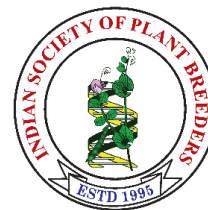


# Electronic Journal of Plant Breeding



## Research Article

### Genetic divergence and clustering studies in advanced breeding lines of rice (*Oryza sativa* L.)

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#### Abstract

Thirty six rice genotypes were used to estimate the magnitude of diversity associated with them using D<sup>2</sup> analysis to discover the diversity of genotypes. The analysis of 17 quantitative traits resulted in 8 different clusters from 36 rice genotypes. The maximum number of genotypes was grouped in Cluster I, whereas only one genotype each was associated with Cluster VII and VIII. Cluster I had maximum cluster mean for the number of grains per panicle and minimum for days to maturity whereas Cluster VII exhibited a better grain type with maximum cluster mean for grain yield. Hence the selection of genotypes from these clusters would give better breeding lines with higher grain yield. The dendrogram analysis helped to choose the improved mutants from the original variety.

#### Key words

Rice, D<sup>2</sup> analysis, inter and intra cluster distance, dendrogram.

#### INTRODUCTION

Rice (*Oryza sativa* L.) belonging to the family Poaceae is an essential cereal food in the world. India is the second largest producer and consumer of rice next to China. For meeting the dietary requirements of increasing population, genetic improvement of rice with higher yield, good grain quality, resistance to biotic and abiotic stresses is the most logical and promising approach. Over 90 per cent of the world's rice is produced and consumed in the Asian Region with six countries namely China, India, Indonesia, Bangladesh, Vietnam and Japan, that account for 80% in the world's production and consumption (Abdullah *et al.*, 2006).

Rice mutation breeding could be successful in obtaining new cultivars and broadening the genetic base of this crop (Domingo *et al.*, 2007). Genetic variation is the basis of plant breeding and provides a great array of genotypes that can be selected to develop new varieties or breeding materials. The divergence can be studied by D<sup>2</sup> statistics developed by Mahalanobis (1936). Knowledge concerning

heritability helps the plant breeders to predict the nature of the succeeding generation, to make an appropriate selection and to assess the magnitude of genetic improvement through selection (Tuhina-Khatun *et al.*, 2007). Genetic diversity determines the inherent potential of a cross for heterosis and frequency of desirable recombinants in advanced generations.

In this study, variability was generated through physical and chemical mutagens and recombinant breeding in rice. The selection was exercised to develop early maturing lines with desirable traits. Further it aims to assess the similarity and diversity among the advanced breeding lines in rice.

#### MATERIALS AND METHODS

The experimental research work was conducted at Agricultural College and Research Institute, Madurai. The research material includes thirty five rice genotypes which embrace three diverse types of breeding materials

along with Anna (R) 4 rice variety as check. Among the 35 genotypes, 12 advanced breeding lines derived from Anna (R) 4, EMS induced mutant lines in  $M_7$  generation, seven genotypes of MDU 6 x *Jaldi Dhan* 6 and one TKM 6 x *Jaldi Dhan* 6 recombinant lines in  $F_5$  generation and 15 entries of Anna (R) 4 irradiated entries which include eight entries from gamma irradiation and seven entries derived from electron beam irradiation in  $M_4$  generation (Table 1). Anna (R) 4 rice variety was included for evaluation and to study the diversity among the genotypes, crop was raised during *Kharif* 2019. In the main field, crop was transplanted with a spacing of 25 cm row to row and 15 cm plant to plant in Randomized Block Design (RBD) with two replications. Each genotype planted with three rows of 3 meter length.

Seventeen quantitative traits (Table 4) were recorded at right crop stages using Standard Evaluation System rice (IRRI, 2013). From each genotype five plants are selected and the followed traits were recorded with these plants. The traits measured are plant height at 50<sup>th</sup> day (cm), days to fifty per cent flowering (days), SPAD meter value, plant height (cm) at mature stage, panicle length (cm), flag leaf length (cm), flag leaf breadth (cm), total tillers, productive tillers, days to maturity (days), panicle weight (g), the number of grains per panicle, the number of filled grains, grain length (cm), grain width (cm), hundred seed weight (g), single plant yield (g). Analysis of variance, clustering of genotypes, per cent contribution of different traits to the total variation, inter and intra cluster distance

