Genetic Divergence Analysis of Rice Genotypes under Salt Stress Environment

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Authors’ contributions

This work was carried out in collaboration among all authors. Author AK designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors DKD and PKB managed the analyses of the study. Authors Shivani, PK, AD and NAK managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The present investigations were conducted in randomized block design with three replications within the net house of the department of PMB&GE, A. N.D.U.A.T, Kumar Ganj, Ayodhya to estimate the genetic divergence under normal and salt stress conditions involving 20 rice genotypes during Kharif 2018-19, on the basis of relative parameters of D2 values, the clustering pattern of 20 rice genotypes under normal and salt stress conditions were grouped into five non-overlapped clusters. Under normal condition, Cluster III having highest 7 rice genotypes, Cluster II having 5 genotypes, cluster V having 4 genotypes and IV having highest 3 rice genotype. Cluster I having only one genotype. Under saline condition, Cluster I having highest rice 6 genotypes, cluster III having 5 genotypes and cluster II & IV having 4 genotypes respectively. Cluster V having only one genotype. It means the genetic similarity was found in the genotypes were expressed within the cluster and the pattern of distribution of genotypes in various clusters exhibited that topographical diversity wasn't

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associated with ancestral diversity as genotypes of same countryside were grouped into different cluster and vice-versa. The highest inter cluster distance was recorded between cluster 2 and cluster 5 (26108.030) followed by between cluster 1 and cluster 5 (18550.010), cluster 3 and cluster 5 (15231.860), cluster 4 and cluster 5 (5335.860) in normal condition and in saline condition the maximum inter cluster distance was existed between cluster 4 and cluster 5 (2344.091) followed by between cluster 3 and cluster 5 (2067.610), cluster 2 and cluster 5 (1447.564), cluster 1 and cluster 5 (1238.095). The results showed extensive variation from one cluster to other in respect of cluster means for all parameters, which represented that genotypes having distinctly different mean performance for various characters were reported into different clusters.

**Keywords:** Rice; genotypes; clusters and $D^2$ analysis.

**1. INTRODUCTION**

Rice (Oryza sativa L.) is one of the most important plants from poaceae. Rice is a cost-effective important food crop with nourishment diversification and useful in poverty eradication more than three billion of world populations. Rice is ranked as the world’s number one human food crop. In India, rice is grown in an area of 43.97 million hectares with the production and productivity levels of 104.32 million tonnes and 2372 kg/ha, respectively, (Indiastat, 2017-2018). Plant breeding programme with different genetic base could encourage a high level of crop yield. The narrow genetic base of semi-dwarf germplasm is likely to build them vulnerable to various biotic and abiotic stresses. Heterogeneity in rice has been contributed to breed high yielding varieties. Germplasm of rice are being assembled over past several decades to use them in crop improvement programmes to improve high yielding, resistant to biotic and abiotic stresses with good adaptability. The success of any breeding programme depends on the exploitation of existing diversity and therefore, it is desirable to assemble, examine and utilize the existed diversity for crop improvement to required a specific ecosystem. $D^2$ analysis among the ancestral is important because a cross involving genetic diversity of parents is likely to produce maximum heterogenetic effect and also more diversity could be expected in segregating generations. Therefore, a meaningful classification of genotypes will enable the breeder to identify the best ancestral with wide $D^2$ and to utilize some of the selected diverse parents in the hybridization programme.

**2. MATERIALS AND METHODS**

Rice germplasm a total twenty rice genotypes were used in this study, which were IR68144-2B-2-2-3-1-120, IR-68144-2B-2-2-3-1-127, IR-91167-99-1-1-1-3, IR-91167-133-1-1-2-3, NUD-3, NDR-359, IR-28, FL-478, NUD-2, CSR-13, AYYAR, NDRK-2008, IR-64, SWARNA, IR-92953-49-1-3, IR-91171-66-3-2-1-3, IR-83668-35-2-2-2, SAMBHA MANSURI, TARAMON and MTU-1010.

