Genetic analysis of rice germplasm suitable for direct and transplanted establishments

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Abstract
Rice is the staple food crop for most of the population in the world especially in Asia. Due to climate change and labour shortage problems, there is a shift in rice cultivation practices in South Asia. The direct seeded rice cultivation is now preferred over transplanted rice owing to its greater advantage in mechanization and management practices. In the present study, 600 rice germplasm lines were evaluated in transplanted and direct seeded conditions (dry and wet). The principal component analysis revealed that, the first three principal components contributed for 76.54, 78.07 and 80.05 per cent for the total divergence in different establishments viz., dry seeding, wet seeding and transplanting, respectively. The traits viz., days to five per cent flowering, days to 50 per cent flowering, days to 100 per cent flowering and days to maturity contributed the maximum for the total divergence. Further, the genotypes were grouped into 10 clusters based on the dissimilarity coefficient. The clustering pattern of the different establishments was different from one another owing to the differential performance of genotypes in different establishments. The genotypes Tundus, Liu 19, Semiocho and Minchechinhsuthou were found to be very diverse from others in the current study as they were placed in the single solitary clusters in different crop establishments. The genotypes viz., White Ponni, La Hung Ku, IR 78875206B4B and Hsung Tieng 2 had performed better in all the three establishments. Hence, these lines can be used for both direct seeded and transplanted conditions.

Key words: Dry seeded, Wet seeded, Transplanted, PCA, Rice, Cluster analysis

INTRODUCTION
Rice (Oryza sativa L.) is one of the most vital and staple food crops in the world for more than 50% of the global population. Rice provides 35–75% of the total calories to more than three billion Asians. It is planted in about 154 million hectares annually or on about 11 per cent of the world’s cultivated land with the production of about 518.4 million tonnes (FAOSTAT, 2021). In South Asia, rice is cultivated in an area of 60 million hectares and produces slightly above 225 million tonnes of paddy, accounting for 37.5 per cent of the global area and 32 per cent of global production (Goswami et al., 2018). Within South Asia, both India and Bangladesh are major rice-growing countries. India occupied the largest rice area in the world with 43.86 million hectares (more than a quarter of the...
There are three principal methods of rice establishments: dry seeding, wet seeding, and transplanting methods. Although these methods vary, each is characterized by distinct salient features. Dry seeding consists of sowing dry seeds on dry (unsaturated) soils. Seeds can be broadcast, drilled, or dibbled. Wet seeding involves sowing pre-germinated seeds in wet (saturated) puddled soils. Because the seeds are sown directly, the dry- and wet-seeding methods are often jointly referred to as direct seeding. Whereas, transplanting is the dominant crop establishment practice in Asia particularly in tropical regions. In this method, the land is puddled and seedlings raised in the nursery are transplanted to the main field (Pandey and Velasco, 2002; Kaur and Singh, 2017). Accurate data on the proportion of rice area established by different methods are scanty. The direct seeded area in Asia is about 29 million ha, which is approximately 21% of the total rice area in the region (Pandey and Velasco, 2002). Assessment of germplasm collections for their suitability to three principal methods of rice establishment: dry seeding, wet seeding, and transplanting is very much necessary to understand genetic diversity present in the collections and its adaptability to different crop establishments is very important for identifying germplasm and developing hybrids. PCA is a non-parametric and straight forward method that provides a roadmap to reduce the baffling datasets by extracting the pertinent information without compromising any information loss (Shinde et al., 2012). The Principal Component Analysis (PCA) is an exciting tool that computes the degree of genetic divergence and delivers the association between the genetic divergence based on the generalized distance at the genotypic level. The Unweighted Pair Group Method with Arithmetic mean (UPGMA) clustering method helps in efficient clustering of genotypes based on similarity or dissimilarity index. Information on the nature and degree of genetic divergence would help the breeder in choosing the right type of parents for purposeful hybridization (Arunachalam, 1981).

**MATERIALS AND METHODS**

The experimental material consisted of 600 diverse accessions of rice germplasm, which are part of Corteva Agriscience rice germplasm collection, which were collected from various rice institutes including International Rice Research Institute (IRRI), Philippines, Barwale Foundation Hyderabad etc. The experiment was carried out during Kharif, 2018 at Rice Research Station, (Latitude: 21.16; Longitude: 81.62) Raipur, Chhattisgarh. The 600 genotypes were evaluated in Randomized Complete Block Design with two replications in three different methods of crop establishments viz., dry seeding, wet seeding, and transplanting. The single seed/seedling per hill was planted in a single row of 12 hills each, with the spacing of 20 cm row to row and 15 cm plant to plant, for all methods. The recommended package of practices was carried out to raise a healthy crop. The observations were recorded on five randomly selected plants in each replication and each germplasm on 10 traits viz., early vegetative vigour (score 1-9 scale), days to five per cent

