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EXPLOITING GENETIC DIVERSITY THROUGH CLUSTER ANALYSIS FOR YIELD IMPROVEMENT IN DRY DIRECT-SEEDED RICE (*ORYZA SATIVA* L.)

V. Sridhar^{1*}, N. Lingaiah², G. Shiva Prasad³ and K. Sumalini⁴

¹Senior Scientist, Plant Breeding, Agricultural Research Station, Kampasagar, Nalgonda, Telangana –508207, India

²Principal Scientist, Plant Breeding & Head, Agricultural Research Station, Kampasagar, Nalgonda, Telangana –508207, India

³Scientist, Plant Breeding, Agricultural Research Station, Kampasagar, Nalgonda, Telangana –508207, India, Telangana –508207, India

⁴Department of Genetics and Plant Breeding, College of Agriculture, Professor Jayashankar Telangana Agricultural University, Rajendranagar, Hyderabad, Telangana – 500030, India

*Corresponding author E-mail: sridharphd@pjetau.edu.in

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ABSTRACT

An experiment was conducted to assess the genetic diversity among thirty rice genotypes, comprising released varieties and advanced breeding lines, for traits associated with dry direct-seeded rice (DDSR). The genotypes were evaluated for thirteen agronomic and physiological characters, and genetic divergence was estimated using Mahalanobis' D² statistics. Based on D² values, the genotypes were grouped into eight distinct clusters. Cluster II was the largest, containing 13 genotypes, followed by Cluster I (8 genotypes) and Cluster III (4 genotypes), while the remaining clusters comprised a single genotype each. The maximum inter-cluster distance was observed between Clusters III and VIII (1822.85), indicating substantial genetic divergence, whereas the highest intra-cluster distance was recorded in Cluster II (91.27). The inter-cluster distances were considerably higher than intra-cluster distances, demonstrating the presence of wide genetic variability among the studied genotypes. Cluster VII recorded the highest mean grain yield, followed by Clusters III and IV. Seedling vigour index was highest in Cluster III, followed by Clusters VII and I. Among the traits studied, grain yield (24.14%), seed vigour index (22.99%), shoot length (20.69%), and number of filled grains per panicle (14.48%) contributed most significantly to total genetic divergence. The results suggest that these traits should be prioritized in selection strategies for improving dry direct-seeded rice. Hybridization between genotypes belonging to highly divergent clusters, particularly Clusters III and VIII, may generate superior segregants with enhanced performance under dry direct-seeded conditions.

Keywords: Rice, Dry DSR, Genetic diversity, Cluster analysis, Vigour Index, Yield traits.

Introduction

Rice (*Oryza sativa* L.) is a principal staple food for more than half of the world's population and plays a vital role in ensuring food security, nutritional stability, and rural livelihoods, particularly in Asia and India. For decades, rice cultivation has predominantly relied on the puddled transplanted rice (PTR) system. Although PTR has contributed substantially to production gains during the Green Revolution, its long-term sustainability is increasingly threatened by escalating labor costs, water scarcity, declining

groundwater tables, soil structural degradation, and increased greenhouse gas emissions. Furthermore, climate variability, characterized by erratic rainfall patterns and rising temperatures, intensifies production risks and necessitates the development of resilient rice production systems (Dey *et al.*, 2025; Jagdeep & Singh, 2024). Dry direct-seeded rice (DDSR) has emerged as a promising resource-conserving and climate-resilient alternative to PTR. In DDSR, seeds are directly sown into non-puddled, dry soil, eliminating nursery raising and transplanting operations. This system substantially reduces water and

labor requirements, lowers production costs, enables mechanization, and shortens crop duration. Additionally, DDSR contributes to reduced methane emissions compared to continuously flooded systems (Ahmed *et al.*, 2025). Despite these advantages, DDSR faces several agronomic constraints, including poor and uneven crop establishment, intense weed competition, nutrient management complexities, lodging, and yield variability across environments. The altered soil–plant–atmosphere interactions under aerobic conditions significantly influence root development, nutrient dynamics, and physiological responses, resulting in differential genotype performance compared to transplanted systems. Genetic improvement is central to enhancing adaptability and yield stability under DDSR conditions. Traits such as rapid early seedling vigor, anaerobic germination tolerance, deep and proliferative root systems, drought tolerance, nutrient-use efficiency, weed competitiveness, and lodging resistance are particularly critical for successful crop establishment and productivity. However, most of these adaptive traits are quantitatively inherited, polygenic in nature, and strongly influenced by genotype \times environment interactions, thereby complicating direct selection. Genetic diversity serves as the cornerstone of crop improvement programs, providing the raw material for selection, recombination, and sustained genetic gain. The narrow genetic base of many modern high-yielding rice cultivars poses a potential risk to resilience under changing climatic scenarios. Therefore, comprehensive assessment of genetic divergence among available genotypes is essential for broadening the genetic base and identifying suitable parents for hybridization. Evaluation of agromorphological and physiological traits offers valuable insights into population structure, genetic relationships, and trait variability. Multivariate statistical techniques, particularly Mahalanobis' generalized distance (D^2) statistics coupled with cluster analysis, are powerful tools for quantifying genetic divergence in plant breeding. These methods enable grouping of genotypes into distinct clusters, estimation of intra- and inter-cluster distances, and identification of genetically diverse parents for strategic crossing. Selection of parents from clusters exhibiting maximum inter-cluster distance enhances the probability of obtaining superior recombinants and transgressive segregants in a self-pollinated crop like rice. In this context, the present investigation was undertaken to assess the magnitude of genetic diversity among thirty rice genotypes under dry direct-seeded conditions using Mahalanobis D^2 statistics and cluster analysis, with the objective of identifying genetically divergent and agronomically

superior genotypes for yield improvement in DDSR-adapted rice breeding programs.

