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Genetic Diversity Analysis of Red Kernel Rice Genotypes using K-means Clustering for Grain Yield, its Components and Cooking Quality Traits

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ABSTRACT

The field experiment was conducted during *kbarif* season (August-November), 2018 to evaluate the available red rice
genotypes for genetic diversity using K-means cluster analysis for grain yield, its components and cookin the College of Agriculture, V. C. Farm, Mandya. University of Agricultural Sciences, Bengaluru, Karnataka, India. Multivariate analysis was carried out in RCBD with 2 replications to understand the nature and magnitude of genetic divergence among sixty-four red rice genotypes. The analysis of variance showed that significant variance was observed among genotypes for all the traits studied. Based on K-means cluster analysis, optimum number of clusters formed were nine. Maximum number of genotypes grouped in cluster III (15) followed by cluster IV (12). Maximum intra-cluster distance was shown by cluster I (55.79) and cluster IX (53.50) indicating wide genetic variation among genotypes belonging to these clusters. Maximum inter-cluster distance (234.57) was recorded between cluster I and cluster IX followed by cluster VI and IX (231.49) revealing that the genotypes of these clusters were highly diverse and can be used as divergent parents for hybridization. The characters, plant height, alkali spreading value, water uptake ratio, gel consistency, days to 50% flowering and grain L/B ratio contributed 82.40% to the total divergence among the genotypes studied. Diversified red kernelled genotypes namely Rajamudi, Bramavara-8, Jenugudu, MSN-10-3, IET-19778, MSN-33-1, RP-1310-326, Champaka, Bramavara-8 and IET-16902 possessing desirable traits may prove useful for incorporation of these traits in the improvement of red rice.

KEYWORDS: Cooking quality, cluster distance, K-means clustering, red rice

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Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

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1. INTRODUCTION

Commercial availability of red rice in markets is limited, however, by a lack of improved varieties at the producer level. Few farmers cultivate red rice using local varieties which are long duration, low yielders and susceptibility to lodging (Islam et al., 2018). To develop promising red rice varieties or hybrids, knowledge of genetic diversity is pre-request for breeder to choose diverse parent among red kernel rice genotypes based on the contribution of different quantitative and qualitative traits for genetic improvement of red rice (Sharma et al., 2022). Genetic diversity is a potential resource for a broad range of genetic research and trait improvement but the genetic diversity in red rice is relatively unexplored. Since success of plant breeding depends on the availability of genetic variation, knowledge about desired traits, and efficient selection strategies, a plant breeder has to identify the source of favorable genes to incorporate them into the breeding populations and select for a combination of desirable traits that might result in the isolation of productive genotypes (Tiwari et al., 2011).

Scientists are looking for rice varieties with outstanding grain quality attributes other than carbohydrates, protein and fat, as lifestyle-related health disorders are on the rise around the world. Rice grain quality is determined by its physical and physicochemical properties. Physical qualities namely milling recovery, head rice recovery, kernel size and shape (Sellappan et al., 2009, Shijagurumayum, et al., 2018); Kernel shape and grain L/B ratio are important features while assessing grain quality (Rita and Sarawgi, 2008). Cooking quality of rice has a profound impact in determining its economic value and consumer acceptability (Bhat and Riar, 2017). The physicochemical traits namely gelatinization temperature, gel consistency, water uptake and volume expansion ratio are directly related to cooking quality (Rani et al., 2019).

Volume expansion ratio has an important bearing for economically backward residents as quantity has an important role in their scheduled life (Bhat and Riar, 2017). The quality desired would vary from one geographical region to the other, for example, in japonica rice eating countries, short grain and low amylose is preferred while, in indica rice consuming countries, long grain with intermediate amylose, soft gel consistency and high-volume expansion is preferred (Hossain et al., 2009, Sharma and Khanna, 2019).

Cluster analysis is a multivariate analysis that have the function of minimizing differences within clusters and maximizing differences between clusters (Oliveira et al., 2016). Genotype selection in large numbers have a high level of difficulty. Cluster analysis can be used to classify genotypes and determine the best cluster (Kozak et al., 2008). Cluster analysis had been done for selection of potential genotypes by Bhati et al. (2015) and Rashmi et al. (2017) based on estimation of genetic diversity among traditional landraces. Nirmala et al. (2015) had conducted cluster analysis on rice genotypes based on grain yield and cooking quality characteristics. Various studies have been reported based on cluster analysis for estimation of genetic diversity in rice (Roy and Sharma, 2014, Pillai et al., 2020, Naik et al., 2021).