**Table 1. Experimental materials and their pedigree of 35 rice genotypes**

S.No	Pedigree	Code	Parent/ Treatment
1	EMS-T <sub>5</sub> 85-6-1-3-30	M <sub>7</sub> -1	100mM (EMS)
2	EMS-T <sub>5</sub> 85-3-2-5-25	M <sub>7</sub> -2	100mM (EMS)
3	EMS-T <sub>5</sub> 85-6-1-3-37	M <sub>7</sub> -3	100mM (EMS)
4	EMS-T <sub>5</sub> 84-1-2-3-27	M <sub>7</sub> -4	100mM (EMS)
5	EMS-T <sub>5</sub> 85-3-2-5-24	M <sub>7</sub> -5	100mM (EMS)
6	EMS-T <sub>6</sub> 151-4-16-8-1	M <sub>7</sub> -6	110mM (EMS)
7	EMS-T <sub>5</sub> 85-6-1-3-34	M <sub>7</sub> -7	100mM (EMS)
8	EMS-T <sub>6</sub> 151-1-15-8-35	M <sub>7</sub> -8	110mM (EMS)
9	EMS-T <sub>5</sub> 81-2-1-5-22	M <sub>7</sub> -9	100mM (EMS)
10	EMS-T <sub>5</sub> 85-3-2-5-33	M <sub>7</sub> -10	100mM (EMS)
11	EMS-T <sub>6</sub> 151-11-1-9-28	M <sub>7</sub> -11	110mM (EMS)
12	EMS-T <sub>6</sub> 151-1-15-8-27	M <sub>7</sub> -12	110mM (EMS)
13	C <sub>13</sub> -10-30-34	F <sub>5</sub> -1	MDU 6 X <i>Jaldi Dhan</i> 6
14	C <sub>13</sub> -10-4-15	F <sub>5</sub> -2	MDU 6 X <i>Jaldi Dhan</i> 6
15	C <sub>13</sub> -10-4-3	F <sub>5</sub> -3	MDU 6 X <i>Jaldi Dhan</i> 6
16	C <sub>13</sub> -10-22-34	F <sub>5</sub> -4	MDU 6 X <i>Jaldi Dhan</i> 6
17	C <sub>13</sub> -12-3-45	F <sub>5</sub> -5	MDU 6 X <i>Jaldi Dhan</i> 6
18	C <sub>13</sub> -19-20-30	F <sub>5</sub> -6	MDU 6 X <i>Jaldi Dhan</i> 6
19	C <sub>13</sub> -19-2-18	F <sub>5</sub> -7	MDU 6 X <i>Jaldi Dhan</i> 6
20	C <sub>17</sub> -6-16-24	F <sub>5</sub> -8	TKM 6 X <i>Jaldi Dhan</i> 6
21	GT <sub>1</sub> -24-3-1-8	M <sub>4</sub> -1	100 Gy Gamma Rays
22	GT <sub>1</sub> -45-7-2-9	M <sub>4</sub> -2	100 Gy Gamma Rays
23	GT <sub>1</sub> -46-3-1-6	M <sub>4</sub> -3	100 Gy Gamma Rays
24	GT <sub>1</sub> -46-4-1-6	M <sub>4</sub> -4	100 Gy Gamma Rays
25	GT <sub>1</sub> -129-15-2-4	M <sub>4</sub> -5	100 Gy Gamma Rays
26	GT <sub>2</sub> -14-15-2-7	M <sub>4</sub> -6	150 Gy Gamma Rays
27	GT <sub>2</sub> -73-17-1-2	M <sub>4</sub> -7	150 Gy Gamma Rays
28	GT <sub>2</sub> -82-1-1-2	M <sub>4</sub> -8	150 Gy Gamma Rays
29	ET <sub>1</sub> -37-6-2-6	M <sub>4</sub> -9	100 Gy Electron Beam
30	ET <sub>1</sub> -64-3-2-4	M <sub>4</sub> -10	100 Gy Electron Beam
31	ET <sub>1</sub> -79-29-2-6	M <sub>4</sub> -11	100 Gy Electron Beam
32	ET <sub>1</sub> -91-23-1-6	M <sub>4</sub> -12	100 Gy Electron Beam
33	ET <sub>2</sub> -51-13-2-7	M <sub>4</sub> -13	150 Gy Electron Beam
34	ET <sub>2</sub> -62-4-1-9	M <sub>4</sub> -14	150 Gy Electron Beam
35	ET <sub>2</sub> -118-19-1-3	M <sub>4</sub> -15	150 Gy Electron Beam
36	Anna (R) 4	Anna (R) 4	Check variety

