Divergence Studies for Early Seedling Vigour Related Traits, Yield and Yield Components in Rice (*Oryza sativa* L.)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

The present study was carried out in the Autumn 2020-21 using a Randomized Complete Block Design (RCBD) with three replications. Forty-two different genotypes of rice were evaluated in this investigation. These genotypes were assessed based on 21 quantitative traits, which includes yield and yield related traits and early seedling vigour related traits. The Mahalanobis D^2 statistic was used for characterizing the genotypes. Based on the D^2 analysis, the 42 rice genotypes were grouped into seven clusters. Among these clusters, cluster I was the largest, consisting of 22 genotypes. Cluster III exhibited the highest intracluster distance (22.04), while the maximum inter-cluster distance was observed between clusters II and III (49.06), followed by clusters III and VII (45.84). These results highlight the significance of the genotypes present in these clusters for harnessing heterosis and exploiting the desirable traits associated with them.

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1. INTRODUCTION

Rice (*Oryza sativa* L.) is a crucial staple food for a significant portion of the global population, providing 23% of calories and accounting for approximately 20% of total grain production worldwide [1]. It serves as the primary source of income and livelihood for over 100 million households in Asia and Africa (FAO, 2004), earning the nickname "Grain of Life" (FAO, 1966). The global rice cultivation area spans around 165.25 million hectares, producing 502.98 million tonnes of rice [2].

In Asia, rice is commonly grown through transplanting in puddled soils, with intermittent flooding, and is considered an upland crop [3,4]. However, the traditional method of transplanting rice has drawbacks such as water and labor shortages, prompting the exploration of alternative approaches. Direct rice seeding has emerged as a viable option to address the challenges associated with traditional cultivation methods [5-7]. This method is increasingly practiced in many Asian countries, offering benefits such as reduced water and labor requirements, as well as overcoming time and soil-related conflicts, thereby promoting sustainable rice production [4]. To optimize crop establishment and productivity in direct-seeded rice (DSR), it is important to select varieties with high germination rates, rapid seedling emergence, and vigorous seedlings. These traits contribute to quick emergence, ensure an adequate crop stand, and suppress weed growth, thereby enhancing the success of direct seeding. Seedling vigor, which is closely linked to seedling development and germination, plays a crucial role in direct-seeded varieties [8].

The genetic diversity among rice genotypes provides ample opportunities for crop improvement. Understanding the genetic diversity within and among closely related rice varieties is essential for effective utilization of genetic resources. It enables genotype monitoring and helps predict genetic gains. Furthermore, quantifying the genetic diversity within and between groups of genotypes is crucial, as crosses between parents with the greatest genetic divergence often exhibit the most favorable response to genetic improvement [9]. This approach allows for the attainment of higher heterosis and superior recombinants [9].

In light of these considerations, This study focuses on augmenting genetic diversity in rice populations to bolster resilience against environmental challenges and enhance adaptability. By identifying diverse genetic rice lines which are suitable for direct-seeded condition types, to meet evolving agricultural demands, thereby contributing to global food security.

2. MATERIALS AND METHODS

In Autumn 2020-21, a study was conducted at the wetland farm of S. V. Agricultural College in Tirupati, Andhra Pradesh. The study used a RCB design with three replications to evaluate forty-two different genotypes. Details of the experimental material is furnished in Table 1. Each entry was grown in three rows, each measuring three meters in length and spacing followed was 20 x 15cm between and within the rows. Standard agronomic practices and recommended fertilizer doses were followed for optimal crop growth. The observations on eight yield components, including plant height, panicle length, number of panicles per plant, 1000-grain weight, number of filled grains per panicle, number of grains per panicle, spikelet fertility, and grain yield per plant were recorded.Also, the number of days it took for the plants to reach 50% flowering was recorded on plot basis. In addition, eleven components related to early seedling vigor were evaluated, such as germination percentage, germination rate, seedling fresh and dry weight, shoot length, root length, seedling height, root to shoot ratio and two seedling vigor indices as per International Seed Testing Association [10]. To analyze the genetic divergence, D^2 statistics proposed by Mahalanobis in 1936 was used. Standard statistical techniques were applied to the data collected [11] and diversity analysis was carried out through D^2 statistics [12].

