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Genetic divergence studies in maintainer lines of rice (*Oryza sativa* L.) for yield and its attributing traits

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Abstract

Aim: To study the genetic divergence studies in maintainer lines of rice for its yield and its attributing traits

Study design: Randomized block design.

Place and Duration of study: Institute of Rice Research, ARI, Rajendranagar, Hyderabad, Telangana, during the kharif season in 2024.

Methodology: The study involved 28 maintainer lines and two checks. Sown in nursery and transplanted with spacing of 30 cm x 15 cm after 25 days. Observations was recorded in yield and its attributing traits. Statistical analysis was conducted using INDOSTAT software for the analysis of variance and genetic divergence for yield and its attributing traits.

Results: Analysis of variance revealed that there was a significant variation ($p < 0.01$) among genotypes for all traits. In Mahalanobis's (1936) D^2 , the 30 genotypes of rice were divided into eight clusters. Cluster I had highest number of rice genotypes (12), followed by Cluster II and VI with seven, six genotypes, respectively. The clusters, III, IV, V, VII and VIII were mono genotypic. Inter cluster distances between clusters VII and VIII were maximum. Cluster VI had also exhibited higher intra-cluster distance. Further, Cluster IV had showed maximum yield per plant, panicle length, kernel length and kernel L/B ratio. Cluster V has highest mean value for maximum number of grains per panicle, stigma exertion% and stigma length. The greatest contribution to genetic divergence was recorded by kernel breadth (19.77%), followed by days to 50% flowering (18.16%), 1000- grain weight (15.86%) and grain yield per plant (13.56%) indicating that selection for these traits was effective.

Keywords: Rice, *Oryza sativa*, maintainer lines, genetic divergence

1. Introduction

'Rice is Life' for billions of people, and rice production has influenced life style, diet, and economic status of humans across the globe. It is being cultivated in over 100 countries worldwide. Asia produces and consumes 90% of the world's rice. It is referred to as 'Global Grain' and is the dominant staple cereal food of world (Bhargavi *et al.*, 2023) [3]. The present rice varieties have reached the yield plateau to overcome this problem there is need to development of novel technology which meets the demand of the growing population by increasing the yield.

The development of hybrid rice varieties using male sterility, maintainer lines and fertility restoration systems has been one of the turning moments in rice history which helps to meet the food security (Hyndhavi *et al.*, 2025) [5]. Whereas "maintainer lines (B lines) are the key genotypes that are used to maintain the sterility and supply yield boosting genes to the female parent (A line) during new female line development in hybrid breeding. Maintainer lines (B lines) are mainly developed using B×B crossing method" (Hossain *et al.*, 2020) [4].

The success of any breeding programme depends on the amount of variability present in the germplasm. The knowledge of genetic divergence is very important in the identification of suitable parents for hybridization. Wider the diversity among the parents, the greater will be the chance of obtaining heterotic combinations and broader would be the spectrum of variability in the segregating generations (Arunachalam., 1981) [2].

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This study estimates the nature and magnitude of genetic divergence in twenty-eight maintainer lines and two checks to maximize utilization of genetic resources and select suitable donor parents (Meena *et al.*, 2017) ^[7].

2. Materials and Methods

2.1 Materials and Planting

The experimental material for the present investigation

comprised of 28 genotypes along with 2 checks. The experiment was done during Kharif season of 2024 in a randomized block design at the Institute of Rice Research, ARI, Rajendranagar, Hyderabad. All the genotypes were initially sown in a nursery bed. After twenty-five days after sowing, the seedlings were transplanted into the field with a spacing of 30 cm between the rows and 15 cm between the plants.