Notes: S.No. 1 to 12 is EMS induced Anna (R) 4 mutants, S.No. 13 to19 is MDU 6 X *Jaldi Dhan* 6, S.No. 20 is TKM 6 X *Jaldi Dhan* 6, S.No. 21to 35 is irradiated mutants of Anna (R) 4 and S.No. 36 is check variety Anna (R) 4.

and cluster mean of different clusters with respect of different traits were analysed by Mahalanobis  $D^2$  analysis and clustering was done by using Toucher's method (Rao, 1952) in GENRES version 7.01. Dendrogram of 36 genotypes were constructed by hierarchical clustering using software DARwin 6.0.21.

## RESULT AND DISCUSSION

The present study consisting of 36 genotypes, exhibited significant difference for 17 observed quantitative characters. This result pointed out the existence of genetic variation among the genotypes, because of the experimental materials was derived from different dose of mutagen and parental crosses. The  $D^2$  analysis clustered the 36 genotypes into 8 different clusters (Table 2) in respective of 17 quantitative traits.

The Cluster I solely occupied 45 per cent of genotypes, which comprises all Anna (R) 4 EMS mutant genotypes with some of the recombinant lines. Findings revealed that the clustering pattern was not influenced by the nature of the genotypes it derived. Distribution of genotypes among different clusters also indicated that the genotypes derived from same mutagen treatment were located with different cluster. So the type of grouping might be due to the similarity of characters expressed in genotypes, environmental effects and selection pressure (Devi *et al.*, 2015).

Second highest number of genotypes about 7 was associated with Cluster III followed by Cluster IV consists 5 genotypes whereas Cluster VII and VIII encompasses only one genotype each.

**Table 2. Cluster of 36 rice genotypes based on 17 quantitative traits in rice**

	I	II	III	IV	V	VI	VII	VIII
I	<b>247.656</b>	466.987	414.936	370.131	504.826	444.897	1055.385	707.614
II		<b>18.996</b>	533.191	192.792	114.55	395.256	993.372	644.202
III			<b>262.298</b>	366.007	496.518	300.78	1532.945	647.190
IV				<b>231.442</b>	213.034	301.938	1099.719	598.458
V					<b>188.612</b>	312.765	1256.717	700.264
VI						<b>324.369</b>	1698.083	814.702
VII							<b>0</b>	1102.873
VIII								<b>0</b>

Inter and intra cluster  $D^2$  value of 8 clusters obtained from 36 rice genotypes is presented in Table 3. This result showed that the Cluster VI (324.36) had a maximum intra cluster distance value followed by Cluster III (262.29) and Cluster I (247.65). The minimum intra cluster distance was associated with Cluster II (18.99). The Cluster with highest intra cluster distance value indicates maximum range of genetic variation may be associated with genotypes presented in that cluster. In this study, the Cluster VI recorded maximum intra cluster distance but it contains only two genotypes namely Anna (R) 4 and its mutant (M<sub>4</sub>-15), which indicates that there is a higher genetic variation caused by mutation.

Inter-cluster distance value of the different clusters is the main factor which determines divergence among the

genotypes associated with those two clusters. Among the different clusters, maximum inter - cluster distance was observed between Cluster VI and VII (1698.08), followed by Cluster III and VII (1532.94) and Cluster V with VII (1256.71). The minimum inter - cluster distance was measured with Cluster II and V (114.55) followed by Cluster II and IV (192.79). The maximum inter-cluster distance between two clusters indicates that the genotypes present between clusters may exhibit a wide range of genetic variation. So crossing between these genotypes may produce more heterosis or useful for recombinant breeding.