| Principal components | Dry seeded rice | Wet seeded rice | Transplanted rice |
|---------------------|-----------------|-----------------|-------------------|
|                     | Eigen values    | Per cent of variance | cumulative per cent of variance | Eigen values | Per cent of variance | cumulative per cent of variance | Eigen values | Per cent of variance | cumulative per cent of variance |
| PC1                 | 4.35            | 43.6            | 43.6              | 4.18            | 41.8            | 41.8              | 4.23            | 42.3            | 42.3              |
| PC2                 | 1.66            | 18.6            | 62.2              | 2.24            | 22.5            | 64.3              | 2.62            | 26.2            | 68.5              |
| PC3                 | 1.43            | 14.3            | 76.5              | 1.38            | 13.8            | 78.1              | 1.16            | 11.6            | 80.1              |
| PC4                 | 0.87            | 8.7             | 85.3              | 0.71            | 7.1             | 85.2              | 0.78            | 7.8             | 87.8              |
| PC5                 | 0.67            | 6.7             | 92.0              | 0.63            | 6.3             | 91.5              | 0.52            | 5.2             | 93.1              |
| PC6                 | 0.44            | 4.5             | 96.4              | 0.55            | 5.5             | 97.0              | 0.45            | 4.5             | 97.6              |
| PC7                 | 0.34            | 3.5             | 99.9              | 0.29            | 2.9             | 99.9              | 0.24            | 2.4             | 99.9              |
| PC8                 | 0.01            | 0.1             | 100.0             | 0.01            | 0.1             | 100.0             | 0.01            | 0.1             | 100.0             |
| PC9                 | 0.00            | 0.0             | 100.0             | 0.00            | 0.0             | 100.0             | 0.00            | 0.0             | 100.0             |
| PC10                | 0.00            | 0.0             | 100.0             | 0.00            | 0.0             | 100.0             | 0.00            | 0.0             | 100.0             |

Table 1. Contribution of principal components to the total divergence in dry, wet and transplanted rice.
flowering, days to 50 per cent flowering, days to 100 per cent flowering, days to maturity, number of tillers per plant, plant height (cm), lodging severity (score 1-9 scales), panicle length (cm) and single plant yield (g). The data were subjected to multivariate analysis like Principal Component Analysis (PCA) and cluster analysis to study the genetic diversity among the genotypes, trait association and identification of suitable genotypes for different establishments and across environments. The PCA and cluster analysis was done utilizing the software R package version 3.3.2 and R studio 1.0.136 (R 2016) using ‘factoextra’ and ‘FactomineR’ packages (Lê et al., 2008).

RESULTS AND DISCUSSION
The PCA helps in breakdown of a large and complex datasets into fewer multidimensional dataset without losing the information by eliminating its redundancy (Jolliffe and Cadima, 2016). In the present study, PCA for ten traits divided the total variation into ten principal components (PC's) in all three establishments. The major principal components were those with eigen values of more than one and it contributes maximum for the total divergence. The first three principal components contributed 76.54, 78.07 and 80.05 per cent for the total divergence in different establishments viz., dry seeding, wet seeding and transplanting, respectively (Fig. 1). The first three principal components (PC1, PC2 and PC3) were considered as the major principal components as their eigen values were more than one. Similarly, Kumari et al. (2021) reported that the first three principal components as a major principal component while studying 121 rice germplasm. The eigen values and contribution per cent towards divergence are presented in Table 1 and Fig.1 for the three establishments. The contribution of different morphological characters to the total divergence explains the amount of variability present for that particular trait. The characters viz., days to five per cent flowering, days to 50 per cent flowering, days to 100 per cent flowering and days to maturity contributed the maximum for the first principal component for all the three establishments. The characters viz., plant height, early vegetative vigour and panicle length attributed the maximum for the second principal component. On the other hand, number of tillers per plant and single plant yield was responsible for the third principal component. A similar result was reported by Sheela et al. (2020) in rice.

The scatter plot was constructed using the first two major principal components that constituted 600 germplasm scatter along with the ten traits (Fig. 2, 3 & 4). The genotypes that are in proximity to the origin of the scatter plot are close to the average value and those away from the origin are the extreme observations or the outliers (Hartmann et al., 2018). It is also reported that, the genotypes were close to each other in the scatter plot were closely related and the genotypes far apart were
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Fig. 1. Eigen values and its contribution over three different rice establishments.