➢ **Estimation and significance of Wilk's criterion**

Analysis of covariance for the character pairs was estimated on the basis of mean values [11] the estimation of Wilk'scriterion [13] was done using the following relationship.

$$\wedge = \frac{(E)}{(E+V)}$$

Table 1. List of 42 rice genotypes included in the study and their pedigree

S. No.	Designation	Pedigree
1	54-3	BPT5204 x NLR33892
2	150-3	BPT5204 x NLR33892
3	187-1	BPT5204 x NLR33892
4	187-3	BPT5204 x NLR33892
5	239-3	BPT5204 x NLR33892
6	419-1	BPT5204 x NLR33892
7	419-3	BPT5204 x NLR33892
8	538-3	BPT5204 x NLR33892
9	BPT5204	(GEB24 x TN1) x MAHSURI
10	NLR33892	NLR 27999 x MTU 4870
11	NDP3	NLR34449 x IR64 Drt1
12	ND13	NLR34449 x IR64 Drt1
13	ND42	NLR34449 x IR64 Drt1
14	ND44	NLR34449 x IR64 Drt1
15	ND60	NLR34449 x IR64 Drt1
16	NLR34449	IR 72 x BPT5204
17	MDP2	MTU1010 x IR64 Drt1
18	MDP3	MTU1010 x IR64 Drt1
19	MDP4	MTU1010 x IR64 Drt1
20	MD4	MTU1010 x IR64 Drt1
21	MD5	MTU1010 x IR64 Drt1
22	MDP6	MTU1010 x IR64 Drt1
23	MTU1010	KRISHNAVENI x IR 64
24	28C1	N22 x IR64
25	46C2	N22 x IR64
26	76C2	N22 x IR64
27	81C	N22 x IR64
28	130C	N22 x IR64
29	153C1	N22 x IR64
30	221C3	N22 x IR64
31	273C2	N22 x IR64
32	405C3	N22 x IR64
33	425C3	N22 x IR64
34	457C2	N22 x IR64
35	476C3	N22 x IR64
36	488C	N22 x IR64
37	NAGINA 22	LANDRACE
38	IR64	IR5657-33-2-1 x IR2061-465-1-5-5
39	SM227	SWARNA MUTANT
40	MTU3626	IR B x MTU 3
41	MTU1121	BPT5204 x MTU BB 8-24-1
42	SMB3	SWARNA MUTANT

where,

∧ = Wilk's criterion
(E) = Determinant of error matrix
(E+V) = Determinant of error + varieties matrix

➢ **Computation of D2 values**

The D^2 value between 'ith' and 'jth' genotypes for 'p' characters was calculated as:

$$D_{ij}^2 = \sum (Y_{it} - Y_{jt})$$

where,

Y_{it} is uncorrelated mean value of ith genotype for character 't'
Y_{jt} is uncorrelated mean value of jth genotype for character 't'
D$^2_{ij}$ is D^2 between ith and jth genotype

> **Grouping of genotypes into various clusters**

The grouping of genotypes into different clusters was done using the Tocher's method as described earlier [14]. The criterion was that the two genotypes belonging to the same cluster at least on an average show a smaller D^2 value than those belonging to different clusters. For this purpose, D^2 values of all combinations of each genotype were arranged in ascending order of magnitude in a tabular form as described earlier [15].

> **Average intra and inter-cluster distance**

For the measurement of intra-cluster distances, the formula used was $\Sigma\ D^2i/n$ where, $\sum D^2 i$ was the sum of distances between all possible combinations (n) of the populations included in a cluster. The square root of the average D^2 value gave the genetic distance 'D' between the clusters. Based on D^2 values (inter-cluster distance), the cluster dia-gram was prepared adopting the scale given earlier [14] for rating of the distance.

> **Cluster means**

The cluster mean for a particular trait is the summation of mean values of the genotypes included in a cluster divided by number of genotypes in the cluster.

> **Contribution of individual characters towards divergence**

In all combinations, each character was ranked based on their contribution towards divergence between two entries.