The experiments were conducted in Randomized Block Design with three replications in normal and salt stress conditions in the green house of the department of PMB&GE, A. N.D.U.A.T, Kumar Ganj, Ayodhya during Kharif 2018-19. The data were recorded on days to 50% flowering, plant height, panicle bearing tillers/plants, panicle length (cm), spikelets/panicle, grains/panicle, spikelet fertility (%), test weight (g), biological yield (g), harvest index (%), grain yield (g) Na⁺ content, K⁺ content (mg g⁻¹) and Na⁺/K⁺ ratio. Observations were recorded on randomly selected five plants from each variety in each replication at maturity except for days to 50% flowering which were recorded on the plot basis at flowering stage.

**2.1 Estimation of Genetic Divergence ($D^2$)**

The genetic divergence analysis of 20 genotypes of rice was worked out using Mahalanobis [1] $D^2$ statistics Rao, [2].

**2.2 The Calculation of $D^2$ Values Involved Following Steps**

1. A set of uncorrelated linear combinations (y’s) was obtained by pivotal condensation of the common dispersion matrix Rao, [2] of a set of correlated variables (x’s). The common dispersion matrix was arranged with the help of error mean sum of squares and mean sum of products.

2. The combination between y’s and x’s the mean values of various genotypes for different characters (X1 to X10) were transformed into the mean values of a set of uncorrelated linear combinations (Y1 to Y10).
3. The $D_2^2$ values between $i^{th}$ and $j^{th}$ genotypes for $k$th characters is calculated as:

$$D_{2ij}^2 = K (Y_{it} - Y_{jt})^2$$

Where, $t = 1$

2.3 The K Components were Calculated Separately and Added to get $D_{2ij}^2$

1. The ‘$K$’ components of ‘$D_{2ij}^2$’ for each combination were ranked in descending order of magnitude.
2. These ranks were added up for each component $D_{2ij}^2$ over all combinations of $i$ and $j$th rank totals were obtained.

2.4 Group Constellation

The $D_2^2$ values were arranged in an increasing order of magnitude. The grouping of the strains into various clusters was done using Tocher's method Rao, [2]. The two widely related groups were chosen and third groups were found which had the smaller average $D_2^2$ value from the first two. Similarly, the fourth was chosen to have the smallest average $D_2^2$ from the first three and so on. The $D_2^2$ value did not fit in with the former group and was, therefore, taken as another cluster.

2.5 Intra and Inter-Cluster Distance

The inter-cluster $D_2^2$ was calculated as the sum of $n(n-1)/2$ genotypes within a cluster divided by total number of combinations. All possible $D_2^2$ values between the groups of two clusters were added and then divided by $n_1 \times n_2$ for computing inter-cluster distance. Where, $n_1$ and $n_2$ = The number of genotypes in two clusters.

2.6 Cluster Mean

The cluster mean for the distinct character is the summation of mean values of the genotypes included in a cluster classified by number of genotypes in the cluster.

3. RESULTS AND DISCUSSION

The selection of appropriate various oldsters for sexual union is a vital feature of any crop breeding programmes as a result of parental diversity in optimum magnitude is needed to get superior genotypes in segregating generations Moll et al. [3]. The importance of genetic divergence in crop improvement has been stressed by many scientists Griffing and Lindstrom, [4]; felon et al., 1962 Arunachalam [5] and Hawkas (1981). $D_2^2$ analysis has been used by many employees for the assessment of genetic divergence in many crops Malhotra and Singh [6].