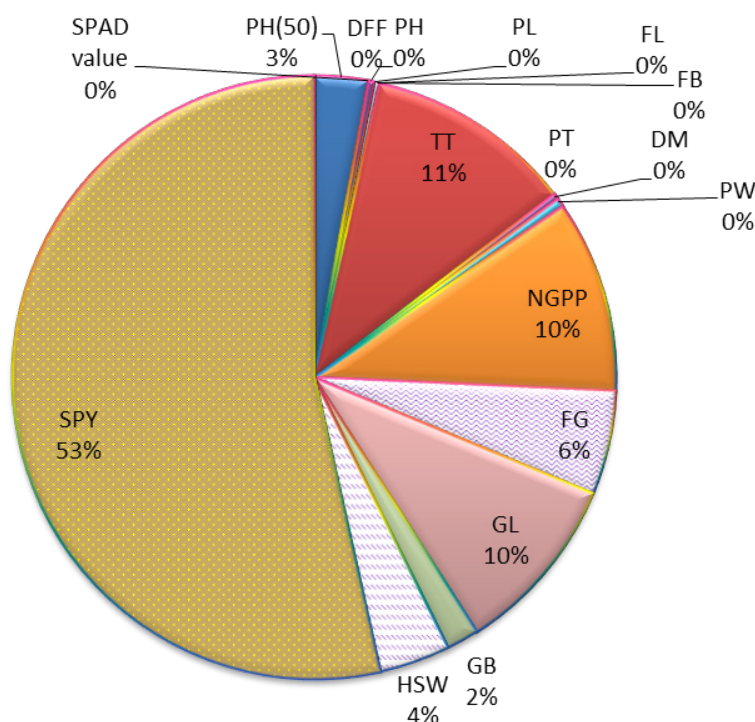
The per cent contribution of 17 quantitative traits in respective of 36 rice genotypes towards the total variation

**Table 3. Inter and intra cluster ( $D^2$ ) distance value of 8 clusters in rice**

Cluster number	No. of genotypes	Genotypes
I	16	M7-1, M7-2, M7-3, M7-4, M7-5, M7-6, M7-7, M7-8, M7-9, M7-10, M7-11, M7-12, F5-1, F5-2, F5-3, F5-4.
II	2	M4-4, M4-5
III	7	F5-5, F5-6, F5-7, F5-8, M4-1, M4-9, M4-14.
IV	5	M4-2, M4-3, M4-6, M4-7, M4-8
V	2	M4-11, M4-13
VI	2	M4-15, Anna (R) 4
VII	1	M4-10
VIII	1	M4-12

is presented in Fig. 1. Among the different traits, single plant yield has contributed to maximum divergence followed by the total number of tillers, the number of grains per panicle, grain length and filled grains. Moreover these traits exhibited maximum range of variation about

90.15%. The remaining traits like plant height, panicle length, flag leaf length, days to maturity did not influenced significantly towards the total divergence. This might be due to the selection pressure excised towards early maturing lines.



**Fig. 1. Per cent contribution of different traits for genetic diversity**

Note: PH (50)= Plant height at 50<sup>th</sup> day (cm), DFF= Days to fifty per cent flowering (days), SPAD value= SPAD meter value, PH= Plant height (cm), PL= Panicle length (cm), FL= Flag leaf length (cm), FB= Flag leaf breadth (cm), TT= Total tillers (nos.), PT= Productive tillers (nos.), DM= Days to maturity (days), PW= Panicle weight (g), NGPP= Number of grains per panicle (nos.), FG= Number of filled grains (nos.), GL= Grain length (mm) , GB= Grain breadth (mm), HSW=Hundred seed weight (g), SPY= Single plant yield (g).

The whole mutant lines in this experimental material were derived from Anna (R) 4 and recombinant lines from the MDU 6 x *Jaldi Dhan* 6 crosses. In this previous generation apart from earliness, the lines were selected based on reduced grain length, high number grains per panicle and more grain yield per plant. The advanced breeding lines were selected based on plant height, early maturity and the number of productive tillers. So, these traits did not contribute to the total variation. Genetic uniformity among these traits due to selection resulted in less variations, which led to least contribution to the genetic diversity in this study.

The superiority among the genotypes have been analysed based on the cluster mean of different traits associated with that cluster. Cluster mean of the eight clusters for 17 quantitative traits is given in Table 4. Among the eight

clusters, genotype M<sub>4</sub>-10 exhibited higher cluster mean value for single plant yield, which comes under the Cluster VII (Table 4), whereas, mutant M<sub>4</sub>-12 had minimum cluster mean for single plant yield.