Table 1: List of maintainer lines and checks used in the study

S. No	Genotypes	Source
1	BCN 2756	CRRRI, Cuttack
2	BCN 2768	RRU, ARI, Rajendranagar
3	BCN 2786	ICAR-IIRR, Rajendranagar
4	BCN 2802	ICAR-IIRR, Rajendranagar
5	BCN 2816	ICAR-IIRR, Rajendranagar
6	BCN 2830	RARS, Jagtial
7	BCN 2846	RRU, ARI, Rajendranagar
8	BCN 2852	IRRI, Philippines
9	BCN 2864	RRU, ARI, Rajendranagar
10	BCN 2874	RRU, ARI, Rajendranagar
11	BCN 2894	RRU, ARI, Rajendranagar
12	BCN 2904	RRU, ARI, Rajendranagar
13	BCN 2922	RARS, Warangal
14	BCN 2934	RARS, Warangal
15	BCN 2944	RARS, Warangal
16	BCN 2950	RARS, Warangal
17	CMS 23B	IRRI, Philippines
18	CMS 59B	IRRI, Philippines
19	CMS 64B	IRRI, Philippines
20	JMS 17B	RARS, Jagtial
21	RMS 1B	RARS, Warangal
22	RMS 2B	ICAR-IIRR, Rajendranagar
23	RMS 3B	PDKV, Maharastra
24	RMS 4B	PDKV, Maharastra
25	RMS 5B	ICAR-IIRR, Rajendranagar
26	RMS 6B	ICAR-IIRR, Rajendranagar
27	RMS 7B	ICAR-IIRR, Rajendranagar
28	RMS 9B	RARS, Jagtial
29	RNR 15048	RRU, ARI, Rajendranagar
30	JGL 24423	RRU, ARI, Rajendranagar

2.2 Data Collection

Observations were recorded randomly on five plants for days to 50% flowering, angle of the flower opening (°), stigma exertion (%), plant height (cm), panicle length (cm), number of productive tillers per plant, number of grains per panicle, 1000-grain weight, grain yield per plant.

Data was recorded on quality parameters such as kernel length (mm), kernel breadth (mm), kernel L/B ratio, hulling (%), milling (%), head rice recovery (%) and amylose content (%) from genotype was recorded. Observations on hulling% and milling% were taken with the help of Satake Company make laboratory huller and polisher. Kernel length and kernel width of 10 hulled rice were measured by means of dial micrometer and length and breadth ratio was computed. Amylose content was estimated by chemical method.

Statistical analysis was conducted using INDOSTAT software

for the estimation of ANOVA. Genetic diversity was estimated by using Mahalanobis's D^2 (1936) ^[6] analysis to calculate the total genetic distance among the maintainer lines and the Tochers' method, as described by Rao was exercised to cluster the maintainer lines.

3. Results and Discussion

The results on genetic divergence of 30 rice genotypes for yield and yield components traits are presented and discussed here under.

3.1 Test with Wilk's criterion 'Λ'

Analysis of variance (ANOVA) for the different traits studied is presented in Table 2. The results revealed significant differences between the genotypes for all traits studied.

Table 2: Analysis of variance for yield attributes, floral and quality traits in 30 genotypes of rice.

Si. No.	Genotypes	Mean Sum of Squares		
		Replications (df =1)	Treatments (df =29)	Error (df = 29)
1	Days to 50% flowering	2.017	114.037***	1.499
2	Plant height (cm)	6.521	118.101***	13.484
3	Panicle length (cm)	9.457	5.108*	2.064
4	Number of productive tillers per plant	1.667	9.944***	1.988
5	Number of filled grains per panicle	361.131	9746.753***	576.815
6	1000- grain weight (g)	0.434	37.478***	0.652
7	Grain yield per plant (g)	11.80	119.369***	4.298
8	Angle of flowering (°)	2.860	11.913***	1.258
9	Stigma exertion (%)	4.532	75.659***	17.040
10	Stigma length (µm)	9798.689	27987.040***	2103.722
11	Hulling (%)	10.626	15.974***	2.258
12	Milling (%)	3.169	64.209***	2.801
13	Head rice recovery (%)	2.961	64.413***	6.339
14	Kernel length (mm)	0.005	1.164***	0.033
15	Kernel breadth (mm)	0.000	0.097***	0.007
16	Kernel L/B ratio	0.001	0.294***	0.036
17	Amylose content (%)	0.131	4.292***	0.940

The Wilk's statistic ' Λ ' was highly significant with value of 24949.83, justifying the calculation of D^2 values. The results are in conformity with the findings of earlier workers (Sudeepthi *et al.*, 2020) ^[14] who also reported ' V ' statistic to be highly significant with value of 2736.30 in their studies on 107 rice genotypes for yield and yield component traits, justifying the need to calculate D^2 values.

