table 3: Cluster means of various characters of rice hybrids under study

Bold figures indicate maximum and minimum values in each character. DFF-days to 50% flowering, DMdays to maturity, PH-plant height, EBT/m²- Effective bearing tillers/m², PL-panicle length, FGP-filled PC1 with eigen grains per panicle, UFGP-unfilled grains per panicle, TGP-total grains per panicle, SF %- spikelet fertility %, TW-test weight, GY- grain yield

The constellation plot based on Wards method (Figure 2) depicts relationship among the 67 hybrids together with eight checks. The hybrids are grouped as end points and every cluster join as a new point with lines drawn will act as membership in constellation plot. The plot divided the total hybrids into 8 clusters with membership of 5,9,12,12,11,15,6 and 5. The Clustering pattern divulged that majority of hybrids congregated in cluster VI (15), followed by Cluster III (12) and Cluster IV (12). The hybrids with longer 50 line representing greater genetic distance between the clusters. Further, the identified hybrids with maximum genetic distance are considered as superior γ and exploited commercially in yield improvement programme in rice.

The PCA an authentic tool utilized for successful sso selection of divergent genotypes in crop improvement programme. The results of PCA revealed the significance of first four PCs in discriminating 67 rice hybrids along with eight checks. The first four PCs, PC1, PC2 PC3 and PC4 exhibited eigen value

variances explained by PCs is furnished in Table 4. greater than one explaining 75.80% of total variation. The eigen values and total cumulative per centage of PC1 with eigen value of 2.724 contribute 24.76% of the total variability, PC2, PC3 and PC4 with eigen value of 2.559, 1.604 and 1.455 attributed 23.62%, 14.54% and 13.22% of the total variability, respectively. The first PC displayed high positive weight to UFGP (0.850), DFF (0.645), TGP (0.624) and DM (0.622). The second PC displayed highest positive loading to DFF (0.569), DM (0.563) and EBT (0.438). Likewise, the third and fourth PCs gave positive loading to GY (0.588) and TW (0.661) , respectively (Table 5).

Figure 2: Constellation plot of 67 hybrids along with eight checks into Eight clusters based on Euclidean distance

Journal of Rice Research 2024, Vol 17, No. 2 \star 39

DFF-days to 50% flowering, DM-days to maturity, PH-plant height, EBT/m²- Effective bearing tillers/m², PL-panicle length, FGP-filled grains per panicle, UFGP-unfilled grains per panicle, TGP-total grains per panicle, SF %- spikelet fertility %, TW-test weight, GY- grain yield

The greater portion of the variance (24.76%) was noticed in PC1and was strongly convinced DFF, DM, PH, PL, FGP, UFGP, TGP. Similarly, PC2 was influenced by DFF, DM, PH, EBT/m², TW and GY. length implying Likewise, PC3 and PC4 are primarily influenced by UFGP, TW and DFF, DM, EBT/m², FGP, SF, respectively. Similar kind of results are in agreement with findings of Nachimuthu et al., (2014), Allam et al., (2017), Riaz et al., (2018), Umadevi et al., PCA-Biplot (2019), Sudeepthi et al., (2020), Singh et al., (2020), Pushpa et al., (2021), Christina et al., (2021), Dhanuja et al., (2021), Venkata Ratnam et al., (2022), Lakshmi et al., (2022), Mushtaq and Kumar (2023), Nayak $\frac{20}{8}$ et al., (2023), in rice.

The interaction between the characters and the $\frac{NRH_2}{NRH_3}$ genotypes that perform better for the traits are depicted in the biplot diagram. The length of the vector for each trait represents its offering to total divergence, longer the vector length, more is the contribution of

, FGP, SF, congruence with the research findings of Lakshmi concerned traits. The biplot depicted the relationship of 67 rice hybrids along with eight checks for 11 traits (Figure 3). The trait TG/P displayed greater vector length implying its contribution to the total divergence followed by DFF, SF %and FGP. These results are in et al., (2022), Tiwari et al., (2022) and Gayathridevi et al., (2023).

Figure 3: Biplot comprising of 67 rice hybrids along with eight hybrids studied for eleven yield and yield attributing traits

 $40 \div$ Journal of Rice Research 2024, Vol 17, No. 2

DFF-days to 50% flowering, DM-days to maturity, PH-plant height, EBT/m²- Effective bearing tillers/ crop improvem m², PL-panicle length, FGP-filled grains per panicle, clusters. NRH UFGP-unfilled grains per panicle, TGP-total grains per panicle, SF %- spikelet fertility %, TW-test weight, GY- grain yield

The angle formed by the vectors of the traits indicates the association between the traits. A right angle (90º) between the vectors denotes no correlation, while an obtuse angle (>90º) denotes a negative correlation and an acute angle (<90º) between vectors suggests a positive correlation. All the traits studied displayed positive correlation with grain yield per plant except UFGP which noticed no correlation. From the biplots, the 11 yield and yield attributing traits were divulged into four groups. GY, TW and EBT were grouped in same cluster. DFF, DM and PH were grouped in same cluster. The traits UFGP, PL, TGP and FGP were grouped in another cluster. Whereas, SF % alone grouped as one cluster. The selection of hybrids with desirable highest score (0.588) for grain yield in PC3 will be desirable for developing high grain yielders in rice. The study showed that NRH 24, NRH 46, NRH 40, NRH 38, NRH 53, NRH 2; Hybrid checks HC2 (US 314), HC4 (HRI 174) and varietal check $\frac{A_{\text{D}}}{A_{\text{D}}}\frac{H}{A_{\text{D}}}\frac{H}{A_{\text{D}}}\frac{H}{A_{\text{D}}}\frac{H}{A_{\text{D}}}\frac{H}{A_{\text{D}}}\frac{H}{A_{\text{D}}}\frac{H}{A_{\text{D}}}\frac{H}{A_{\text{D}}}\frac{H}{A_{\text{D}}}\frac{H}{A_{\text{D}}}\frac{H}{A_{\text{D}}}\frac{H}{A_{\text{D}}}\frac{H}{A_{\text{D$ VC1 (BPT 5204) were located at extreme ends of distinct quadrants of the plot. Hence, theses hybrids and checks were recognized as highly divergent and found to be potential for exploitation in hybridization programme to enhance heterotic potential in rice crop. These results are in congruence with findings $sativa L$.). Elements of R_{ch} (2012). Regular $sl(1)$: 269-278. of Rahimi et al., (2013), Pandit et al., (2016), Sharafi et al., (2018), Divya et al., (2022), in rice.

Conclusion

PCA concluded that the first four PCs with eigen values more than one describing 24.76%, 23.26%, 14.54 and 13.22%, respectively attributed 75.80% of total variation. The cluster analysis exhibited $40(4)$: 201-208.

high genetic diversity, indicating a great chance for crop improvement by employing hybrids from other clusters. NRH 24, NRH 46, NRH 40, NRH 38, NRH 53, NRH 2 were identified as promising hybrids and can be used in developing diverse and heterotic inbred lines. Besides, the hybrids viz., NRH 5 (49.89%), NRH 14 (28.54%), NRH 11(24.09%), NRH 16 (22.01%), NRH 18 (19.02%), NRH 36 (17.90%), NRH 27 (16.57%), NRH 47 (16.52%) were recognized as best heterotic hybrids for yield over best hybrid check HC4 (HRI 174) that governed by dominant genes and hence these hybrids can be advanced to evaluate under multi-location trails and further forwarded for commercial exploitation.

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- 42 ★ Journal of Rice Research 2024, Vol 17, No. 2

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Journal of Rice Research 2024, Vol 17, No. 2 \star 43