Fig. 2. Scatter plot of 600 rice germplasm based on first two principal components in dry seeded rice

Fig. 3. Scatter plot of 600 germplasm based on first two principal components in wet seeded rice

Fig. 4. Scatter plot of 600 germplasm based on first two principal components in transplanted rice
Fig. 3. Scatter plot of 600 germplasm based on first two principal components in wet seeded rice

Fig. 4. Scatter plot of 600 germplasm based on first two principal components in transplanted rice

Fig. 5a. Loadings plot with association of ten traits in 600 rice germplasm (Directed seeded - Dry)
Fig. 5b. Directed seeded - Wet

Fig. 5c. Transplanting
more diverse (Walle et al., 2019). The genotypes viz., Tsung Mi Z 31, Semiocho, Shuang Ai 4 and Anlintsao were fall on the four corners of a scatter plot indicated that they were more diverse genotypes in the dry seeded establishment. The genotypes viz., Semiocho, Kunming Tiao Ku, Panghsiaichao and IR72667161BB3 were more diverse in the wet seeded establishment. On contrary, China 97512, Putus Tolo, Shuang Ai 6 and Tungcanromao were highly diverse genotypes in the transplanted establishment.

The loading plot of PC1 Vs PC2 represents the correlation of the morphological characters (Hartmann et al., 2018). The loading plot for first two principal components is given in Fig.5 for all three establishments. The traits viz., days to five per cent flowering, days to 50 per cent flowering, days to 100 per cent flowering and days to maturity were oriented with the axes of PC 1, indicating their higher contribution to PC1. The traits viz., early vegetative vigour, single plant yield and plant height were directed towards axes of PC2. The longer the vector in the loading plot, higher variability of the variables was explained by the two principal components. The shorter vectors are explained better in other dimensions. Moreover, the variables that are the same direction represents the positive correlation between the traits and the variables that were opposite direction were negatively correlated. In the present study, days to five per cent flowering, days to 50 per cent flowering, days to 100 per cent flowering and days to maturity were highly positively correlated in all the three establishments. On contrast, the traits viz., plant height and lodging severity were negatively correlated. Similarly, Sudeepthi et al. (2020) reported a positive association between number of tillers per plant, single plant yield and days to fifty per cent flowering in rice.

The clustering of the genotypes based on the dissimilarity index helps in the identification of the diverse genotypes that can be effectively used in hybridization programs to avail higher genetic variability (Amegan et al., 2020). In the present study, 600 genotypes were grouped into ten clusters in all three establishments (Table 2; Fig. 6, 7 & 8). The clustering patterns of the different establishments were different from one another owing to the differential

Fig. 6. Clustering of 600 rice germplam into 10 clusters based on ten traits in dry seeded establishment
Table 2. Clustering of genotypes based on the dissimilarity index for three different establishments in rice

| Clusters     | Number of genotypes | Dry seeded rice | Wet seeded rice | Transplanted rice |
|--------------|---------------------|-----------------|-----------------|-------------------|
| Cluster I    |                     | 196             | 156             | 172               |
| Cluster II   |                     | 296             | 65              | 50                |
| Cluster III  |                     | 10              | 52              | 28                |
| Cluster IV   |                     | 9               | 252             | 302               |
| Cluster V    |                     | 43              | 2               | 2                 |
| Cluster VI   |                     | 1               | 52              | 10                |
| Cluster VII  |                     | 22              | 10              | 7                 |
| Cluster VIII |                     | 21              | 8               | 27                |
| Cluster IX   |                     | 1               | 1               | 1                 |
| Cluster X    |                     | 1               | 2               | 1                 |

Fig. 7. Clustering of 600 rice germplam into 10 clusters based on ten traits in wet seeded establishment
performance of genotypes in different establishments (Kesh et al., 2021). In the dry seeded rice, the cluster II had the highest number of genotypes (296) in it. On the other hand, cluster IV had the highest number of genotypes 252 and 302 in wet seeded and transplanted rice establishments, respectively. The genotypes with in the same cluster were closely related for the morphological traits studied, while genotypes placed in the different cluster were more diverse. The presence of single solitary clusters or clusters with single genotypes represents the higher level of variability present in the particular genotype under study (Ulaganathan and Nirmalakumari, 2015). In the present study, the solitary clusters were cluster IX and cluster X in dry seeded and transplanted establishments, while cluster IX was in the wet seeded establishment. The genotypes Tundus, Liu 19, Semiocho and Minchechinhsuthou were found to be very diverse from others in the current study as they were placed in solitary clusters in different crop establishments. Hence, the selection of these diverse parents would be helpful for better hybridization programs.

In India and most of the South Asian countries, rice is usually transplanted from the nursery into the puddled
field. But in recent times, the concept of direct seeded rice (wet and dry) is in need of the hour owing to climate change, less water and less labour availability (Alam et al., 2018). Hence, the identification of suitable genotypes for the direct seeded and transplanted condition is vital for yield improvement and other future breeding programs. In the present study, the genotypes White Ponni and Shuabatsan were best for the dry seeded establishments. The genotypes viz., Hsung Tieng 2, La Hung Ku and Chinkachang were highly suitable for the wet seeded establishment (Table 3). The genotypes like White Ponni, La Hung Ku, IR76875206B4B and Hsung Tieng 2 performed better in all three establishments. Hence, these lines can be used for both direct seeded and transplanted conditions.

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