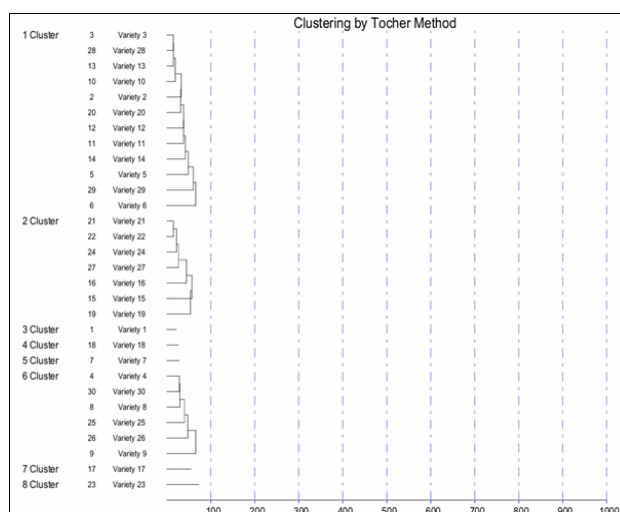
3.2 Grouping of genotypes into various clusters

The 30 genotypes of rice were grouped into eight clusters based on D^2 values using Torcher's method (Rao, 1952) ^[11]. The composition is presented in Table 3 and Fig 1. The cluster I

comprising 12 genotypes (BCN 2786, RMS 9B, BCN 2922, BCN 2874, BCN 2768, BCN 2904, BCN 2894, BCN 2934, BCN 2816, RNR 15048, BCN 2830, JMS 17B) was the largest group followed by cluster II comprising of 7 genotypes (RMS 1B, RMS 2B, RMS 4B, RMS 7B, BCN 2950, BCN 2944, CMS 64B), cluster VI with 6 genotypes (BCN 2802, JGL 24423, BCN 2852, RMS 5B, RMS 6B, BCN 2864), cluster III (BCN 2756), IV (CMS 59B), V (BCN 2846), VII (CMS 23B), VIII (RMS 3B) were represented by single genotype indicating high degree of heterogeneity among the genotypes. The results in conformity with the reports of earlier worker (Sri Lakshmi *et al.*, 2021) ^[13].

Table 3: Clustering pattern among 30 genotypes of rice.

Cluster number	Number of genotypes	Name of genotypes
Cluster I	12	BCN 2786, RMS 9B, BCN 2922, BCN 2874, BCN 2768, BCN 2904, BCN, 2894, BCN 2934, BCN 2816, RNR, 15048, BCN 2830, JMS 17B
Cluster II	7	RMS 1B, RMS 2B, RMS 4B, RMS 7B, BCN 2950, BCN 2944, CMS 64B
Cluster III	1	BCN 2756
Cluster IV	1	CMS 59B
Cluster V	1	BCN 2846
Cluster VI	6	BCN 2802, JGL 24423, BCN 2852, RMS 5B, RMS 6B, BCN 2864
Cluster VII	1	CMS 23B
Cluster VIII	1	RMS 3B

**Fig 1:** Clustering Pattern of 30 rice genotypes by Torcher's Method

3.3 Average Intra Cluster and Inter Cluster Distances

The average intra and inter cluster D^2 distance values among eight clusters are presented in Table 4 and Fig. 2. Intra cluster D^2 values were zero for III, IV, V, VII and VIII as these clusters were containing single genotypic and as they are more divergent. The intra cluster distance were highest for cluster VI (161.89) compared to other clusters followed by cluster II (143.87) revealing that some genetic divergence still existed among the genotypes of the cluster. The intra cluster distance lower than the inter cluster distances, indicating that there was genetic diversity among the genotypes of different clusters, which could be made use of in the yield improvement through recombination breeding. The highest inter cluster distance was distance was observed between cluster VII and VIII (861.28) compared to other clusters followed by cluster VI and VIII (757.64) than by cluster IV and VIII (602.42), cluster II and VI (586.72) and cluster II and VII (551.90). It is assumed that maximum amount of heterosis will be present in cross combination involving the