To ensure farmers' livelihoods and nutritional security of the population, there is a need to develop red rice hybrids using diverse parents which have potential to yield better than open pollinated genotypes and good nutrient profiles so that people consuming rice diets are supplied with adequate minerals, vitamins, proteins, carbohydrates and other health promoting agents that would benefit the farming community by increasing income and also health benefits. In this context, this research program was envisaged to evaluate the available red rice genotypes for genetic diversity using cluster analysis for grain yield, its components and cooking quality traits.

2. MATERIALS AND METHODS

64 red kernel rice genotypes including 2 checks (Jyothi and MSN-100) were utilized for diversity analysis in the present study (Table 1). The experimental material is analyzed in RCBD with 2 replications during *kharif* season (August−November, 2018) at College of Agriculture, V. C. Farm, Mandya. University of Agricultural Sciences, Bengaluru, Karnataka, India. It is located at latitude of 12°30'N, longitude of 76°50'E and altitude of 694.65 m above mean sea level (MSL) with red sandy loam soil. All the recommended package of practices are followed to raise the healthy crop. Observations are recorded on five randomly selected plants for traits such as days to 50% flowering, plant height (cm), number of productive tillers plant⁻¹, panicle length (cm), number of spikelets panicle-1, spikelet fertility (%), 1000-grain weight (g), grain yield plant⁻¹ (g), grain L/B ratio, milling $%$ and head rice recovery (%). Characterization of red kernel rice genotypes for cooking quality traits such as alkali spreading value, volume expansion ratio, water uptake ratio and gel consistency are indicated below.

2.1. Alkali spreading value (ASV)

It involves incubating six kernels of whole rice in 10 ml of 1.7% KOH for 23h as per Jennings et al. (1979). It measures the degree of spreading using a 7-point scale (1- intact, 7- greatly dispersed).

2.2. Gel consistency (mm)

Dispersing 100 mg of rice flour in 0.2 ml of 95% ethanol containing 0.025% thymol blue in test tube followed by adding 2 ml of 0.2N KOH. The contents were heated on *International Journal of Bio-resource and Stress Management* 2022, 13(8):845-853

boiling water, cooling in an ice-water bath, and measuring gel length. The gel consistency values are classified as soft (>61 mm), flaky rice with medium (41–60 mm) or very flaky with hard (26–40 mm) (Jennings et al., 1979).

2.3. Water uptake ratio

Estimated as ratio of final cooked weight to uncooked weight (Bhattacharjee and Kulkarni, 2000).

2.4. Volume expansion ratio

Estimated as ratio between cooked volume to the uncooked volume (Pillaiyar and Mohandas, 1981).

2.5. Statistical analysis using k-means clustering

The red kernel rice genotypes were classified following 'k-means clustering' model as explained by Macqueen (1967) and Forgy (1965) to unravel organization of variability using R software VER., 4.1.3.

J= ∑^m i=k ∑^k k=1ωik II X2 - μk II² ………….. (1)

Where,

IIX²- μ_kII²- Indicator of distance between n data point from their respective cluster center.

xi: Number of data points.

μk: Number of cluster centre.

3. RESULTS AND DISCUSSION

3.1. Analysis of variance (ANOVA) among red kernel rice genotypes

Analysis of variance revealed highly significant mean sum of squares attributable to sixty-four red rice genotypes for all the traits and the same results were presented in Table 2. These results suggest that existence of spectrum of genetic variability among the genotypes. The characters such as days to 50% flowering, spikelet fertility (%), plant height (cm), number of productive tillers plant⁻¹, number of spikelets panicle-1, panicle length (cm), panicle weight (g), 1000-grain weight (g) and grain yield plant⁻¹ (g) were highly significant. Similarly, the physical quality traits such as grain L/B ratio, milling (%) and head rice recovery (%) and cooking quality traits such as volume expansion ratio, water uptake ratio,

gel consistency and alkali spreading value also showed significant difference among genotypes studied. Earlier workers (Dushyanthakumar, 2008; Nirmala et al., 2015; Pillai et al., 2020 and Rukmini Devi et al., 2020) had also reported the presence of high degree of genetic diversity in red rice for both grain yield and quality traits. Although the analysis of variance revealed sufficient variability among the genotypes, but the extent of genetic diversity present among the genotypes could not be explained, therefore, cluster analysis was performed to measure the genetic divergence between any two genotypes or group of genotypes.

