Assessing the genetic diversity for yield traits in rice (*Oryza sativa* L.) genotypes using multivariate analysis under controlled and water stress conditions

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The genetic diversity of yield and yield attributing characteristics was explored in this research. In the topical study, fifty-two rice genotypes including four checks were used under three environmental conditions i.e. irrigated (IR), rainfed (RF) and terminal stage drought (TSD) conditions. The prevalence of genetic divergence was evaluated using clustering and Principal component analysis (PCA) was used to determine the relative contribution of various traits. To fulfill the aim of the study, fifty-two genotypes were grouped into three distinct and non-overlapping clusters among these 3 clusters, cluster-I was the largest with the highest number of genotypes i.e. 47, 49 and 49 under IR, RF and TSD conditions, respectively. The highest average intra-cluster distance was observed in cluster-I, also the genotypes showed high variability under all three conditions. The highest inter-cluster distance between the cluster-II and cluster-III (IR and TSD) and cluster-I and cluster-II (RF) was observed, indicated that genotypes from the group should be considered for direct use as parents in hybridization programme to produce high yield. Only five of the 13 principal components (PCs) have been considered in the study based on the Eigen values and variability criteria. From the complex matrix it was revealed that the first-PC accounted for the highest variability. Genotypes which fall under a common PC were observed to be the most important factor for grain yield.

Introduction

Rice (*Oryza sativa* L.) is a major staple crop that feeds more than half of the world’s population (Ricepedia, 2020; USDA, 2020). Rice genotypes from Chhattisgarh are critical for preserving and maintaining rice biodiversity. Rice germplasm is a valuable resource that must be protected. In order to produce superior hybrid and desirable transgressive segregants, genetic diversity plays a critical role in selecting suitable parents for the hybridization programme (Burman *et al.*, 2019). Cluster analysis is a numerical approach used for measuring genetic divergence in the germplasm lines. Although yield is a complex trait that is influenced by a variety of factors and the environment, principal component analysis was used to discover and minimize the number of traits for effective selection (Gaur *et al.*, 2019). Because it is a simple, non-parametric method for extracting crucial data from confusing data sets, PCA has become a standard tool in modern data analysis. It is a mathematical process that converts a set of (potentially) correlated variables into a set of
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(smaller) uncorrelated variables known as principal components. It decreases the data’s dimensionality while preserving the majority of data set variation. The first principal component accounts for as much variability as possible. Multivariate analysis (PCA) has been widely used in the selection of diverse parents in any hybridization programme. The main advantage of PCA is that it quantifies the value of each dimension in characterizing the variability of a data set (Raj et al., 2020). The current study was conducted to assess genetic divergence and PCA in 52 rice germplasm lines to discover yield related characteristics whose selection would result in an increase in rice grain yield.

Material and Methods
The current research was conducted at the Research Farm, Department of Genetics and Plant Breeding, Indira Gandhi Agricultural University, Raipur (Chhattisgarh) during kharif 2018 and kharif 2019 using Randomized Block Design (RBD) with two replications. The experimental material consists of 52 germplasm lines which were grown in two rows with row to row and plant to plant spacing of 20 × 20 cm maintained under three environmental conditions (IR, RF and TSD). In IR and TSD conditions, seeds were sown in nurseries and seedlings were transplanted as a single plant after twenty-one days and under RF condition, seeds were sown directly in both the seasons. The collected data were pooled over the season and the data recorded for thirteen yield traits were days to 50% flowering, plant height in cm, flag leaf length in cm, flag leaf width in cm, number of tillers per \( m^2 \), panicle length in cm, biological yield per plot in g, grain yield per plot in g, harvest index in per cent, thousand grain weight in g, number of filled grains per panicle, number of unfilled grains per panicle and total number of grains per panicle. Cluster analyses for the above characters were done by following Agglomerative hierarchical clustering (AHC) using XLSTAT. The Hierarchical clustering method’s structure is represented by a dendrogram. For the traits, intra and inter cluster distances, as well as mean cluster performance were calculated (Sudeepthi et al., 2020). Similarly, Multivariate Analysis (PCA) was performed by following Pearson correlation type (Kumari et al., 2019) using XLSTAT. For the traits, Eigen values, factor loading and principal component scores were calculated.