clusters with most divergent. But for a plant breeder, the objective may not only high heterosis but other quality characters also. Hence it is suggested that the genotypes RMS 3B (cluster VIII), could be crossed with the genotypes CMS 23B (cluster VII) followed by crossing with genotypes such as BCN 2802, JGL 24423, BCN 2852, RMS 5B, RMS 6B, BCN 2864 of cluster VI and with CMS 59B of cluster IV likewise genotypes of cluster II such as RMS 1B, RMS 2B, RMS 4B, RMS 7B, BCN 2950, BCN 2944, CMS 64B could be crossed with the genotypes BCN 2802, JGL 24423, BCN 2852, RMS 5B, RMS 6B, BCN 2864 (cluster VI) and genotypes CMS 23B (cluster VII) to achieve wider and desirable recombination. While the lowest inter cluster distance was noticed between cluster IV and V (105.40) followed by cluster V and VI (144.66) and cluster III and VII (173.29) describing that the genotypes included in these clusters are closely related as result they are not divergent. Similar results were similar with reports of earlier workers (Akhtar *et al.* (2022) ^[1] and Bhargavi *et al.* (2023) ^[3].

Table 4: Intra and inter cluster distance (D^2 values) of 30 genotypes of rice Bold: Intra cluster values

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII	Cluster VIII
Cluster I	136.74	233.12	231.30	220.63	255.79	428.50	414.95	379.46
Cluster II		143.87	348.46	350.20	423.93	586.72	551.90	469.58
Cluster III			0.00	353.94	280.68	424.09	173.29	478.26
Cluster IV				0.00	105.40	179.58	407.09	602.42
Cluster V					0.00	144.66	375.10	503.16
Cluster VI						161.89	331.89	757.64
Cluster VII							0.00	861.28
Cluster VIII								0.00

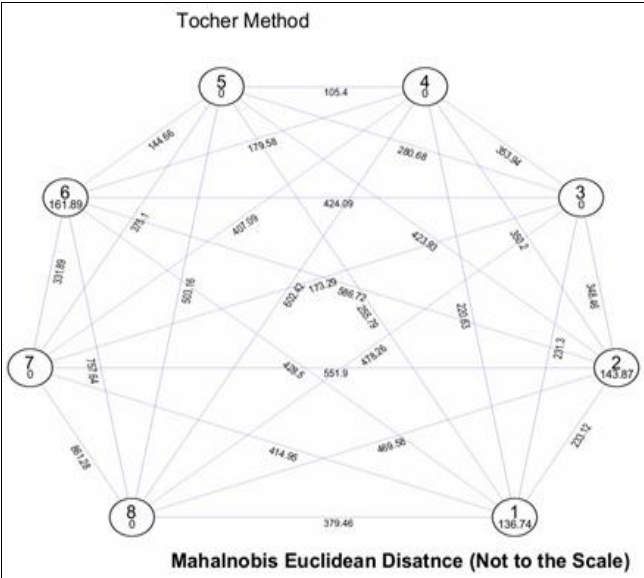


Fig 2: Average inter and intra cluster distance between 8 cluster by Torcher’s method

3.4. Cluster means of the characters

The estimation of cluster means gives details on suitable donors for a specific trait. Mean performance of clusters for yield and yield parameters traits for the 30 rice genotypes were evaluated in the experiment and presented in Table 5. Cluster II had highest cluster mean value (106.14) for days to 50% flowering whereas cluster III and VII showed lowest mean value (85.00). Plant height had showed highest mean value in cluster V (102.35cm) whereas lowest mean was recorded in cluster VII (78.55cm). while panicle length recorded highest cluster mean in IV and V (26.05cm) clusters and lowest panicle

