Estimating genetic divergence in rice (Oryza sativa L.) using $D^2$ analysis

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Abstract
Rice is the staple food across Asia, where around half of the world’s poorest people live and hence that necessitates breeding for high yielding genotypes. Genetic diversity present in the germplasm is pre-requisite for crop improvement as it helps in the development of superior recombinants. Knowledge about genetic diversity between the genotype can be utilized for the selection of diverse parents in hybridization programmes. In this study, using Mahalanobis $D^2$ statistics twenty four advanced genotypes were grouped into five different clusters based on their level of divergence. The Cluster III displayed the maximum intra-cluster distance with six genotypes followed by cluster II with ten genotypes. The cluster II and IV exhibited the maximum inter-cluster distance. Among the traits, days to 50% flowering contributed the highest towards total divergence.

Key words
Rice, Genetic diversity, $D^2$ statistics.

INTRODUCTION
The first step before initiating any breeding programme is the assessment of diversity among the set of genotypes. The success of plant breeding programme depends on the development of high yielding, input responsive, better quality and disease and insect resistant varieties. This in turn depends on the selection of suitable plants to be utilized in breeding programme. Genetic diversity present in the germplasm is pre-requisite for crop improvement as it helps in the development of superior recombinants. Knowledge about genetic diversity between the genotype can be utilized for the selection of diverse parents in hybridization to exploit the heterosis. Mahalanobis $D^2$ statistics assist in grouping the genotypes into different clusters based on their divergence level. This analysis takes into account the contribution of each character towards the total divergence. So, that provides the idea about the total genetic divergence among the genotypes with the estimates of Inter-cluster and Intra-cluster distance. Hybridization involving genetically diverse parents belonging to different clusters would provide an opportunity for bringing together gene constellations of diverse nature, promising hybrid derivatives resulting probably due to the complementary interaction of divergent genes in parents (Ovung et al., 2012). Therefore, helps to pick up appropriate genotypes for utilization in the hybridization programme.

MATERIALS AND METHODS
The present investigation was conducted during the Kharif season 2016-2017 at the Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi using twenty four rice genotypes which were received from IRRI Networking Project, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi (Table 1.). The experiment was laid out in randomized block design (RBD) with three replications. Twenty five days old seedlings were transplanted in main research plot. Each plot consisted of five rows of 1.5 m length with spacing 15 × 20 cm. The crop was maintained as per the standard agronomic practices. The field observations were recorded for 19 quantitative traits including Days to 50% Flowering, Days to Maturity, Plant Height (cm),...
Hulling %, Milling %, Kernel Length (mm), Kernel Breadth (mm), L/B Ratio, Panicle Length (cm), Tillers/Plant, Effective Tillers/Plant, Spikelet/ Panicle, Fertile Grain/ Panicle, Spikelet Fertility (%), Sterile Spikelet/ Panicle, 1000 Grain Weight(g), Grain Weight/Panicle(g), Grain Yield/ Plant(g) and Grain Yield/ Plot(g). Analysis of variance is used for testing whether there is significant difference among the treatments or not by following the procedure of randomized block design (RBD) analysis (Panse and Sukhatme, 1967). The genetic divergence was estimated based on Mahalanobis' generalized distance as described by Rao (1952). The genotypes were grouped into a number of clusters by Tocher's method described by Rao (1952).

Table 1. List of Genotypes studied with their sources.