3.2. Genetic diversity study using k-means clustering

K-means clustering is an iterative algorithm that intends to partition the dataset into k distinct non-overlapping clusters where each data point belongs to the cluster with nearest mean. K-means is a centroid-based clustering algorithm. 'K' represents the number of clusters, and it is also an input parameter (Kanavi et al., 2020). In K-means clustering, the optimal clustering is the one with the smallest amount of variation within clusters, which is calculated using the within-clusters sum of squares. It assigns data points to a cluster such that the sum of the squared distance between

the data points and the cluster's centroid (arithmetic mean of all the data points that belong to that cluster) is at the minimum. The less variation we have within clusters, the more homogeneous (similar) the data points are within the same cluster. This method produces exactly k different clusters of greatest possible distinction (Meirmans and Van Tienderen, 2004).

3.3. Clustering pattern and composition of group among red kernel rice genotypes

Clustering of 64 red kernel genotypes using K-means cluster analysis (Nbclust R package) based Mahalanobis genetic distance resulted in nine distinct clusters is represented in Table 3 and Figure 1. The genotypes within each cluster were closer to each other than the genotypes belonging

IX 3 CR-2711-144-1, MSN 20-3 and MSN-15-15

Figure 1: K-means clustering pattern of red kernel rice genotypes (K=9) based on mean data of grain yield, its components and cooking quality traits

to different clusters. Out of nine clusters, cluster III had maximum number of genotypes *i.e.,* 15 followed by cluster IV with 12 genotypes, cluster II with 8 genotypes, cluster V, VII and VIII with 6 genotypes each, cluster VI with 5 genotypes and cluster I and XI with 3 genotypes each. The mode of distribution of genotypes from different geographical regions into various clusters was at random indicating that the genotypes originating from different agro-climatic / geographical regions grouped together into different clusters showing no parallelism between genetic diversity and geographical distribution. Similar kind of results were observed by Pandey and Kaushik (2011) and Rukmini Devi et al. (2020).

Shanmugam and Rangasamy (1982) reported that grouping of material of same geographical origin into different clusters was an indication of the broad genetic base of genotypes belonging to that origin.

The average intra and inter-cluster D^2 values with their corresponding intra and inter-cluster distance are presented in Table 4. Higher intra cluster distance was recorded in cluster I (55.79) followed by cluster IX (53.50) indicating wide genetic variation among the genotypes belonging to these clusters. The chances of developing good segregants by crossing the genotypes of the same cluster showing high values for intra-cluster distance is very high.

Maximum inter cluster distance was recorded between the clusters I and IX (234.57) followed by cluster VI and IX (231.49) revealing the existence of maximum difference among the genotypes falling into these clusters. Therefore,

for different traits									
Cluster		$_{\rm II}$	III	IV	V	VI	VII	VIII	IX
\bf{I}	55.79	162.94	81.39	104.14	124.18	86.94	118.25	104.08	234.57
$_{\rm II}$		36.17	117.90	74.65	56.00	158.63	75.78	99.60	82.75
III			35.67	56.41	83.24	71.05	104.73	69.80	190.99
IV				32.72	47.06	97.70	72.82	58.33	146.82
$\rm V$					36.89	129.96	56.41	82.81	121.37
VI						45.10	144.89	74.84	231.49
VII							38.25	98.61	131.24
VIII								42.70	168.82
IX									53.50

Table 4: Intra cluster (Diagonal) and inter cluster distances for 9 clusters formed by 64 red kernelled genotypes of rice for different traits

the genotypes with higher mean values for desirable traits belonging to these clusters separated by high cluster distance could be used in breeding programme for obtaining wide spectrum of variation among the segregants (Anurag et al*.*, 2017). Hence, selection of genotypes within these clusters holds great promise as parents for obtaining promising elite lines through inter varietal hybridization and also to create further variability for these traits (Mishra and Pravin, 2004, Anurag et al*.*, 2017).

In contrast, least value of inter-cluster distance was recorded between cluster IV and V (47.06), indicating homogenous nature of the genotypes for most of the traits with less deviation between the genotypes and hence, may not be emphasized upon to be used in hybridization programme. Parallel findings were found by Priyanka et al. (2015), Nirosha et al. (2016) and Shivangi et al. (2021).