length recorded in cluster VII (21.80 cm). Cluster II recorded the highest mean value for number of productive tillers per plant (17.43) and lowest mean was recorded by cluster VII with the value of 11.20. while 1000-grain weight recorded highest value in cluster VI (24.18 g) and lowest in cluster VIII (14.06 g). Whereas maximum number of filled grains per panicle recorded in the cluster V (243.30) however cluster III recorded lowest number of grains with mean value of (121.90). Highest grain yield per plant was recorded in cluster IV (38.20 g) whereas lowest grain yield per plant was recorded in cluster III (13.50 g). Angle of flower opening has shown the highest mean value for cluster VIII (37.88) and lowest mean was shown by cluster IV (28.60). Cluster V (87.14%) has highest mean value for stigma exertion% and cluster III (67.57%) has shown lowest cluster value. Maximum stigma length was recorded in cluster V (1298.63 μ m) and lowest length was observed in cluster VII (878.64 μ m). While Cluster VII (82.92%) has showed highest mean value for hulling% and lowest mean value was shown by cluster VIII (76.67%). Milling% has shown highest mean value in cluster VI (71.18%) whereas lowest recorded in VIII (45.42%). Whereas head rice recovery% has highest mean value for cluster VII (65.05%) and lowest mean values for cluster VIII (37.92%). Kernel length recorded highest mean value in cluster IV (7.36 mm) whereas lowest mean value was observed in cluster I and II (5.58 mm). Maximum kernel breadth recorded highest in cluster VI (2.20 mm) and lowest in cluster VIII (1.70 mm). whereas kernel L/B ratio recorded highest in cluster IV (4.08) and lowest in cluster VII (4.08). Highest cluster mean was observed in cluster VIII (25.21%) and lowest mean was observed in cluster VII (20.45%) for amylose content. Similar results were observed in earlier reports (Prasad *et al.*, (2018) ^[10], Shanmugam *et al.* (2023) ^[12] and Mondal *et al.* (2024) ^[8].

Hence cluster V genotype BCN 2846 has good outcrossing traits with long slender grain intermediate range of amylose content and comparative yield per plant with checks (RNR 15048 and JGL 24423) which can be used as a parent in breeding programme for these traits and cluster IV genotype CMS 59B has shown medium slender grain type with low amylose content. BCN 2756 genotype of cluster III had shown short duration with

moderate height but susceptible to blast and also showed less yield. While genotype of cluster II BCN 2950 showed higher yield and resistance blast.

Therefore, it is necessary to carry out hybridization between the selected genotypes from divergent clusters to combine all the traits in to one hybrid.

Table 5: Cluster means of 30 genotypes of rice

SI. No	Characters	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII	Cluster VIII
1	Days to 50% flowering	94.33	106.14	85.00	94.00	93.00	92.42	85.00	96.00
2	Plant height (cm)	95.33	91.81	92.51	100.55	102.35	101.43	78.55	96.30
3	Panicle length (cm)	23.55	22.94	24.60	26.05	26.05	25.63	21.80	23.00
4	Number of productive tillers per plant	16.83	17.43	13.70	16.90	15.00	15.40	11.20	15.40
5	Number of filled grains per panicle	240.03	234.57	121.90	148.80	243.30	132.23	76.80	210.10
6	1000- grain weight (g)	15.28	15.15	17.86	21.63	23.85	24.18	20.53	14.06
7	Grain yield per plant (g)	30.15	25.91	13.50	38.20	32.90	30.50	15.00	34.70
8	Angle of flowering (°)	30.95	31.53	35.73	28.60	34.68	32.45	31.38	37.88
9	Stigma exertion (%)	75.77	76.37	67.57	75.65	87.14	75.55	76.15	82.86
10	Stigma length (µm)	1074.30	1084.65	1249.80	1000.03	1298.63	947.96	878.64	1065.19
11	Hulling (%)	78.52	80.00	82.09	80.84	77.50	80.97	82.92	76.67
12	Milling (%)	67.10	66.25	67.09	68.75	68.75	71.18	73.34	45.42
13	Head rice recovery (%)	57.83	56.13	55.00	53.75	59.17	58.54	65.05	37.92
14	Kernel length (mm)	5.58	5.58	5.78	7.36	6.80	7.03	5.88	5.69
15	Kernel breadth (mm)	1.78	1.82	2.06	1.81	2.10	2.20	2.17	1.70
16	Kernel L/B ratio	3.14	3.08	2.81	4.08	3.27	3.25	2.70	3.36
17	Amylose content (%)	24.07	24.62	22.27	23.06	23.10	24.13	20.45	25.21

3.5 Relative contribution of individual characters towards divergence

The relative contribution of various characters towards divergence was reported to aid the breeder in the choice of parents for hybridization and effective selection. The 17 traits studied for genetic divergence have given ranks according to the contribution with respect to number of times appeared first are presented in the Table 6 and Fig 3.