The genotype M<sub>4</sub>-10 exhibited not only a higher grain yield but also recorded a lower grain length and breadth, which indicates that this genotype is, comes under high yielder with short slender grain type. Among the different clusters, in Cluster VI ( M<sub>4</sub>-15 and Anna (R) 4) the traits grain length and grain yield was recorded as the second highest cluster mean along with the maximum cluster mean for the traits such as grain breadth and 100 seed weight. The reason for maximum intra- cluster distance trapped with Cluster VI is due to the significant differences present between the mutants for the traits like grain length and breadth as compared with Anna (R) 4.

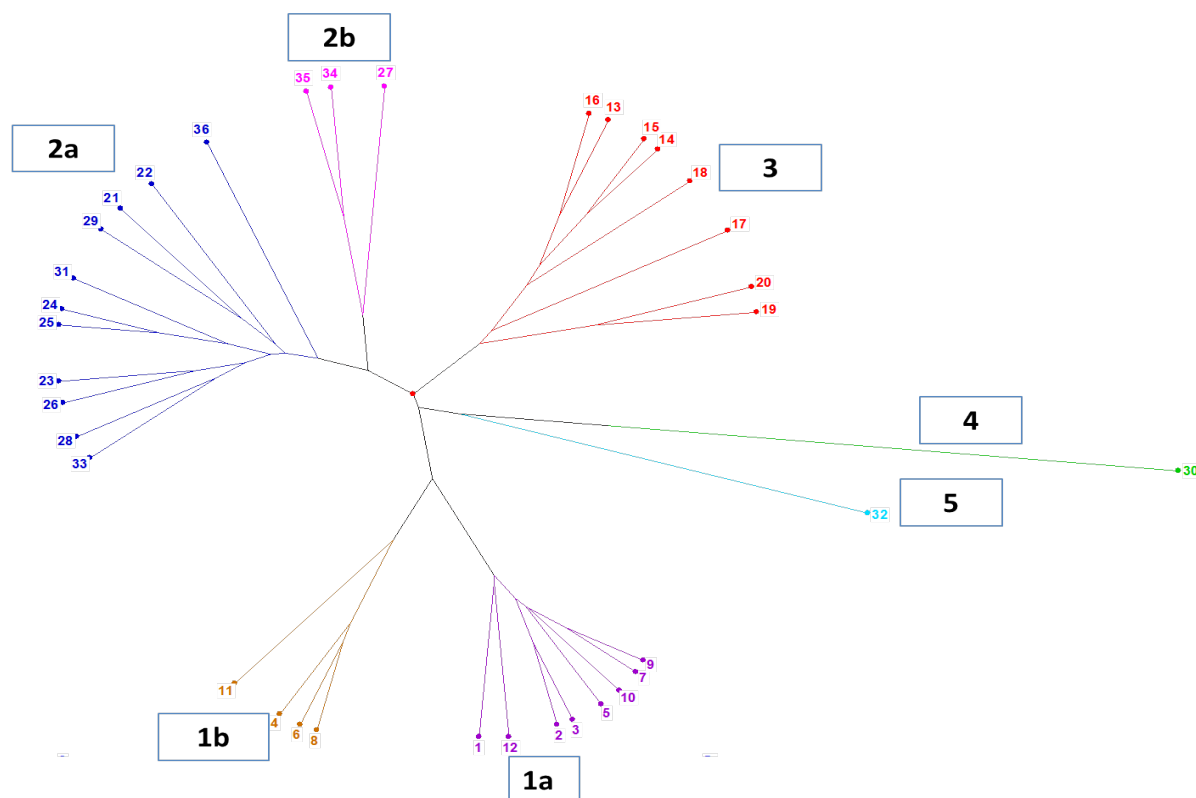
**Table 4. Cluster mean of eight clusters for 17 quantitative traits from 36 rice genotypes**