Results and Discussion
Cluster analysis
Cluster analysis divides the fifty-two rice genotypes into 3 clusters under three conditions (Table 1) and dendrogram showed in Figure 1. Cluster-I with 47 genotypes was the biggest cluster followed by cluster-II with 2 genotypes, while cluster-III was mono-genotypic under irrigated condition. Cluster-I with 49 genotypes had the most genotypes under rainfed and TSD conditions, followed by cluster-II, which had 2 genotypes and cluster-III was mono-genotypic. As per the topical study the intra and inter cluster under irrigated, rainfed and TSD conditions are shown in Table 2. The highest intra-cluster distance under all three conditions were found in cluster-I (IR-14.41), (RF-8.23), (TSD-8.18), and crossing between the genotypes of cluster-I produces better segregants with greater genetic diversity and genetic advance. The highest inter-cluster distance between the clusters-II and cluster-III under irrigated (30.56) and TSD (12.46) conditions and between the cluster-I and cluster-II under rainfed (13.38) condition, followed by cluster-I and cluster-II (26.96), cluster-I and cluster-III (15.87) under irrigated condition; cluster-II and cluster-III (10.87), cluster-I and cluster-III (9.91) under rainfed condition; cluster-I and cluster-III (10.53), cluster-I and cluster-II (8.21) under TSD condition, revealed greater diversity among these clusters and may be used in hybridization for the development of germplasm lines. Based on mean performance of three clusters (Table 3), the traits which showed high mean values were biological yield per plot, grain yield per plot, number of tillers per \( m^2 \) and total number of grains per panicle. The highest percent contribution (Table 4) were showed by the traits, harvest index under irrigated (16.104) and rainfed (13.598) conditions and thousand grain weight under TSD (11.800) condition. Kali Mai was the only genotype commonly observed in all three clusters. The genotypes falling in the same cluster (intra-cluster) are more closely related and less divergent than those which are placed in different clusters (inter-cluster).
Table 1: Pooled clustering pattern of fifty two rice genotypes in different water regimes during Kharif 2018 and 2019.

| Cluster No. | No. of genotypes | Name of genotypes | No. of genotypes | Name of genotypes | No. of genotypes | Name of genotypes |
|-------------|------------------|-------------------|------------------|-------------------|------------------|-------------------|
|             | Irrigated (Pooled) |                 | Rainfed (Pooled) |               | Terminal Stage Drought (Pooled) |
| I           | 47               | Bega hudi, Aajan, Banko, Barangi, Khurabal, Peelee Luchai, Nagbel, Bangoli-5, Byalo, Duggi, Saja chhilau, Surmatia, Baja bhog, Dhusari, Gandhak, Cross 116, IR 62266, Laloo-14, Aganni, Safri 17, Tarunbhog, Chepti Gurmatia (3011), Basmati 370, Kalanamak, Moroberekan, Pakshi Raj, Dokra Dokri, Parmal, Tedesi, Bisni, Dhaniya Phool, Tulsi Manjar, Sarai Phool, Bharma Tripal, Dudd Malai, Shonth, Chhind Guchchhi, Nayaikin Jhaba, Ramali Chonch, Roti, Hathi Panjara, Nangodar, Soth, Bajarang Bali, Kurso bhog, Swarna, Maheshwari | 49 | Bega hudi, Aajan, Banko, Barangi, Khurabal, Peelee Luchai, Nagbel, Bangoli-5, Byalo, Duggi, Saja chhilau, Surmatia, Baja bhog, Dhusari, Gandhak, Cross 116, IR 62266, Laloo-14, Aganni, Safri 17, Tarunbhog, Chepti Gurmatia (3011), Basmati 370, Kalanamak, Moroberekan, Nagina-22, Pakshi Raj, Dokra Dokri, Parmal, Tedesi, Bisni, Dhaniya Phool, Tulsi Manjar, Sarai Phool, Kharani, Bharma Tripal, Dudd Malai, Shonth, Chhind Guchchhi, Nayaikin Jhaba, Ramali Chonch, Roti, Hathi Panjara, Nangodar, Soth, Bajarang Bali, Kurso bhog, Swarna, Maheshwari, MTU 1010 |
| II          | 4                | Nagina-22, Kharani, Mahamaya, MTU 1010 | 2               | Basmati 370, Swarna | 2               | Bisni, Dhaniya Phool |
| III         | 1                | Kali Mai          | 1               | Kali Mai          | 1               | Kali Mai          |

Figure 1: Dendrogram of fifty-two rice genotypes in different conditions.