3. RESULTS AND DISCUSSION

The results on genetic divergence of 42 rice genotypes for early seedling vigour related traits, yield and yield components are presented and discussed hereunder.

> **Test with Wilk's criterion 'ᴧ'**

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. The Wilk's statistic 'ᴧ' was highly significant with value of 126189.18, justifying the calculation of D^2 values.

> **Grouping of Genotypes into Clusters**

All the 42 genotypes of rice were grouped into seven clusters using Tocher's method [14]. The distribution of genotypes in clusters was random. Cluster I was the largest comprising of 22 genotypes followed by cluster II with 12 genotypes and cluster III with 4 genotypes. The clusters IV, V, VI and VII were represented by a single genotype indicating a high degree of heterogeneity among the genotypes. This finding has also been reported by of Mishra et al. [16], Devi et al. [17] and Alamir et al. [18] and the distribution of genotypes into seven clusters is presented in Table 3 and Fig. 1. The grouping of genotypes revealed that there was no direct relationship between genetic diversity and geographical diversity as genotypes from different geographical origin were included in one cluster and genotypes collected from the same place were found in different clusters. This kind of clustering of genotypes from different eco-geographic locations into one cluster was attributed to the free exchange of genotypes from one region to another and also be due to the character constellation that might be practiced in several regions resulting in segregation of genotypes irrespective of their geographic region. The results in conformity with the reports of earlier workers [19], who also reported random clustering of 22 rice genotypes into 8 clusters, with no relation to geographic diversity.

> **Intra-cluster and inter-cluster D^2 and Cluster means**

The average values of intra-cluster and inter-cluster D^2 and D distances were presented in Table 4, and the corresponding cluster diagram is shown in Fig. 2 Intra-cluster distances ranged from 0.00 to 22.04. Cluster III exhibited the highest intra-cluster distance (22.04), followed by cluster II (20.20) and cluster I (17.81). Clusters IV, V, VI, and VII had zero intra-cluster distances since they consisted of only one genotype. The maximum inter-cluster distance was observed between cluster II and III (49.06), followed by cluster III and VII (45.84) and cluster II and V (39.21). Conversely, the minimum inter-cluster distance was found between cluster V and VI (21.29), followed by cluster I and IV (23.54). These results align with previous findings by Devi et al. [20], Chamundeswari [21], and Bendi et al. [22].

Table 2. Analysis of variance (ANOVA) for 21 quantitative characters in 42 genotypes of rice.

| Sl. No.	Character	Mean sum of squares		
		Replications (df:2)	Genotypes (df:41)	Error (df:82)
1.	Days to 50% flowering	2.45	610.75**	1.58
2.	Plant height (cm)	33.39	996.21**	18.87
3.	Panicle length (cm)	0.28	27.31**	0.51
4.	Number of panicles plant^{-1}	0.88	19.42**	0.46
5.	1000-grain weight (g)	0.42	60.51**	0.34
6.	Number of filled grains panicle^{-1}	14.21	19,354.81**	14.03
7.	Number of grains panicle^{-1}	5.47	23,965.46**	22.19
8.	Spikelet fertility (%)	3.26	64.79**	2.742
9.	First count of germination (%)	3.07	157.51**	2.99
10.	Final count of germination (%)	3.02	102.01**	3.36
11.	Rate of germination	0.00001	0.0019**	0.00018
12.	Germination index	0.294	149.33**	1.353
13.	Seedling fresh weight(mg)	4.29	352.98**	6.68
14.	Seedling dry weight(mg)	0.06	7.89**	0.18
15.	Shoot length (cm)	0.55	11.87**	0.24
16.	Root length (cm)	0.51	27.311**	0.24
17.	Seedling height(cm)	36	55.53**	0.15
18	Root to shoot ratio	0.03	0.397**	0.01
19.	Seedling vigour index-I	8775.2	650578.91**	3092.80
20.	Seedling vigour index-II	821.70	83555.62**	1779.85
21.	Grain yield plant^{-1}(g)	0.20	137.02**	2.97