Clusters	PH(50)	DFF	SPAD value	PH	PL	FL	FB	TT	PT	DM	PW	NGPP	FG	GL	GB	HSW	SPY
I	55.03	73.71	41.091	102.98	23.83	30.27	1.25	19.35	17.18	103.62	3.20	181.20	158.32	6.13	1.94	1.972	31.17
II	60.87	76.25	35.913	105.11	26.10	34.55	1.60	11.60	11.05	107.75	3.73	175.58	131.16	6.497	2.19	2.447	26.52
III	55.78	73.71	37.272	97.85	24.79	30.78	1.43	22.29	20.05	103.42	3.55	136.53	115.50	6.42	2.136	2.48	28.56
IV	56.99	77.10	38.38	106.04	26.09	33.89	1.49	16.22	15.24	107.00	3.45	157.75	122.84	6.453	2.051	2.46	32.26
V	58.93	77.25	41.313	107.01	26.89	33.18	1.69	13.40	11.90	107.50	3.49	167.75	118.91	6.815	2.16	2.59	24.80
VI	56.53	75.50	42.00	100.07	25.51	30.08	1.56	15.35	14.30	107.75	4.02	157.16	134.30	6.725	2.22	2.69	33.14
VII	52.85	88.50	35.675	105.79	22.41	29.77	1.74	16.90	15.60	122.50	3.25	156.00	132.83	5.40	1.60	1.21	38.90
VIII	63.03	82.00	36.025	108.67	20.03	29.54	1.30	18.70	15.70	110.50	2.65	102.16	94.33	6.37	2.115	2.22	23.06

Note: PH (50)= Plant height at 50<sup>th</sup> day (cm), DFF= Days to fifty per cent flowering (days), SPAD value= SPAD meter value, PH= Plant height (cm), PL= Panicle length (cm), FL= Flag leaf length (cm), FB= Flag leaf breadth (cm), TT= Total tillers (nos.), PT= Productive tillers (nos.), DM= Days to maturity (days), PW= Panicle weight (g), NGPP= Number of grains per panicle (nos.), FG= Number of filled grains (nos.), GL= Grain length (mm) , GB= Grain breadth (mm), HSW=Hundred seed weight (g), SPY= Single plant yield (g).

Some of the mutants and cross derivatives clustered with Cluster III, which showed higher cluster mean for total tillers and productive tillers with minimum mean value for days to maturity and plant height. So, these genotypes are more useful for short duration with semi- dwarf plant type and high tillering ability. Apart from this, the Cluster I consists the entire EMS induced mutant lines with some recombinant lines. These genotypes exhibited maximum cluster mean value for traits such as the number of grains

per panicle and filled grains. The genotypes associated with this cluster also had lower cluster mean for days to maturity with better grain yield per plant. Similarly Oladosu *et al.* (2014) clustered 45 mutant lines into five different clusters with 13 morphological traits, while Tiruneh *et al.* (2019) formed 6 clusters by using 36 rice genotypes for 15 quantitative traits. Ashok *et al.* (2017) evaluated the genetic divergence among 64 genotypes of rice for yield and quality traits.

**Fig. 2. UPGMA clustering of 36 rice genotypes for 17 quantitative characters**

Unweighted Pair Group Method with Arithmetic Mean (UPGMA) of hierarchical clustering showed five major clusters with subgroups (Fig. 2). The genotypes of the first cluster were divided into two sub groups (1a & 1b), which contains all EMS derived mutants from Anna (R) 4. Whereas second group consist of all the irradiated mutants of Anna (R) 4 rice variety and the cross derivatives are completely grouped in third cluster. In group 2 the selected mutants derived from gamma rays and electron beam showed more similarity to Anna (R) 4, which indicates these mutants has higher chances to retain the Anna (R) 4 characters with improved grain quality and drought tolerance. So these genotypes are more useful for the further improvement of Anna (R) 4 variety. But the genotypes M<sub>4</sub>-10 and M<sub>4</sub>-12 solitarly clustered as 4 and 5. Because M<sub>4</sub>-10 recorded as mid early duration (122 days), short slender grain type with better grain yield whereas M<sub>4</sub>-12 recorded as minimum panicle length, panicle weight, the number of grains per panicle and the number of filled grains per panicle with low grain yield per plant. So, this may be the rationale for these two genotypes individually separated as solitary cluster. Jeng *et al.* (2011) also observed reduced plant height from the tall plant type of SA-586 rice genotype by using sodium azide induced mutation and Sushmitharaj *et al.* (2019) also reported the effect of recurrent EMS treatment on reduced plant height in mutants as compared with Anna (R) 4 variety.

The wide range of diversity was observed with 36 rice genotypes for 17 quantitative traits. Among the different clusters, Cluster VI and VII had a maximum inter cluster distance due to existence of more variation among genotypes. The genotypes associated with Cluster I and VI exhibited higher mean value for grain related traits like grain length, breadth and seed weight but the genotypes composed by Cluster VII exhibited short slender grain type. So the selection from these clusters helps to improve the grain qualities, earliness and grain yield of rice with early maturity.

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