The contribution of kernel breadth highest towards genetical divergence (19.77%) by 86 times ranking first followed by days to 50% flowering (18.16%) by 79 times ranking first. Whereas 1000- grain weight contributed 15.86% by ranking 69 times first and grain yield per plant ranked first by 59 times by contributing 13.56% towards total divergence. while quality parameters such as hulling%, milling%, head rice recovery% and amylose content ranked first by 25, 42, 1, 18 times respectively and contributed 5.74%, 9.65%, 0.22%, 4.13% respectively towards

total divergence. whereas grain dimensions parameters such as kernel length, kernel breadth, kernel L/B ratio contributed 6.66%, 19.77%, 1.83% respectively by ranking 29, 86, 8 times first.

Floral traits such as angle of flower opening, stigma exertion%, stigma length were contributed 2.52%, 0.22%, 1.14% and ranked first 11, 5, 25 times. The number of filled grains per panicle contributed 0.45% towards total divergence and ranked first for 2 times. Plant height, panicle length and number productive tillers per plant have not contributed to total divergence.

Kernel breadth, days to 50% flowering, 1000- grain weight, grain yield per plant and milling% together contributed 77% towards divergence. Therefore, importance should be given to these parameters in hybridizing programmes. The similar reports were reported by earlier workers (Tiwari *et al.* (2022) [15] and Nikhil *et al.* (2023) [9].

Table 6: Relative contribution of different characters to genetic diversity in 30 genotypes of rice

Si. No	Characters	Times Ranked First	Contribution (%)
1	Days to 50% flowering	79.00	18.16
2	Plant height (cm)	0	0
3	Panicle length (cm)	0	0
4	Number of productive tillers per plant	0	0
5	Number of filled grains per panicle	2.00	0.45
6	1000- grain weight (g)	69.00	15.86
7	Grain yield per plant (g)	59.00	13.56
8	Angle of flowering (°)	11.00	2.52
9	Stigma exertion (%)	1.00	0.22
10	Stigma length (µm)	5.00	1.14
11	Hulling (%)	25.00	5.74
12	Milling (%)	42.00	9.65
13	Head rice recovery (%)	1.00	0.22
14	Kernel length (mm)	29.00	6.66
15	Kernel breadth (mm)	86.00	19.77
16	Kernel L/B ratio	8.00	1.83
17	Amylose content (%)	18.00	4.13

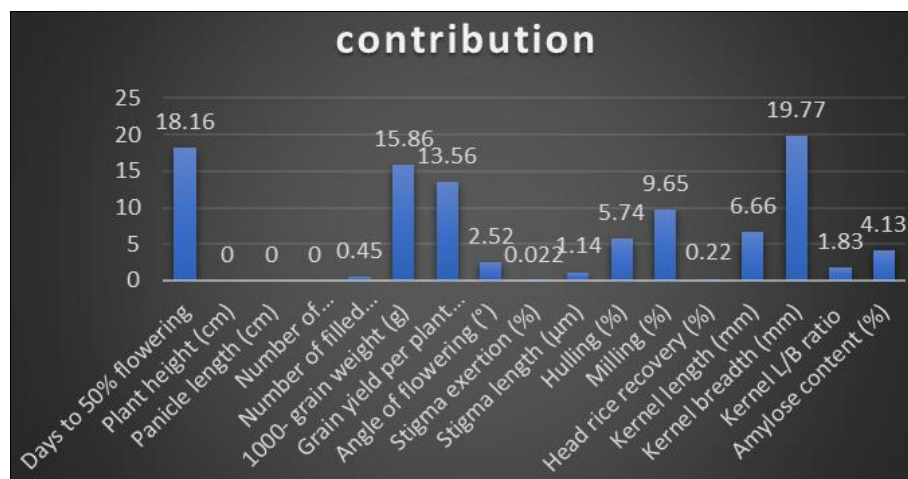


Fig 3: Relative contribution of individual characters towards divergence in 30 genotypes of rice

4. Conclusion

According to the findings, considerable variation exists among the maintainer lines considered for the current study. The emergence of different yield attribute clusters implies the possibility of divergence among the lines used. Recombination breeding for Cluster II maintainer lines could increase the yield potential and had significant inter cluster distance. Clusters V had good outcrossing genotypes with long slender intermediate amylose content and IV has good quality trait maintainer lines and III had short duration maintainer lines with most significant inter-cluster distance. The maintainer lines in these clusters can be employed in crosses, which will be fruitful due to favourable recombination throughout the selection process.

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Competing interests

Authors have declared that no competing interest exists.

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