*Significant at 5% level; ** Significant at 1 % level

Table 3. Clustering composition of 42 rice genotypes based on Tocher's method

Cluster number	No. of genotypes	Genotypes
I	22	130C, 425C3, 405C3, 273C2, 488C, 457C2, 81C, 76C2, ND13, 153C1, 46C2, ND44, MDP6, ND60, 28C1, ND42, NAGINA 22, MDP2, 476C3, MD4, MD5, NLR34449
II	12	419-1, 538-3, 150-3, 239-3, 419-3, 187-3, 150-3, 54-3, MTU1121, SM227, 221C3, NDP3
III	4	MDP3, MTU1010, MDP4, IR64
IV	1	BPT5204
V	1	SMB3
VI	1	MTU3626
VII	1	NLR33892

Table 4. Average intra (diagonal) and inter-cluster distances (D2 values) for seven clusters of rice genotypes

Cluster	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII
Cluster I	317.31 (17.81)	1114.36 (33.38)	692.75 (26.32)	553.61 (23.53)	663.35 (25.76)	645.61 (25.41)	1129.21 (33.60)
Cluster II		408.06 (20.20)	2406.55 (49.06)	976.17 (31.24)	1537.81 (39.21)	1292.00 (35.94)	680.82 (26.09)
Cluster III			485.63 (22.04)	960.94 (31.00)	1252.57 (35.39)	1400.46 (37.42)	2101.14 (45.84)
Cluster IV				0.00 (0.00)	1347.04 (36.70)	1268.62 (35.62)	870.53 (29.50)
Cluster V					0.00 (0.00)	453.10 (21.29)	1412.14 (37.58)
Cluster VI						0.00 (0.00)	1234.54 (35.14)
Cluster VII							0.00 (0.00)
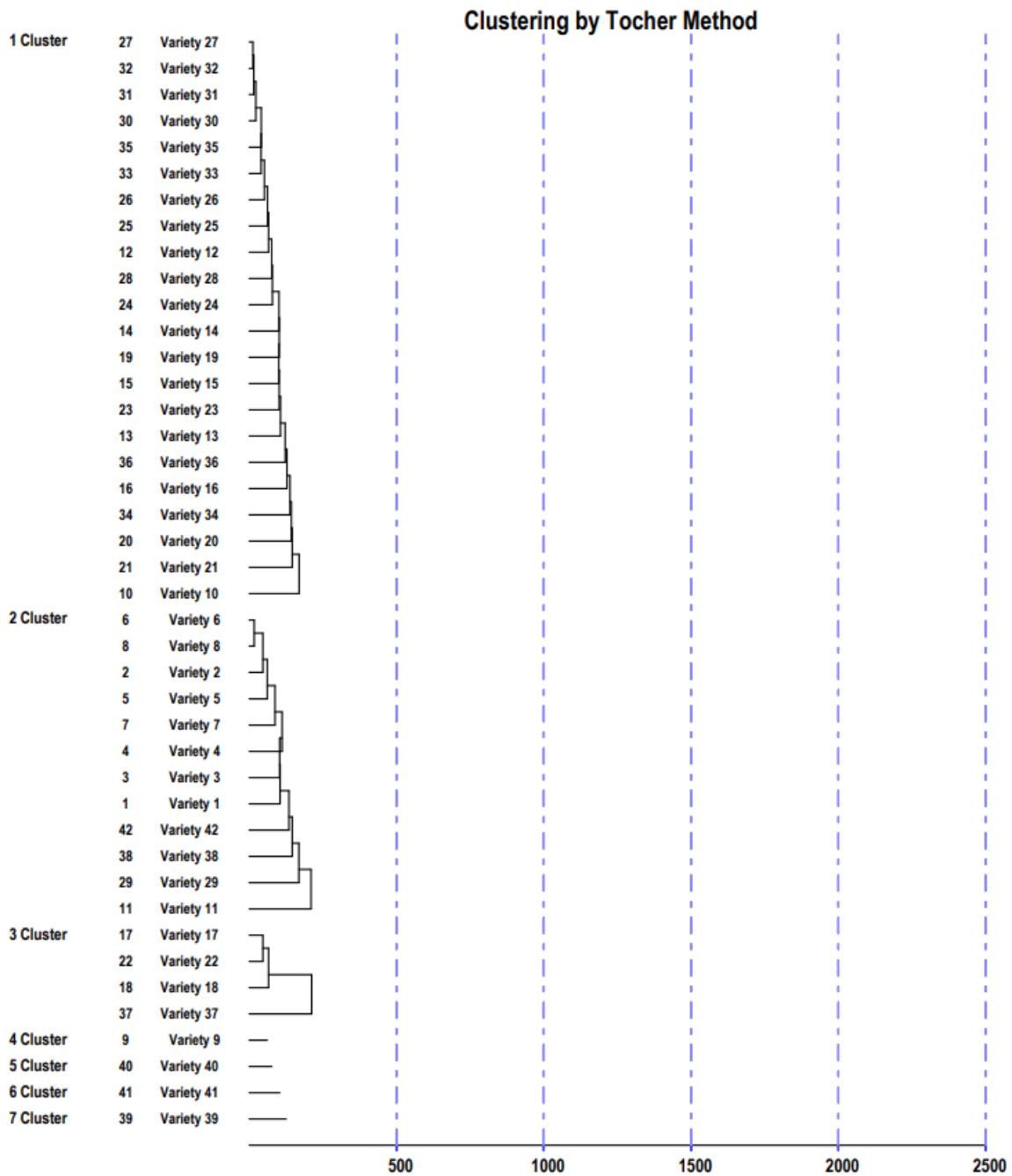
Fig. 1. Clustering of 42 rice genotypes obtained through Tocher's method of classification