In the present study, the twenty genotypes of rice were grouped into five different non-overlapping clusters under normal and saline conditions (Table 1a and b), suggesting considerable amount of genetic diversity in the materials. under control condition Yadav, Pratibha, et al. [7]. Cluster III having highest rice genotypes (7) namely NDR-359, IR-29, CSR 13, AYYAR, NDRK2008, IR-92953-49-1-3 and IR-83668-35-2-2-2, Cluster II having five genotypes i.e. IR-68144-2B-2-2-3-1-120, FL-478, NUD-2, TARAMON and MTU-1010. Cluster V having four genotypes i.e. IR-68144-2B-2-2-3-1-127, NUD-3, IR-64 and SWARNA, Cluster IV having three genotypes i.e. NDR-359, IR-91171-66-3-2-1-3 and SAMBA MASURI. Cluster I having only one genotype i.e. IR-91167-133-1-1-2-3. While in saline condition Cluster I having highest rice genotypes (6) namely, IR-68144-2B-2-2-3-1-120, IR-91167-133-1-1-2-3, NDR-359, IR-29, NUD-2 and CSR 13, Cluster III having five genotypes i.e. IR-68144-2B-2-2-3-1-127, NUD-3, NDRK-2008, SAMBA MASURI and TARAMON, Cluster II & IV having four genotypes i.e. FL-478, AYYAR, IR64, SWARNA and IR-91167-99-1-1-1-3, IR-92953-49-1-3, IR-83668-35-2-2-2, IR-91171-66-3-2-1-3, respectively. Cluster V having one genotypes i.e. MTU-1010. It means the general genetic similarity was found within the genotypes were reported within the cluster and therefore the pattern of distribution of genotypes in various clusters exhibited that topographical diversity wasn’t related with genetic diversity as genotypes of same countryside were grouped into various cluster and vice-versa, as supported by earlier finding of Devi et al. [8]; Devi M, et al. [9]; Devi Shashi and Dwivedi, [10]; Devi et al. [11]; Mall et al. [12] and Ovung et al. [13].

The estimates of average intra and inter cluster distances for presented in Table 2a and Table 2b revealed that the maximum intra cluster distance was exhibited by the genotypes of cluster 5 followed by cluster 4, cluster 3, cluster 2 and cluster 1. The highest inter cluster distance was recorded between cluster 2 and cluster 5 (26108.030) followed by between cluster 1 and cluster 5 (18550.010), cluster 3 and cluster 5 (15231.860), cluster 4 and cluster 5 (5335.860) under normal condition while, under saline condition the highest intra cluster distance was...
recorded by cluster 5 followed by cluster 4, cluster 3 and cluster 2. The highest inter cluster distance was recorded between cluster 4 and cluster 5 (2344.091) followed by between cluster 3 and cluster 5 (2067.610), cluster 2 and cluster 5 (1447.564), cluster 1 and cluster 5 (1238.095) reported good diversity between them and genotypes in these clusters could be used as ancestral in breeding programme to improve desirable line because crosses between genetically divergent lines will generate heterotic segregates. As heterosis can be best exploited and chances of getting transgressive segregates are high when generating divergent lines are hybridized Zaman et al. [14] and Saxesena et al., [15]. Many researchers in various crops have also reported that selection of ancestral for breeding should be done from two clusters having wide inter-cluster distance to get maximum diversity in segregating generations. Heterosis is generally attributed to genetic divergence among the ancestral lines involved in the cross. Nevertheless, the genetic divergence for the maximum expression of the heterotic effects has a limit Moll et al. [3] and Arunachalam et al., [16].

Table 1a. Clustering pattern of 20 rice genotype on the basis on D² analysis for 14 characters in controlled condition

| Cluster No. | No. of genotypes | Genotypes                                                                 |
|-------------|------------------|---------------------------------------------------------------------------|
| I           | 1                | IR-91167-133-1-1-2-3                                                    |
| II          | 5                | IR-68144-2B-2-2-3-1-120, FL-478, NUD-2, TARAMON, MTU-1010               |
| III         | 7                | NDR-359, IR-29, CSR 13, AYYAR, NDRK-2008, IR-92953-49-1-3, IR-83668-35-2-2-2 |
| IV          | 3                | NDR-359, IR-91171-66-3-2-1-3, SAMBA MASURI                             |
| V           | 4                | IR-68144-2B-2-2-3-1-127, NUD-3, IR-64, SWARNA                         |

