

Estimation of Genetic Diversity in Rice (*Oryza sativa* L.) CMS Lines for High out Crossing Potential and Quality Traits

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Authors' contributions

This work was carried out in collaboration among all authors. Author KK designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors YCM and VGS managed the analyses of the study. Authors CVDR and LK managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

In the present study genetic diversity among 40 rice genotypes was evaluated using Mahalanobis D² statistic based on morphological markers. Based on cluster analysis, the germplasm were grouped into 4 clusters of which Cluster I comprising of 23 genotypes was the largest group followed by the cluster II comprising of 15 genotypes, the clusters III and IV were represented by single genotype indicating high degree of heterogeneity among the genotypes. Maximum intra cluster distance observed in cluster II is (146.8) followed by cluster I (112.9) indicating the existence of variability among the genotypes within these clusters. The highest inter-cluster distance was observed between cluster IV and III (606.5), While the lowest was noticed between

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cluster II and III (222.9), describing that the genotypes included in these clusters were closely related. The kernel length, kernel length after cooking, kernel breadth, gel consistency, days to 50% flowering and plant height together contributed 90.1% towards total divergence. Therefore, these characters should be given importance during hybridization and selection of segregating populations. The genotypes falling in different clusters with the high mean for grain yield and other component characters can be utilized for the hybridization programme to obtain elite segregants.

Keywords: Rice; genetic diversity; stigma exertion; angle of glume opening.

1. INTRODUCTION

Rice (*Oryza sativa* L.) is the staple food crop of Asia where it is consumed by more than half of the world's population. It is mainly grown in large areas of Asia, Latin America and Africa that are characterized by a semitropical climate with alternating rainy and dry seasons [1]. In India rice is the main food source for more than 60 per cent of the population and it is contributing about 45 per cent of total cereal production [2]. To meet the challenge of producing more rice from limited land area, we need varieties or hybrids which grow better under adverse conditions and possess high yield potential [3]. Hybrid rice is the proven technology for breaking the yield barrier of inbred varieties. Hybrid rice can out yield the promising varieties and it is a key technology to meet the increasing global demand for rice [4,5,6]. The success of hybrid rice breeding programme not only depends on the development of potential hybrids but also the availability of seeds of these hybrids in adequate quantities to the farmer which in turn is dependent on out crossing rates of female lines. Several traits contribute to the hybrid seed production efficiency, among which the stigma exertion and angle of flower opening are important for Cytoplasmic Male Sterile lines, account for improved seed set [7]. Though several new CMS lines were developed indigenously, the majority of them were poor in outcross potential [8]. Hence there is an immense need to develop quality CMS lines with better out cross potential. It is mandatory to develop high yielding hybrids of good quality suiting the needs of the market. About 90% of the existing rice hybrids have been developed from IRRI based CMS lines which have poor cooking quality especially low in amylose (less than 20%), [9]. The amylose content of starches usually ranges from 15 to 35%. High amylose content rice shows high volume expansion and high degree of flakiness. The harder gel consistency is associated with harder cooked rice and this feature is particularly evident in high-amylose rice.

Genetic diversity is the pre-requisite for any crop improvement programme because it helps in the development of superior recombinants [10], through the selection of parents having wider variability for different characters [11]. Since, the last few centuries, rice has faced loss in diversity due to the replacement of native varieties with high yielding varieties [12]. Genetic divergence analysis evaluates the genetic distance among the selected genotypes and shows the relative contribution of specific traits towards the total divergence [13]. Higher heterosis could be achieved from crosses between genetically distant parents.

Therefore, the present investigation aimed to assess the nature and magnitude of genetic divergence across the 40 rice genotypes and for selection of suitable genotypes with high outcross potential and better quality for utilisation as parents in development of heterotic hybrids.

2. MATERIALS AND METHODS

The experiment was carried out with 40 genotypes (Table 1) during 2018, in a Randomized Block Design with three replications at Rice Research Centre, Agriculture Research Institute, Rajendranagar, Hyderabad, Telangana State. The seed was sown in the nursery on raised beds on *kharif* and thirty days old seedlings were transplanted in two rows of 4 m length plot at a spacing of 20 x 15 cm, recommended package of practices was followed to raise a healthy crop.