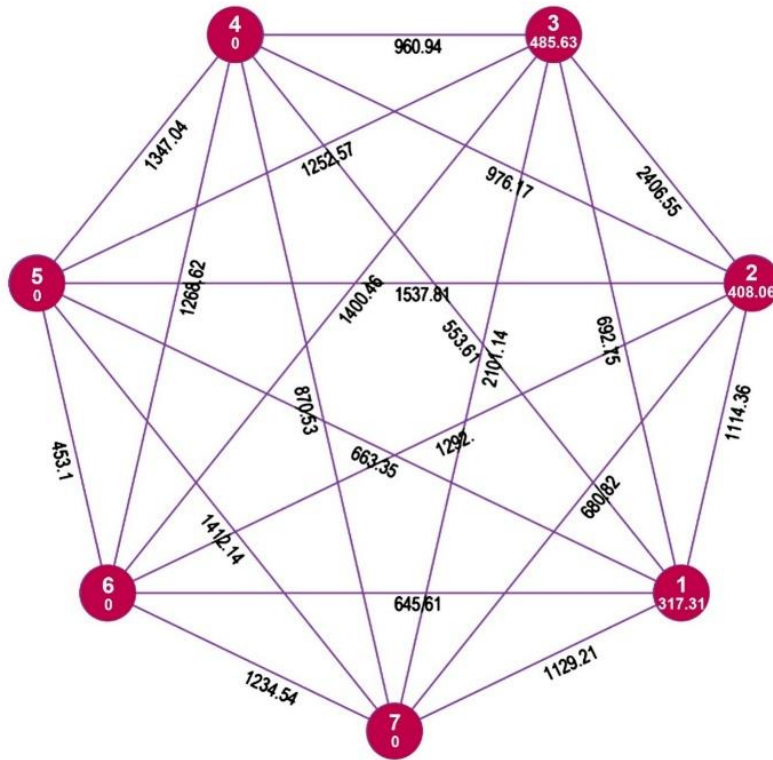|					
1:54(3)	8:538(3)	15:ND60	22:MTU1010	29:221C3	36:NAGINA 22
2:150(3)	9:BPT5204	16:MDP2	23:28C1	30:273C2	37:IR64
3: (1871)	10:NLR34449	17:MDP3	24:46C2	31:405C3	38:SM227
4:187(3)	11:NDP3	18:MDP4	25:76C2	32:425C3	39:NLR33892
5:239(3)	12:ND13	19:MDP6	26:81C	33:457C2	40:SMB3
6:419(1)	13:ND42	20:MD4	27:130C	34:476C3	41:MTU3626
7:419(3)	14:ND44	21:MD5	28:153C1	35:488C	42:MTU1121