Table 2: Average intra (diagonal and bold) and inter cluster distance for irrigated, rainfed and terminal stage drought.
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Table 3: Cluster mean value for different traits under different conditions.

| Cluster | Irrigated | | | Rainfed | | | TSD | | |
|---------|-----------|---|---|---------|---|---|---|---|---|
|         | I         | II | III | I       | II | III | I   | II  | III |
|         | 14.41     | 26.96 | 15.87 | 8.23 | 13.38 | 9.91 | 8.18 | 8.21 | 10.53 |
|         | 13.25     | 30.56 | 5.35  | 10.87 | 7.62  | 12.46 | 0.00 | 0.00 | 0.00 |

Note: DTF= days to 50% flowering, PH= plant height (cm), FLL= flag leaf length (cm), FLW= flag leaf width (cm), NT= number of tillers m⁻², PL= panicle length (cm), BY= biological yield/plot (g), GY= grain yields per plot (g), HI= harvest index (%), TGW= thousand grain weight (g), NFG= number of filled grains per panicle, NUFG= number of unfilled grains per panicle, TNG= total number of grains per panicle

Table 4: Percent contribution of each character under various conditions

| Traits | IRRIGATED | RAINFED | TSD |
|--------|-----------|---------|-----|
| DTF    | 3.771     | 4.192   |     |
| PH     | 6.353     | 6.292   |     |
| FLL    | 5.715     | 7.054   |     |
| FLW    | 5.945     | 6.519   |     |
| NT     | 5.118     | 6.128   |     |
| PL     | 5.087     | 3.554   |     |
| BY     | 8.364     | 6.356   |     |
| GY     | 5.620     | 9.803   |     |
| HI     | 16.104    | 11.733  |     |
| TGW    | 6.730     | 11.800  |     |
| NFG    | 9.749     | 9.238   |     |
| NUFG   | 12.187    | 10.107  |     |
| TNG    | 9.256     | 7.225   |     |

Note: DTF= days to 50% flowering, PH= plant height (cm), FLL= flag leaf length (cm), FLW= flag leaf width (cm), NT= number of tillers m⁻², PL= panicle length (cm), BY= biological yield/plot (g), GY= grain yields per plot (g), HI= harvest index (%), TGW= thousand grain weight (g), NFG= number of filled grains per panicle, NUFG= number of unfilled grains per panicle, TNG= total number of grains per panicle
| Accessions | Score | Irigrated | Rainfed | TSD |
|------------|-------|-----------|---------|-----|
| Bega budri | -2.766 | 0.138 | 0.021 | -1.135 |
| Ajan      | -1.475 | -1.374 | 1.305 | -1.770 |
| Banko     | -0.526 | -2.000 | 0.272 | 1.221 |
| Bariangi  | 0.233  | -1.948 | 1.292 | 2.254 |
| Khurabali | 0.478  | 0.376 | -0.364 | -1.077 |
| Peelee Luchai | -0.217 | 0.333 | 0.774 | -0.672 |
| Nagbel    | 1.190  | 1.397 | 1.176 | 0.190 |
| Bangoli-5 | -0.314 | -2.955 | 0.771 | 0.713 |