Five random plants for each genotype per replication were selected to record data on plant height (cm), number of effective tillers per plant, panicle length (cm), number of grains per panicle, kernel length (mm), grain yield per plant (g), ten random plants of each genotype in each replication were selected to record data on the angle of flowering opening and stigma exertion. Days to 50% flowering was recorded on whole plot basis whereas a random sample in each plot was used to record kernel length (mm), kernel

Table 1. List of rice genotypes (maintainer lines) utilized for the study

S. no.	Genotype	Source
1	CMS 11B	IRRI, Philippines
2	CMS 14B	IRRI, Philippines
3	CMS 23B	IRRI, Philippines
4	CMS 46B	IRRI, Philippines
5	CMS 52B	IRRI, Philippines
6	CMS 59B	IRRI, Philippines
7	CMS 64B	IRRI, Philippines
8	JMS 11B	RARS, Jagtial
9	JMS 13B	RARS, Jagtial
10	JMS 14B	RARS, Jagtial
11	JMS 17B	RARS, Jagtial
12	JMS 18B	RARS, Jagtial
13	JMS 20B	RARS, Jagtial
14	JMS 21B	RARS, Jagtial
15	RNR 21280	RRC, ARI, Hyderabad
16	WGL 44	RARS, Warangal
17	TELLAHAMSA	RRC, ARI, Hyderabad
18	RP 5950-24-6-2-1-1-B	IIRR, Hyderabad
19	GNV 14-25	IIRR, Hyderabad
20	GNV 14-05	IIRR, Hyderabad
21	R 1919-537-1-160-1	IIRR, Hyderabad
22	RP 4993-183-9-2-1-1	IIRR, Hyderabad
23	SYE 160-7-19-7-23-16	IIRR, Hyderabad
24	MTU 1216	RARS, Maruteru
25	TP 30494	IRRI, Philippines
26	JGL 1798	RARS, Jagtial
27	HMT Sona	RRC, ARI, Hyderabad
28	IR 10 F 388	IIRR, Hyderabad
29	CT – 18615-1-S-1-2-4	IIRR, Hyderabad
30	TULASI	IIRR, Hyderabad
31	RNR 26119	RRC, ARI, Hyderabad
32	RNR 26032	RRC, ARI, Hyderabad
33	RNR 26061	RRC, ARI, Hyderabad
34	RNR 26075	RRC, ARI, Hyderabad
35	OR 2573-11	IIRR, Hyderabad
36	Sharbati	NRRI, Cuttack
37	IR – BLZ-F4	IIRR, Hyderabad
38	RNR 26992	RRC, ARI, Hyderabad
Checks		
39	RNR 15048	RRC, ARI, Hyderabad
40	MTU1010	RARS, Maruteru

breadth (mm), L/B ratio, 1000-grain weight (g), hulling percentage, milling percentage, head rice recovery percentage, amylose content, gel consistency and kernel length after cooking. Mean data of each character were subjected to Mahalanobis D^2 analysis [14]. The germplasms were grouped into several clusters by Tocher's method described by Rao [15]. Each character was ranked based on the values in all the combination of genotype for estimation of the contribution of individual characters towards divergence.

3. RESULTS AND DISCUSSION

3.1 Grouping of Genotypes into Various Clusters

The analysis of variance for yield and its components revealed highly significant differences among the genotypes for all the 17 traits indicating the existence of genetic variability among the experimental material. The 40 genotypes of rice were grouped into four clusters based on D^2 values using Tocher's

method [15], (Table 2) Cluster I comprising of 23 genotypes was the largest group followed by the cluster I comprising of 15 genotypes, whereas the clusters III and IV, were represented by single genotype indicating high degree of heterogeneity among the genotypes. Among the cluster I and cluster II which have a greater number of genotypes (38 genotypes), 1000 grain weight and the number of grains per panicle were found to be contrasting and played a major contribution in partitioning these genotypes in different groups.