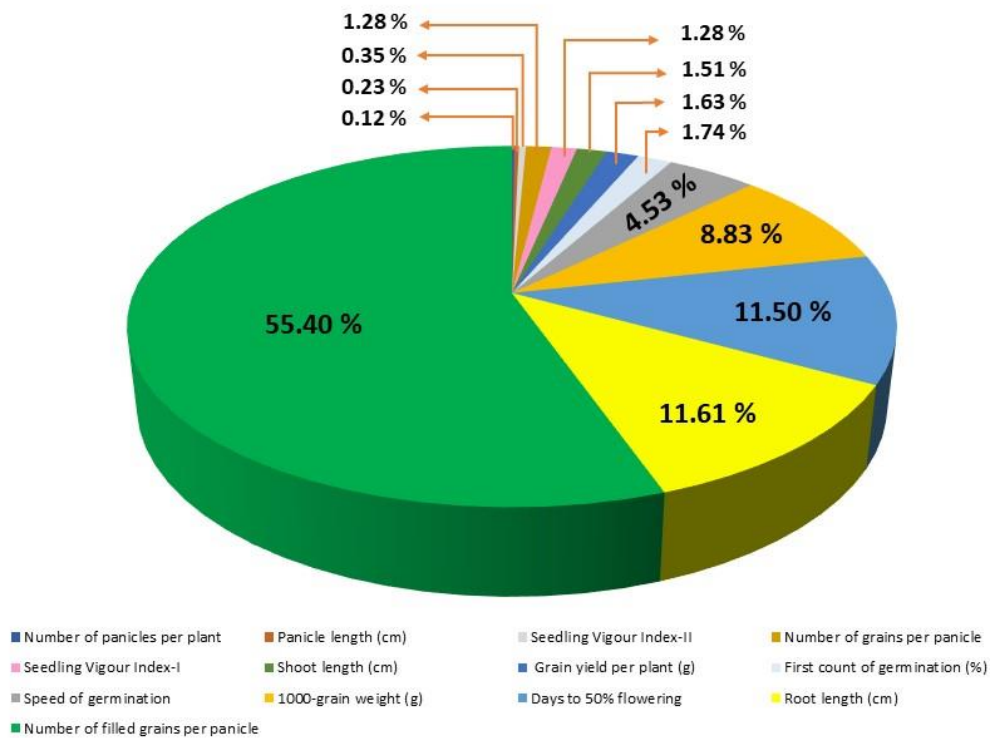**Fig. 2. Clustering diagram of 42 genotypes**

Fig. 3. Relative percentage contribution of 21 characters to total genetic diversity in 42 genotypes of rice

Table 5. Cluster means for yield, yield attributes and early seedling vigour related traits in rice

Character Cluster	Days to 50 % flowering	Plant height (cm)	Panicle length (cm)	Number of panicles plant[-1]	1000-grain weight (g)	Number of filled grains panicle[-1]	Number of grains panicle[-1]	Spikelet fertility (%)	First count of germination (%)	Final count of germination (%)	Rate of germination
Cluster I	88.45	105.78	24.67	13.21	22.24	196.29	223.27	87.69	87.95	91.32	96.00
Cluster II	109.06	124.11	26.42	11.69	17.85	326.33	373.67	87.04	84.72	89.14	95.00
Cluster III	79.67	91.67	22.36	14.65	20.19	107.53	122.39	88.10	88.83	91.75	97.00
Cluster IV	107.00	122.33	20.74	8.53	14.19	200.13	219.33	91.26	96.00	98.00	98.00
Cluster V	91.00	158.40	33.00	8.67	26.98	214.56	231.56	92.66	83.33	88.33	94.00
Cluster VI	98.00	139.13	27.00	13.67	35.70	235.11	251.78	93.39	97.33	98.00	99.00
Cluster VII	140.67	127.27	29.00	13.33	19.35	261.56	289.00	90.52	93.33	95.00	98.00
Mean	101.98	124.10	26.17	11.96	22.36	220.22	244.43	90.09	90.21	93.07	97.00

Table 5. Contd..