Note: DTF= days to 50% flowering, PH= plant height (cm), FLL= flag leaf length (cm), FLW= flag leaf width (cm), NT= number of tillers m\(^{-2}\), PL= panicle length (cm), BY= biological yield/plot (g), GY= grain yields per plot (g), HI= harvest index (%), TGW= thousand grain weight (g), NFG= number of filled grains per panicle, NUFG= number of unfilled grains per panicle, TFN= total number of grains per panicle.
| Variety | Chilled Maturity | Chilled Yield | Chilled Yield | Basmati 370 | IR 62266 | Basmati 370 | IR 62266 |
|---------|-----------------|--------------|--------------|-------------|------------|-------------|------------|
| Byalo   | 1.268           | 0.388        | 2.848        | 2.819       | 0.162      | 0.178       | 0.215      |
| Duggi   | 0.354           | 0.579        | 0.730        | 0.344       | 0.344      | 0.302       | 0.312      |
| Sama    | 0.821           | 0.440        | 0.101        | 0.049       | 0.152      | 0.145       | 0.145      |
| Cross   | 1.956           | 0.450        | 2.053        | 2.136       | 0.650      | 0.786       | 0.842      |
| IR 62266| 0.035           | 0.056        | 0.172        | 0.215       | 0.007      | 0.339       | 0.816      |
| Basmati | 0.050           | 0.114        | 0.244        | 0.049       | 0.086      | 0.030       | 0.030      |
| IR 62266| 0.005           | 0.114        | 0.244        | 0.049       | 0.086      | 0.030       | 0.030      |
| Basmati | 0.005           | 0.114        | 0.244        | 0.049       | 0.086      | 0.030       | 0.030      |
| Basmati | 0.005           | 0.114        | 0.244        | 0.049       | 0.086      | 0.030       | 0.030      |
| Basmati | 0.005           | 0.114        | 0.244        | 0.049       | 0.086      | 0.030       | 0.030      |
| Basmati | 0.005           | 0.114        | 0.244        | 0.049       | 0.086      | 0.030       | 0.030      |
| Basmati | 0.005           | 0.114        | 0.244        | 0.049       | 0.086      | 0.030       | 0.030      |
| Basmati | 0.005           | 0.114        | 0.244        | 0.049       | 0.086      | 0.030       | 0.030      |
| Basmati | 0.005           | 0.114        | 0.244        | 0.049       | 0.086      | 0.030       | 0.030      |
| Basmati | 0.005           | 0.114        | 0.244        | 0.049       | 0.086      | 0.030       | 0.030      |
| Basmati | 0.005           | 0.114        | 0.244        | 0.049       | 0.086      | 0.030       | 0.030      |
| Basmati | 0.005           | 0.114        | 0.244        | 0.049       | 0.086      | 0.030       | 0.030      |
| Basmati | 0.005           | 0.114        | 0.244        | 0.049       | 0.086      | 0.030       | 0.030      |
| Basmati | 0.005           | 0.114        | 0.244        | 0.049       | 0.086      | 0.030       | 0.030      |
| Basmati | 0.005           | 0.114        | 0.244        | 0.049       | 0.086      | 0.030       | 0.030      |
| Basmati | 0.005           | 0.114        | 0.244        | 0.049       | 0.086      | 0.030       | 0.030      |
| Basmati | 0.005           | 0.114        | 0.244        | 0.049       | 0.086      | 0.030       | 0.030      |
| Basmati | 0.005           | 0.114        | 0.244        | 0.049       | 0.086      | 0.030       | 0.030      |
| Basmati | 0.005           | 0.114        | 0.244        | 0.049       | 0.086      | 0.030       | 0.030      |
| Basmati | 0.005           | 0.114        | 0.244        | 0.049       | 0.086      | 0.030       | 0.030      |
| Basmati | 0.005           | 0.114        | 0.244        | 0.049       | 0.086      | 0.030       | 0.030      |
| Basmati | 0.005           | 0.114        | 0.244        | 0.049       | 0.086      | 0.030       | 0.030      |
| Basmati | 0.005           | 0.114        | 0.244        | 0.049       | 0.086      | 0.030       | 0.030      |

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The greater the distance between two clusters, greater is the divergence (Singh and Narayanan, 2013). If crossing takes place among genotypes between clusters, they produce more diverse and better progenies when compared to the crossing of genotypes within the same cluster. Those genotypes can be used as parents in future crossing programmes. The results were found in agreement with Amegan et al., 2020; according to Bekis et al., 2021 the highest inter-cluster distance was recorded between cluster II & III. The results depicted that cross-genotype from cluster II & III, cluster I & III to get genotypes of rice with high grain yield and early maturing genotypes; Burman et al., 2019; Iqbal et al., 2018 and Shrestha et al., 2021 revealed that cluster II & cluster IV showed the highest distance between cluster centroids. The genotypes in cluster II would be grown for higher grain yield. Genotypes in clusters of different conditions with high cluster mean value may be directly used for adaptation, or intercrossing may be recommended to produce the wide spectrum of variability, followed by effective selection for those traits according to the results.