High intra cluster distance observed in cluster II is (146.8) followed by cluster I (112.9) revealing that some genetic divergence still existed among the genotypes of the cluster. The intra cluster distance was lower than inter-cluster distances, indicating the existence of genetic diversity among the genotypes of different clusters, which could be made use of in the yield improvement through recombination breeding (Table 3, Fig. 1).

The highest inter-cluster distance was observed between cluster IV and III (606.5), followed by cluster I and IV (505.9), cluster I and III (359.3), cluster II and IV (296.1). Hence it is suggested that the genotype RNR 26061 (cluster IV) and OR 2573-11 (cluster III) could be crossed with the genotypes of cluster I would give wider and desirable recombination. While the lowest was

noticed between cluster II and III (222.9), describing that the genotypes included in these clusters were closely related.

Selection of the parents based on grain yield of cluster IV and III, cluster I with IV, cluster I with III, cluster II with IV would produce encouraging results. The genotypes of these clusters may be used as parents in the hybridization programme for yield improvement. These findings are in conformity with the findings of Chakma et al. [16].

The data indicated that the cluster means for days to 50% flowering was highest in cluster III (106) and the lowest in cluster II (93) (Table 4). For the number of effective tillers per plant cluster mean was highest in cluster II (18.0) and lowest in cluster I (16.0). Plant height was highest in cluster IV (117.3 cm) and lowest in cluster I (95.6 cm). Cluster IV recorded the maximum panicle length (29.8 cm) and the lowest panicle length was in cluster I (24.4 cm). Cluster I recorded the maximum number of grains per panicle (257) and the lowest was recorded in cluster IV (143). The 1000 grain weight was highest in cluster III (26.4 g) and the lowest in cluster I (15.1 g). Highest grain yield per plant was recorded in cluster III (39.7 g) followed by cluster I (30.2 g), cluster II (28.0 g), while lowest grain yield per plant was recorded in cluster IV (15.9 g). The angle of flower opening

Table 2. Clustering pattern among 40 rice genotypes

Cluster number	Number of genotypes	Name of genotypes
Cluster I	23	CMS 11B, CMS 14B, CMS 64B, JMS 13B, JMS 14B, JMS 17B, JMS 18B, JMS 20B, RNR 21280, WGL 44, TELLAHAMSA, RP 5950-24-6-2-1-1-B, GNV 14-25, GNV 14-05, R 1919-537-1-160-1, RP 4993-183-9-2-1-1, SYE 160-7-19-7-23-16, JGL 1798, HMT Sona, IR 10 F 388, RNR 26119, RNR 26992, RNR 15048.
Cluster II	15	CMS 23B, CMS 46B, CMS 52B, CMS 59B, JMS 11B, JMS 21B, MTU 1216, TP 30494, CT – 18615-1-S-1-2-4, TULASI, RNR 26032, RNR 26075, Sharbati, IR – BLZ-F4, MTU1010.
Cluster III	1	OR 2573-11
Cluster IV	1	RNR 26061

Table 3. Intra (diagonal) and inter cluster distances (D² values) of 40 rice genotypes

	Cluster I	Cluster II	Cluster III	Cluster IV
Cluster I	113	265.7	359.3	505.9
Cluster II		146.8	222.9	296.1
Cluster III			0.00	609.5
Cluster IV				0.00