| Sl no. | Genotypes     | Source of genotypes |
|--------|---------------|---------------------|
| 1      | IR 92522-47-2-1-4 | IRRI                |
| 2      | IR 92522-45-3-1-4 | IRRI                |
| 3      | IR 91648-B-85-B-1-1 | IRRI               |
| 4      | IR 91648-B-215-B-2-1 | IRRI            |
| 5      | IR 91648-B-58-B-7-3 | IRRI               |
| 6      | IR 96321-315-402-B-1 | IRRI           |
| 7      | IR 96321-327-300-B-1-1 | IRRI     |
| 8      | IR 94391-587-1-2-B | IRRI               |
| 9      | IR 85604-19-2-3-2-2 | IRRI            |
| 10     | IR 82475-110-2-2-1-2 | IRRI          |
| 11     | IR 96248-16-3-3-1-B | IRRI              |
| 12     | IR 96248-16-3-3-2-B | IRRI              |
| 13     | IR 97477-110-3-1-B | IRRI               |
| 14     | IR92978-192-1-2 (R-306) | IRRI       |
| 15     | IR91953-141-2-1-2 (R-119) | IRRI     |
| 16     | IR92937-178-2-2 (R-155) | IRRI     |
| 17     | MTU1010         | APRRI, Maruteru, AP |
| 18     | Swarna Sub1     | IRRI                |
| 19     | Swarna          | APRRI, Maruteru, AP |
| 20     | Lalat           | OUAT, Orissa       |
| 21     | R-RHZ-7         | IGKV, Raipur       |
| 22     | CGZR-1          | IGKV, Raipur       |
| 23     | IR64            | IRRI                |
| 24     | Sambha Mahsuri  | APRRI, Maruteru, AP |

Table 2. Analysis of variance (ANOVA) for 19 quantitative traits in twenty four rice genotypes

| Source of variation | df | Source of variation | df | Source of variation | df | Source of variation | df |
|---------------------|----|---------------------|----|---------------------|----|---------------------|----|
|                     |    | Days to 50% Flowering |    | Plant Height (cm) |    | Hulling % |    |
| Replicate           | 2  | 0.0972              | 23 | 460.25 R**         |    | 1.1294     |    |
| Treatments          | 23 | 0.3472              |    | 3.2349              |    | 1.2294     |    |
| Error               | 46 | 0.7349              |    | 1.1443              |    | 6.7736     |    |
|                     |    | Days to Maturity    |    | 10.1274             |    | 122.3610   |    |
| Replicate           | 2  | 1.0976              | 23 | 3.94**              |    | 61.9182    |    |
| Treatments          | 46 | 1.0976              |    | 181.5182            |    | 288.7524   |    |
| Error               |    | 1.0976              |    | 23                |    | 3.94**     |    |

*Significant at p<0.05; **Significant at p<0.01
RESULTS AND DISCUSSION

It is noticeable from the analysis of variance that genotypes differed significantly for all the nineteen characters studied, which is presented in Table 2. This specified that there is significant variability for grain yield and yield components traits studied.

In the present study, D² analysis revealed the presence of considerable diversity in the set of 24 rice genotypes. Based on their level of divergence, the genotypes formed five different clusters (Table 3). The intra-cluster distance for cluster IV was observed to be zero since that contain only one genotype. Cluster III displayed the maximum intra-cluster distance with six genotypes followed by cluster II with ten genotypes. The genotypes with close relationship fall in the same cluster and the genotypes which show relatively more divergence were contained in the different clusters. Therefore, it could be probable that genotypes gathered together in one cluster were less divergent than those which are positioned in different clusters. The cluster II and IV exhibited the maximum inter-cluster distance followed by cluster I and II signifying that the hybridization between the genotypes fall in diverse clusters would yield desirable segregates with the accumulation of favorable genes in the segregating generations (Fig 1.). The greater the distance between two clusters, higher is the expected genetic advance between them. Therefore, clustering pattern provide the proper picture about the diversity among the genotypes and useful in selecting diverse parents for hybridization program. However, while selecting parents for hybridization programmes their yield potential should not be overlooked (Singh et al. 1987).

A study of cluster means revealed the presence of considerable differences in the mean values of different characters (Table 4). The distribution of highest and lowest mean values for different characters in distinct cluster indicated the traits contributing to the total divergence. In this regard, days to 50% flowering contribute highest towards divergence followed by grain yield per plot, spikelet per panicle and grain yield per plant (Table 5). These results were in agreement with the findings of Bose and...
### Table 3. Grouping of twenty four rice genotypes into five clusters (by Tocher method)