Character Cluster	Germination index	Seedling fresh weight (mg)	Seedling dry weight (mg)	Shoot length (cm)	Root length (cm)	Seedling height (cm)	Root to shoot ratio	Seedling vigour index-I	Seedling vigour index-II	Grain yield plant[-1] (g)
Cluster I	33.56	66.90	7.73	10.11	16.69	26.80	1.70	2449.47	706.79	27.84
Cluster II	31.57	58.93	6.31	8.42	14.72	23.14	1.76	2073.04	565.89	30.24
Cluster III	34.37	65.80	6.99	9.61	18.41	28.01	1.97	2591.31	648.23	18.07
Cluster IV	41.59	55.60	5.86	9.17	11.13	20.30	1.21	1989.24	573.81	24.55
Cluster V	28.72	92.58	11.12	14.94	22.69	37.63	1.53	3324.28	982.40	25.73
Cluster VI	39.88	86.25	11.90	13.23	20.26	33.49	1.53	3282.23	1165.81	41.92
Cluster VII	26.70	62.71	7.44	8.33	21.22	29.56	2.55	2807.77	707.27	30.77
Mean	33.77	69.82	8.19	10.54	17.87	28.42	1.75	2645.33	764.31	28.45

Table 6. Per cent contribution of various characters towards genetic diversity in 42 genotypes of rice

Sl. No.	Character	Times Ranked first	Contribution (%)
1.	Days to 50 % flowering	99	11.50
2.	Plant height	0	0.00
3.	Panicle length	2	0.23
4.	Number of panicles plant^{-1}	1	0.12
5.	1000-grain weight	76	8.83
6.	Number of filled grains panicle^{-1}	477	55.40
7.	Number of grains panicle^{-1}	11	1.28
8.	Spikelet fertility	0	0.00
9.	First count of germination	15	1.74
10.	Final count of germination	0	0.00
11.	Rate of germination	0	0.00
12.	Germination index	39	4.53
13.	Seedling fresh weight	0	0.00
14.	Seedling dry weight	0	0.00
15.	Shoot length	13	1.51
16.	Root length	100	11.61
17.	Seedling height	0	0.00
18.	Root to shoot ratio	0	0.00
19.	Seedling Vigour Index-I	11	1.28
20.	Seedling Vigour Index-II	3	0.35
21.	Grain yield plant^{-1}	14	1.63

By employing Mahalanobis's method of clustering based on inter-cluster distances, it was determined that the pairs of clusters II vs III, III vs VIII, II vs V, V vs VII, III vs VI, IV vs VI, III vs V, and VI vs VIII exhibited increasing levels of genetic distances. The computation of intra and inter-cluster D^2 values from the divergence analysis indicated that the inter-cluster distances exceeded the intra-cluster distances, indicating the presence of significant genetic diversity among the accessions. Table 5 presents the results of cluster mean for 21 yield-related traits and early seedling vigor attributes in rice. Analysis of the data revealed significant differences between clusters for most of the studied characters. A closer look at the cluster means for different traits demonstrated considerable variations among the clusters (Table 5).