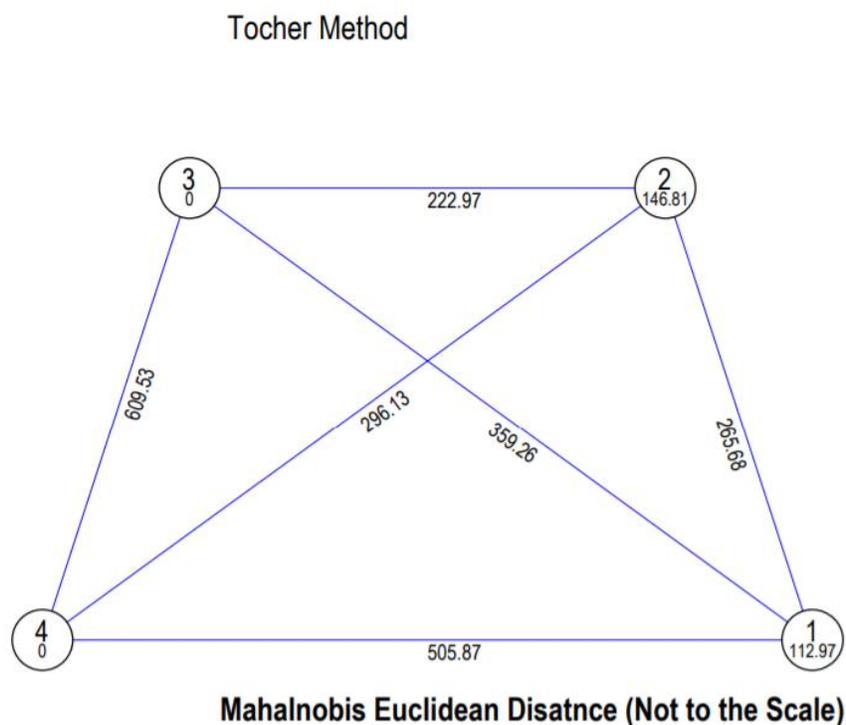


Fig. 1. Average inter and intra cluster distance

recorded highest in cluster III (30.7°), lowest was recorded in cluster I (26.2°). Stigma exertion recorded highest in Cluster III (47.4%) followed by cluster I (44.4%) while lowest in cluster I (43.3%).

Highest hulling percentage was recorded in cluster III (86.7%) and the lowest in cluster IV (61.7%). For milling percentage cluster mean was highest in cluster III (71.5%) and lowest in cluster IV (54.3%). Cluster I recorded the highest head rice recovery percentage (58.0%) and the lowest in cluster IV (41.4%). High kernel length was recorded in cluster IV (7.45 mm) whereas it was low in cluster I (5.82 mm). High kernel breadth was recorded in cluster III (2.41 mm) whereas it was low in cluster IV (1.62 mm). High amylose content was recorded in cluster III (25.7%) and low in cluster IV (17.5%). Cluster IV was recorded high gel consistency (69.0 mm) whereas it was low in cluster III (32.6 mm). Kernel length after cooking was recorded highest in cluster IV (10.50 mm) and low in cluster I (8.09 mm).

It is observed that no cluster contained at least one genotype with all the desirable traits, which ruled out the possibility of selecting directly one genotype for immediate use. Therefore,

hybridization between the selected genotypes from divergent clusters is essential to judiciously combine all the targeted traits.

It is very clear that cluster III (OR 2573-11) was found to be desirable with respect to angle of glume opening and stigma exertion, however it had higher amylose and more height. In contrast, the cluster I (Table. 6) had intermediate amylose, less height, slender grain and poor outcross traits. In this context, it could be desirable to cross OR 2573-11 with the genotypes of cluster I for developing superior lines possessing high out cross traits coupled with short height, less test weight and more quality. Similarly, the genotype, OR 2573-11 could be crossed to genotypes of cluster II for deriving improved lines with high out crossing, less height, more test weight and quality.

3.2 The Relative Contribution of Characters towards Genetic Divergence

The number of times that each of the 17 characters appeared in first rank and its respective per cent contribution towards genetic divergence is presented in Table 5. The results showed that the contribution of kernel length was

highest towards genetic divergence (39.3%), followed by kernel length after cooking (12.9%), kernel breadth (12.8%), gel consistency (12.7%), days to 50% flowering (7.0%), plant height (5.4%), amylose content (4.9%), grain yield per plant (1.8%), 1000-grain weight (1.4%), hulling percentage (0.9%), number of grains per panicle (0.5%), panicle length (0.3%) and angle of flower opening (0.1%) to the genetic divergence.

The kernel length, kernel length after cooking, kernel breadth, gel consistency, days to 50% flowering and plant height, together contributed

90.1% towards total divergence. Similar results were reported Garg et al. [17]. Therefore, these characters should be given importance during hybridization and selection of segregating populations derived from the materials used in the present study. Stigma exertion, number of effective tillers per plant, milling percentage, and head rice recovery percentage did not contribute to genetic diversity. Similar results were reported by Archana et al. [18] for number of effective tillers per plant, Radha et al. [19], Devi et al. [20] for head rice recovery and Sanju Kumari et al. [21] for grain yield per plant.