**Principal Component Analysis (PCA)**
PCA was used in the topical study to analyze thirteen yield and yield-related parameters in 52 rice germplasm accessions (Table 5). Biplot graph representing the active variables and observations taking PC1 and PC2 under different conditions presented in Figure 2. The PC with Eigen value >1 that described at least 5% of the fluctuations in the data was evaluated in the current investigation, according to the criteria provided by Brejda et al. (2000) and Dhakal et al. (2020). The PC with the highest Eigen values and variables with the highest factor loading was deemed to be the most representational of system characteristics. Only five of the 13 principal components (PCs) had an Eigen value greater or nearer to 1. As a result, these five PCs were given due consideration for additional explanation. For the variables under research, the PC-1, PC-2, PC-3, PC-4, and PC-5 genotypes showed 29.82%, 22.489%, 9.008%, 8.366%, and 7.007% variability, in irrigated; variability 19.281%, 17.258%, 12.214%, 10.662% and 8.806% in rainfed; variability 20.056%,
16.165%, 13.144%, 12.268% and 8.905% in TSD conditions respectively. Each subsequent component accounts for as much of the remaining variability in the data as possible, with the first and second PCs accounting for as much as possible in all three conditions.

The factor loading for thirteen yield-related traits showed in Table 6. Only the most highly loaded factors were retained for further analysis within each PC. From the complex matrix it was revealed that the PC-1 (first PC) accounted for the highest variability (29.82%) was mainly related to traits like biological yield per plot and plant height in irrigated condition; variability in rainfed (19.281%) and TSD (20.056%) was mainly related to traits like total number of grains per panicle and number of filled grains per panicle in both rainfed and TSD conditions. Table 7 showed the top ten (bold values).

Principal Component scores for all genotypes, split down into five principal components. These scores can be used to develop exact selection indices, the intensity of which is determined by the variability described by each principal component. A high PC score for a given accession in a certain component indicates that the variables in that genotype have high values. It was revealed in the results that Bharma tripal (2.976), Kali Mai (3.131) and Khurabal (3.598) had the best PC score in PC-1; Kali Mai (6.564), Tedesi (3.120) and Bisni (4.482) in PC-2; Basa bhog (2.824), Swarna (13.872) and Khurabal (2.183) in PC-3; Mahamaya (3.118), Hathi panjara (3.260) and Roti (3.186) in PC-4, and Moroberekan (2.053), Roti (3.435) and Barangi (2.484) in PC-5 under irrigated, rainfed and TSD conditions respectively.

The results were found in agreement with Raj et al., 2020 and Burman et al., 2021 revealed that first PC showed the most variability among the five principal components, all of the principal components contributed positively to yield and its contributing traits. As a result, Tarunbhog, Safri 17 and Basmati 370 are the common genotypes with high PC1 scores and highly correlated with yield component traits under RF and TSD conditions. Hence, selecting these genotypes would result in higher yield and yield related traits under drought condition.

The PCA emphasizes the features with the maximum variability. As a result, intensive selection processes can be developed to improve yield and yield-related traits rapidly. PCA can also be used to rank genotypes based on PC scores in the corresponding component. The results showed that the selected accessions might be utilized as donors in a varietal development programme to improve yield attributes.

Conclusion

Both multivariate statistical analysis tools showed the existence of the wide genetic diversity among the germplasm lines in the study. In accordance with the current findings, the cluster-I have more genetic variability in specific conditions. Hence, the genotypes present in this cluster could be selected as parents in future breeding programmes. The traits biological yield per plot, grain yield per plot, number of tillers per m$^2$ and total number of grains per panicle revealed that, they play a crucial role in genetic divergence among fifty-two rice genotypes and we would select these traits of rice lines for the diversity purpose. PCA revealed that, PC1 was dominated by the yield and yield contributing traits such as biological yield per plot and plant height under IR condition and the traits total number of grains per panicle and number of filled grains per panicle under both RF and TSD conditions. So, selecting the germplasm lines with a high score in PC1 could result in greater yield and yield related characters.

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Conflict of interest

The authors declare that they have no conflict of interest.
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