3.4. Cluster mean analysis

The diversity among genotypes was also supported by the appreciable amount of variation among the cluster means for different characters. The cluster mean values showed a wide range of variation for majority of the characters undertaken in the present study (Table 5). These are helpful to assess the superiority of clusters during the improvement of characters through hybridization programme. Clusters VI and VIII contains traditional red rice genotypes recorded lowest mean values for days to 50% flowering (91.00 and 92.00 days respectively) while genotypes of same clusters recorded highest mean value for plant height. In contrast, cluster III recorded lowest mean value for plant height (86.65 cm) followed by cluster V (90.94 cm). Similarly, for character number of productive tillers plant⁻¹, the higher mean value (18.85) was recorded by genotypes Mallige-1 and lowest value (6.33) by Krishnaleela in cluster VI and VIII, respectively which are separated by inter-cluster distance of 74.84. The genotype MSN-20-3 in Cluster IX recorded highest mean value (26.67 cm) and that lowest (18.00 cm)

in cluster III by AC39000 for panicle length. The genotype Aishwarya-5 recorded highest spikelet fertility (100.00%) in cluster I and that of lowest (63.91%) by Duddoge in cluster VIII. The genotypes CR-2711-144-1 and MSN 20-3 in cluster IX recorded high mean values for panicle weight (5.36 g). The genotype MSN-15-15 in cluster IX had highest number of spikelets panicle⁻¹ (247.59). Character 1000-grain weight recorded highest mean value (30.61 g) in cluster VI and III by genotypes Rajamudi (31.16g) and Jenugudu (30.91g) respectively and lowest (16.28 g) by MSN-17 in cluster II. Cluster VII recorded highest mean value (31.36 g) by RL-106 for grain yield plant⁻¹ and that lowest (12.52 g) in cluster VI.

Similarly, for grain quality traits, the genotype Rajamudi included in cluster VI was important for grain L/B ratio (5.14) and water uptake (4.30 ml g^{-1}) . Genotype KCMS29A/ MSN78B from cluster III having highest mean value for head rice recovery (76.39%) while cluster V contained genotype IET-16902 recorded highest milling% (90.02%). Character volume expansion ratio and gel consistency recorded highest mean values (2.22 and 121.6 mm) respectively in cluster IV by genotype Champaka. Comparative assessment of cluster means showed that for improving specific characters, the genotypes should be selected from the cluster having high mean value for that particular character. Based on genetic distance, cluster means and *per se* performance, the red kernelled genotypes such as Rajamudi, Bramavara-8, Jenugudu, MSN-10-3, IET-19778, MSN-33-1, RP-1310-326, Champaka, Bramavara-8 and IET-16902 identified as desirable genotypes and can be used in future hybridization programme to develop red rice varieties or hybrids. Ravindra Babu et al*.* (2006) and Subudhi et al*.* (2009) also proposed to choose diverse parents for quality traits such as head rice recovery%, volume expansion ratio, gel consistency, water uptake ratio and alkali spreading value from the most divergent clusters so that they produce larger

variability and desirable segregants that would be productive in rice quality breeding program. Most of the minimum and maximum cluster means were distributed in relatively distant clusters. The hybridization between genotypes of distant clusters yields desirable genotypes (Bose et al., 2011). Recombination breeding between genotypes of different clusters having higher genetic distance has been reported by Sinha et al. (1991) and Maruthi (2018).

3.5. Contribution of traits towards genetic diversity

Contribution of different grain yield and cooking quality characters to total divergence. The maximum contribution in the manifestation of genetic divergence was exhibited by plant height (26.10%) followed by cooking quality traits such as alkali spreading value (21.00%), water uptake ratio (13.8%) and gel consistency (8.10%) further followed by days to 50% flowering (7.60%) and grain L/B ratio (5.80)

Hence, plant height, alkali spreading value and water uptake ratio were found to be potential contributors to genetic divergence in the studied red rice genotypes therefore, selection for these characters may be fruitful for recognizing genetically diverse genotypes for breeding good cooking quality traits. These observations corroborate well with those of earlier researchers (Sandhyakishore et al*.,* 2007, Patil et al*.,* 2005).

Ravindra Babu et al*.* (2006) evaluated cooking quality traits to study genetic divergence pattern among rice genotypes and reported that the cooking quality traits such as alkali spreading value, volume expansion ratio, gel consistency and water uptake contributed maximum towards genetic divergence. However, Dushyantha kumar (2008) carried out genetic diversity analysis among 71 red rice genotypes and found plant height, panicle length and grain yield contributed significantly for the genetic diversity and reported that these traits should be given importance during hybridization and selection of segregating populations because as genetically diverse parents are known to produce high heterotic response and new desirable recombinants.

4. CONCLUSION

A nalysis of variance among genotypes indicating the ample amount of genetic variation for all the traits. Based on the K-means clustering, all the sixty-four genotypes were grouped into nine distinct clusters. The diversified red kernelled genotypes namely Rajamudi, Bramavara-8, Jenugudu, MSN-10-3, IET-19778, MSN-33-1, RP-1310-326, Champaka, Bramavara-8 and IET-16902 possessing different desirable traits from the distinct clusters may prove potential in the future hybridization programme to develop red rice hybrids or varieties with higher grain yield and good cooking quality traits.

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