Means of Clusters varied from 79.67 days (Cluster III) to 140.67 (Cluster VII) for days to 50 per cent flowering; 91.67 cm (Cluster III) to 158.40 cm (Cluster V) for plant height; 20.74 cm (Cluster IV) to 33.00 cm (Cluster V) for panicle length; 14.65 (Cluster III) TO 8.53 (Cluster IV) for number of panicles per plant.14.19 g (Cluster IV) to 35.70 g (Cluster VI) for 1000 grain weight. 107.53 (Cluster III) to 326.33 (Cluster II) for number of filled grains per panicle;122.39 (Cluster III) to 373.67 (Cluster II) for number of grains per panicle. 87.04 percent (Cluster II) to 93.39 percent (Cluster VI) for spikelet fertility; 83.33 % (Cluster V) to 97.33 % (Cluster VI) for first count of germination; 88.33% (Cluster V) to 98.00% (Cluster IV); 94.00 (Cluster V) and 99.00 (Cluster VI) for germination rate; from 26.70 (Cluster VII) to 41.59 (Cluster IV) for germination index; 55.60 mg (Cluster IV) to 92.58 mg (Cluster V) for seedling fresh weight; 5.86 mg (Cluster IV) to 11.90 mg (Cluster VI) for seedling dry weight; 8.33 cm (Cluster VII) to 14.94 cm (Cluster V) for shoot length; 11.13 cm (Cluster IV) to 22.69 cm (Cluster V) for root length; 23.14 cm (Cluster II) to 37.63 cm (Cluster V) for seedling height; 1.21 (Cluster IV) to 2.55 (Cluster VI) for root to shoot ratio; 1989.24 (Cluster IV) to 3324.28 (Cluster V) for seedling vigor index-I and 565.89 (Cluster II) and 1165.81 (Cluster VI) for seedling vigor index-II.

A perusal of cluster means for different characters revealed the considerable differences among the clusters for all the characters (Table 5). Cluster VI recorded maximum grainyieldplant-1 (41.92 g) in addition to 1000-grain weight, spikelet fertility, first count of germination, final count of germination, rate of germination, seedling dry weight and seedling vigour index-II. Cluster II registered maximum values for number of filled grains panicle-1 and number of grains panicle-1. Cluster III exhibited high cluster mean for number of panicles plant-1 and low values for days to 50 per cent flowering, plant height, panicle length, number of filled grains panicle-1, number of grains panicle-1 and grain yield per plant. However, no single cluster had recorded desirable values for all the traits studied. Similar findings were reported earlier [23]. In the context of hybridization programmes aimed at improvement of the desired traits, the selection of genotypes from clusters with high mean values is suggested. Therefore, it is recommended to combine between the selected genotypes from the divergent clusters in order to carefully integrate all the desired traits. The genotypes drawn from divergent clusters namely SM227, MTU1121 and NDP3 from cluster II, IR64 and MDP3 from cluster III, SMB3 from cluster V and NLR33892 from cluster VII could be utilized as potential parents and crossing among themselves would result in high heterotic expression for yield component and early seedling vigour traits and wider segregation among the progenies. The superior recombinants can be obtained by involving such genotypes as parents in hybridization programmes.

➢ **Relative contribution of individual characters towards divergence**

"Information on the relative contribution of various characters towards divergence was reported to aid the breeder in the choice of parents for hybridization and effective selection [24]". Table 6 and Fig. 3 reveal the results of per cent contribution of all the traits included under current study. These findings showed that the number of filled grains per panicle (55.40%) had contributed the maximum to-wards genetic diversity followed by Root length (11.61%), days to 50 percent flowering (11.50%), 1000-grain weight (8.83%) and germination index (4.53%). The findings regarding the significant contribution of the number of filled grains per panicle to genetic diversity align with previous studies by Vennila et al. [25], Bhati et al. [26], Nagavath et al. [27].

4. CONCLUSION

Mahalanobis D^2 analysis resulted in the grouping of 42 rice genotypes into seven clusters. Cluster I was observed to be the largest with 22

genotypes followed by cluster II with 12 genotypes. followed by cluster II (20.20) and cluster I (17.81). Maximum inter-cluster distance was observed between genotypes of cluster II and III (49.06) while intra-cluster distance was noticed to be the maximum for the genotypes in cluster III. Cluster VI had recorded maximum grain yield per plant , 1000-grain weight, spikelet fertility, first count of germination, final count of germination, rate of germination, seedling dry weight and seedling vigour index-II. Further, maximum contribution towards divergence was noticed by number of filled grains per panicle followed by root length and days to 50 per cent floweringalamr. The genotypes drawn from divergent clusters namely SM227, MTU1121 and NDP3 from cluster II, IR64 and MDP3 from cluster III, SMB3 from cluster V and NLR33892 from cluster VII could be utilized as potential parents in breeding programmes aimed to develop high yielding genotypes combined with early seedling vigour for direct seeded rice cultivation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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