Table 1b. Clustering pattern of 20 rice genotype on the basis on D² analysis for 14 characters in saline condition

| Cluster No. | No. of genotypes | Genotypes                                                                 |
|-------------|------------------|---------------------------------------------------------------------------|
| I           | 6                | IR-68144-2B-2-2-3-1-120, IR-91167-133-1-1-2-3, NDR-359, IR-29, NUD-2, CSR 13 |
| II          | 4                | FL-478, AYYAR, IR-64, SWARNA                                             |
| III         | 5                | IR-68144-2B-2-2-3-1-127, NUD-3, NDRK-2008, SAMBA MASURI, TARAMON         |
| IV          | 4                | IR-91167-99-1-1-1-3, IR-92953-49-1-3, IR-83668-35-2-2-2, IR-91171-66-3-2-1-3 |
| V           | 1                | MTU-1010                                                                 |

Table 2a. Estimation of average inter cluster D² value under control condition

| Cluster no. | Cluster 1 | Cluster 2 | Cluster 3 | Cluster 4 | Cluster 5 |
|-------------|-----------|-----------|-----------|-----------|-----------|
| Cluster 1   | 710.163   | 2072.070  | 2170.370  | 4805.715  | 18550.010 |
| Cluster 2   | 1276.286  | 867.018   | 9190.650  | 3981.772  | 26108.030 |
| Cluster 3   |           |           | 811.451   | 5335.071  | 15231.860 |
| Cluster 4   |           |           |           | 0.000     |           |
| Cluster 5   |           |           |           |           |           |

Table 2b. Estimation of average inter cluster D² value under saline condition

| Cluster no. | Cluster 1 | Cluster 2 | Cluster 3 | Cluster 4 | Cluster 5 |
|-------------|-----------|-----------|-----------|-----------|-----------|
| Cluster 1   | 422.428   | 823.908   | 825.924   | 1072.397  | 1238.095  |
| Cluster 2   | 361.387   | 927.603   | 1455.013  | 1447.564  |           |
| Cluster 3   | 522.126   | 1195.375  | 2067.610  |           |           |
| Cluster 4   | 837.769   | 2344.091  |           |           |           |
| Cluster 5   |           |           |           |           | 597.118   |
### Table 3a. Cluster mean of 20 rice genotypes under control condition

| Characters                  | Days to 50% flowering | Plant height (cm) | Panicle bearing tillers/plant | Panicle length (cm) | Spikelet/panicle | Grains/panicle | Spikelet fertility (%) | Test weight (g) | Biological yield/plant (g) | Harvest index (%) | Na’ | K’ | Na’/K’ | Grain yield/plant (g) |
|-----------------------------|------------------------|-------------------|--------------------------------|---------------------|------------------|---------------|------------------------|-----------------|--------------------------|------------------|-----|----|--------|----------------------|
| Cluster 1                   | 90.74                  | 85.56             | 5.86                           | 20.73               | 170.41           | 143.58        | 84.17                  | 22.85           | 47.86                    | 3.69             | 25.98 | 0.14| 19.62   | 47.86                 |
| Cluster 2                   | 95.63                  | 105.90            | 6.81                           | 20.93               | 138.16           | 114.93        | 82.81                  | 23.16           | 49.85                    | 3.71             | 23.58 | 0.15| 21.29   | 49.85                 |
| Cluster 3                   | 94.76                  | 114.09            | 6.30                           | 21.60               | 146.82           | 122.50        | 82.98                  | 25.94           | 44.67                    | 3.64             | 28.32 | 0.12| 18.62   | 44.67                 |
| Cluster 4                   | 94.77                  | 86.00             | 6.61                           | 21.80               | 140.38           | 121.20        | 86.19                  | 23.24           | 41.90                    | 3.23             | 32.95 | 0.09| 17.03   | 41.90                 |
| Cluster 5                   | 98.86                  | 92.77             | 8.91                           | 16.85               | 195.99           | 173.85        | 88.89                  | 22.81           | 57.20                    | 3.53             | 39.56 | 0.08| 17.82   | 57.20                 |