Table 4. Cluster means of 40 rice genotypes for yield, yield components and quality traits

Sl. no	Characters	Cluster I	Cluster II	Cluster III	Cluster IV
1.	Days to 50% flowering	98	93	106	94
2.	No. of effective tillers per plant	16.0	18.0	17.4	16.6
3.	Plant height (cm)	95.6	103.7	112	117.3
4.	Panicle length (cm)	24.4	25.2	26.1	29.8
5.	No. of grains per panicle	257	155	154	143
6.	1000 grain weight (g)	15.1	21.9	26.4	21.0
7.	Grain yield per plant (g)	30.2	28.6	39.7	15.9
8.	Angle of flower opening (°)	26.2	29.1	30.7	28.2
9.	Stigma exertion (%)	43.3	44.4	47.4	45.6
10.	Hulling percentage (%)	76.0	74.7	86.7	61.7
11.	Milling percentage (%)	66.3	63.3	71.5	54.3
12.	Head rice recovery (%)	58.0	52.8	56.7	41.4
13.	Kernel length (mm)	5.82	6.98	7.37	7.45
14.	Kernel breadth (mm)	1.90	2.10	2.41	1.62
15.	Amylose content (mm)	22.6	21.8	25.7	17.5
16.	Gel consistency (mm)	50.2	58.8	32.6	69.0
17.	Kernel length after cooking (mm)	8.09	9.91	9.44	10.50

Table 5. Relative contribution of different characters to genetic diversity in rice genotypes

Sl. no	Characters	Times ranked first	Contribution (%)
1.	Days to 50% flowering	55	7
2.	No. of effective tillers per plant	0	0
3.	Plant height (cm)	42	5.4
4.	Panicle length (cm)	2	0.3
5.	No. of grains per panicle	4	0.5
6.	1000 grain weight (g)	11	1.4
7.	Grain yield per plant (g)	14	1.8
8.	Angle of flower opening (°)	1	0.1
9.	Stigma exertion (%)	0	0
10.	Hulling percentage (%)	7	0.9
11.	Milling percentage (%)	0	0
12.	Head rice recovery (%)	0	0
13.	Kernel length (mm)	306	39.3
14.	Kernel breadth (mm)	100	12.8
15.	Amylose content (%)	38	4.9
16.	Gel consistency (mm)	99	12.7
17.	Kernel length after cooking (mm)	101	12.9

Table 6. Promising genotypes having outstanding mean values for yield and quality

Cluster	Promising Traits	Genotypes
Cluster IV	Better stigma exertion and angle of glume opening.	RNR 26061
Cluster I	Intermediate amylose, less height, slender grain	CMS 11B, CMS 14B, CMS 64B, JMS 13B, JMS 14B, JMS 17B, JMS 18B, JMS 20B, RNR 21280, WGL 44, TELLAHAMSA, RP 5950-24-6-2-1-1-B, GNV 14-25, GNV 14-05, R 1919-537-1-160-1, RP 4993-183-9-2-1-1, SYE 160-7-19-7-23-16, JGL 1798, HMT Sona, IR 10 F 388, RNR 26119, RNR 26992, RNR 15048.
Cluster III	Grain yield per plant, more test weight and high stigma exertion and angle of glume opening	OR 2573-11

4. CONCLUSION

The significant range of variation was evident among forty rice genotypes studied. The forty rice genotypes were grouped into four clusters with the maximum inter-cluster distance was showed by cluster IV and III, followed by cluster I against IV and III, cluster II and IV. The crosses involving genotypes from these clusters would be expected to manifest high heterosis along with, accumulation of favorable genes in subsequent segregating generations. Cluster III had higher mean values of stigma exertion and angle of glume opening poor in grain quality parameters where as cluster I had good quality traits viz., intermediate amylose, less height, slender grain and poor outcross traits. In this context, it could be desirable to cross OR 2573-11 with the genotypes of cluster I for developing superior lines possessing high out cross traits coupled with dwarf height, low test weight and better quality.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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