| Clusters | Genotypes                                                                 | Number |
|----------|---------------------------------------------------------------------------|--------|
| I        | IR 96321-315-402-B-1, IR 96321-327-300-B-1-1, Swarna, IR 97477-110-3-1-B, IR 91648-B-85-B-1-1 | 05     |
| II       | MTU1010, IR84, IR 94391-587-1-2-B, IR 85604-19-2-3-2-2, IR 92522-47-2-1-4, IR 92522-45-3-1-4, CGZ-1, IR 96248-16-3-3-2-B, IR 96248-16-3-3-1-B, IR 82475-110-2-2-1-2 | 10     |
| III      | IR 91648-B-215-B-2-1, Swarna Sub 1, Lalat, IR92978-192-1-2 (R-306), IR92937-178-2-2 (R-155), IR91953-141-2-1-2 (R-119) | 06     |
| IV       | Sambha mahsuri                                                           | 01     |
| V        | IR 91648-B-58-B-7-3, R-RHZ-7                                             | 02     |

### Table 4. Mean values of different characters of 24 rice genotypes grouped in five clusters.

|                      | Cluster I | Cluster II | Cluster III | Cluster IV | Cluster V |
|----------------------|-----------|------------|-------------|------------|-----------|
| Days to 50% Flowering| 121.733   | 95.167     | 112.278     | 122.000    | 112.333   |
| Days to Maturity     | 147.667   | 120.967    | 138.778     | 146.667    | 138.000   |
| Plant Height (cm)    | 95.854    | 98.411     | 103.037     | 89.289     | 104.100   |
| Hulling %            | 76.207    | 76.137     | 77.218      | 73.300     | 77.975    |
| Milling %            | 71.707    | 67.790     | 72.154      | 73.410     | 72.258    |
| Kernel Length (mm)   | 5.611     | 7.023      | 6.587       | 5.227      | 6.587     |
| Kernel Breadth (mm)  | 2.087     | 2.101      | 2.017       | 1.803      | 2.017     |
| L/B Ratio            | 2.703     | 3.828      | 3.274       | 2.903      | 3.313     |
| Panicle Length (cm)  | 23.902    | 25.560     | 26.241      | 21.589     | 27.059    |
| Tillers/ Plant       | 10.022    | 11.100     | 10.944      | 14.222     | 10.000    |

### Table 5. Percentage contribution of each character towards total genetic divergence in 24 genotypes

| Source       | Contribution % |
|--------------|----------------|
| 1 Days to 50% Flowering | 64.49 |
| 2 Days to Maturity       | 0.98 |
| 3 Plant Height (cm)      | 1.09 |
| 4 Hulling %              | 1.09 |
| 5 Milling %              | 0.01 |
| 6 Kernel Length (mm)     | 0.36 |
| 7 Kernel Breadth (mm)    | 0.01 |
| 8 L/B Ratio              | 0.01 |
| 9 Panicle Length (cm)    | 1.09 |
| 10 Tillers/ Plant        | 0.01 |
| 11 Effective Tillers/ Plant | 0.01 |
| 12 Spikelet/ Panicle     | 7.25 |
| 13 Fertile Grain/ Panicle| 1.09 |
| 14 Spikelet Fertility (%)| 0.01 |
| 15 Sterile Spikelet/ Panicle | 0.01 |
| 16 1000 Grain Weight(g)  | 0.01 |
| 17 Grain Weight/ Panicle | 0.01 |
| 18 Grain Yield/ Plant(g) | 3.99 |
| 19 Grain Yield/ Plot(g)  | 18.48 |
Pradhan (2006); Banumathy et al. (2010); Sabesan et al. (2010); Ovung et al. (2012), Kumar et al. (2014) and Dey et al. (2020) who have also studied divergence in rice using 35, 53, 26, 70, 29 and 29 genotypes respectively.

In the present group of rice genotypes considered, based on the information attained from D² analysis, genotypes belonging to the most divergent clusters viz., cluster II and IV with proper mean values and with desirable traits can be utilized for hybridization programme to accumulate favorable alleles and desirable traits into single background and hence effective in developing improved cultivars.

ACKNOWLEDGEMENTS
Authors thankfully acknowledge the IRRI Networking Project, IAS, BHU for providing the requisite germplasm to get this work accomplished.

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