### Table 3b. Cluster mean of 20 rice genotypes under saline condition

| Characters                  | Days to 50% flowering | Plant height (cm) | Panicle bearing tillers/plant | Panicle length (cm) | Spikelet/panicle | Grains/panicle | Spikelet fertility (%) | Test weight (g) | Biological yield/plant (g) | Harvest index (%) | Na’ | K’ | Na’/K’ | Grain yield/plant (g) |
|-----------------------------|------------------------|-------------------|--------------------------------|---------------------|------------------|---------------|------------------------|-----------------|--------------------------|------------------|-----|----|--------|----------------------|
| Cluster 1                   | 88.08                  | 89.03             | 5.54                           | 21.47               | 97.47            | 80.48         | 82.37                  | 17.80           | 27.56                    | 3.29             | 25.77 | 0.12| 10.90   | 27.56                 |
| Cluster 2                   | 95.87                  | 90.94             | 5.11                           | 22.70               | 165.10           | 143.43        | 86.91                  | 20.51           | 30.82                    | 3.01             | 26.32 | 0.11| 12.26   | 30.82                 |
| Cluster 3                   | 102.37                 | 85.60             | 4.64                           | 24.18               | 118.71           | 98.82         | 83.51                  | 21.67           | 31.57                    | 3.61             | 26.06 | 0.14| 11.01   | 31.57                 |
| Cluster 4                   | 91.65                  | 88.87             | 5.94                           | 19.17               | 122.33           | 98.29         | 80.78                  | 18.63           | 29.29                    | 3.02             | 34.69 | 0.08| 11.60   | 29.29                 |
| Cluster 5                   | 89.21                  | 103.8             | 4.62                           | 19.15               | 95.49            | 77.43         | 80.79                  | 18.63           | 36.76                    | 3.24             | 26.22 | 0.12| 14.21   | 36.76                 |
The comparison of cluster means revealed considerable differences among the clusters of various characters under control and salty soil (Table 3a and 3b). Under control condition, cluster mean for days to 50% flowering ranged from 90.74 (cluster 1) to 98.86 days (cluster 5). Early flowering genotypes were grouped in cluster 1 (90.74) followed by cluster 2 (95.67) and cluster 5 (98.86), while in salt stress, cluster mean for days to 50% flowering ranged from 88.08 (cluster 1) to 102.37 days (cluster 3). Early flowering genotypes were grouped in cluster 1 (88.08) followed by cluster 5 (89.21), cluster 4 (91.65) and cluster 2 (95.87). The results showed wide diversity from one cluster to another in respect of cluster means for all characters, which indicated wide variation from one cluster to another in respect of cluster means for all characters, which indicated

4. CONCLUSION

In the present study, the genetic divergence for fourteen characters of 20 genotypes of rice were grouped into five different non-overlapping clusters under normal and saline conditions, suggesting considerable amount of genetic diversity in the materials. Under control condition, Cluster III having highest seven rice genotypes, while in saline condition, Cluster I having highest six rice genotypes. It means the general genetic similarity was found within the germplasm were presented within the cluster the estimates of average intra and inter cluster distances for 20 rice genotypes. The maximum intra cluster distance was exhibited by the genotypes of cluster 5 followed by cluster 4, cluster 3, cluster 2 and cluster 1 under normal condition while, under saline condition the highest intra cluster distance was recorded by cluster 5 followed by cluster 4, cluster 3 and cluster 2. suggesting wide diversity between them and germplasm in these clusters could be used as parents in hybridization programme to develop desirable type because crosses between genetically divergent lines will generate heterotic segregates. The comparison of cluster means revealed considerable differences among the clusters of different characters, cluster mean of days to 50% flowering showed considerable difference Under both conditions. The results showed wide variation from one cluster to another in respect of cluster means for all characters, which indicated
that varieties having distinctly different mean performance for various characters.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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