Materials and Methods

The experimental material for the present study comprised of 30 diverse rice genotypes including released cultivars and advanced cultures, procured from different rice institutes and maintained at Agricultural Research Station, Kammasagar. The experiment was conducted following Randomised Block Design with 3 replications during *Kharif*, 2021 at Agricultural Research Station, Kammasagar, Nalgonda, Telangana situated between 16.8536 N latitude and 79.4569 longitudes at 152 m above mean sea level. Each genotype was directly sown by dibbling in unpuddled soil, at a distance of 15 cm between plants and 20 cm between rows with a plot size of 10.5 m² each. Normal agronomical practices and plant protection measures with external inputs such as supplementary irrigation and fertilizers were given at appropriate time to maintain a consistent and healthy crop stand. Data were recorded on a total of thirteen morphological traits. Data on plant height, no. of effective tillers/ m², panicle length, no. of filled grains/panicle, grain filling percentage, root length, shoot length, seedling length seedling vigour index were recorded on five randomly selected plants in each plot. Days to 50 % flowering, germination, grain yield were recorded on plot basis and test weight was recorded per replication in each genotype and data was subjected to statistical analysis. The data collected on all the characters were subjected to standard methods of analysis of variance (Panse and Sukhatme, 1985).

Assessment of genetic divergence was done using Mahalanobis D^2 statistic and the rice genotypes were grouped into different clusters following Tocher's method (Kumar *et al.*, 2017). as described using Genes statistical package. Divergence was estimated by the multivariate analysis using Mahalanobis' (1936) and D^2 statistic as described by Rao (1952). On the basis of D^2 values obtained, the variables were grouped into different clusters by employing Tocher's method (Rao, 1952). The percent contribution of each character to the total divergence was calculated by ranking each character on the basis of transformed uncorrelated values. Finally, the percent contribution for each character was calculated by taking total number of ranks of all the characters to hundred. The data were analyzed statistically using the software WINDOSTAT, developed by INDOSTAT services Ltd. Hyderabad, India.

Results and Discussion

The analysis of variance showed highly significant differences among the genotypes for all the characters studied indicating the presence of considerable variability in the experimental material (Table-1). Similar findings were reported by (Sneha Malikedi *et al.*, 2024 and Mounika Reddy *et al.*, 2022).

Table 1 : Analysis of variance for mean squares of thirteen morphological traits in rice

Variables	Replication	Genotype	Residuals
degree of freedom (df)	2	29	58
Days to 50% flowering	0.14	289.10**	5.55
Plant height (cm)	2.13	473.53**	66.13
No. of effective tillers/m ²	22.53	19558.7**	174.05
Panicle length (cm)	7.97	19.94**	4.39
No. of filled grains/panicle	16.23	2589.08**	291.33
Test weight (g)	0.36	68.10**	8.99
Grain filling percentage (%)	30.1	226.69***	56.05
Germination (%)	1.36	471.06**	5.85
Root length (cm)	0.01	35.92**	0.09
Shoot length (cm)	0.01	7.08**	0.09
Seedling length (cm)	0.48	60.23**	0.31
Seed Vigour Index	1051.18	1017275.7	3984.66
Grain yield (kg/ha)	975844	2701707.43**	1106719

** 1% level of significance

Principal component analysis and grouping of genotypes

Partitioning of total variance through principal component analysis showed that three principal components viz PC I, PC II and PC III contributed about 88.45% of total variance for the rice genotypes studied. These three PCs i.e. PC I, PC II and PC III contributed 53.10, 25.93 and 9.41 % of total variance (Table 2) and (Fig. 1). These results were in agreement with the findings of Preeti *et al.*, 2023 and Ojha *et al.*, 2017. The results obtained from PCA were further corroborated by cluster analysis using UPGMC (Unweighted Paired Group Method using Centroids). The thirty rice genotypes were grouped into eight distinct clusters. Cluster II is the largest with a maximum number of thirteen (13) genotypes followed by cluster I and cluster III with 8 and 4 genotypes each, cluster IV, V, VI, VII and VIII are with a single genotype each and stood as solitary entities, highlighting a considerable degree of heterogeneity within these clusters. (Table 3) (Fig. 2). Similar results were noted by Janaki Venkata Yashwanth *et al.* (2025). Grouping of genotypes into different clusters had suggested the presence of ample amount of divergence in present experimental material studied and allotment of genotypes from various sources to same cluster and genotypes developed from same geographical area into different clusters indicates that grouping pattern does not relate to geographical origin. Murty and Arunachalam (1966) stated that genetic drift and

selection in different environments could cause greater diversity than geographical distance. These findings are similar to the reports of Srinivas *et al.*, 2021, Sowmiya and Venkatesan (2017). The results of D² analysis helped to identify diverse accessions from the available genotype lines for use in crop improvement programmes. The varieties of these clusters may be used as parents in the crossing programme to generate breeding material with high diversity.

Table 2: Principal component analysis for yield component traits in rice genotypes

	Traits	1 Vector	2 Vector	3 Vector
	Eigene value (Root)	428.52150	209.30260	75.97277
	% Var. Exp.	53.10352	25.93734	9.41475
	Cum. Var. Exp.	53.10352	79.04086	88.45561
1	Days to 50% flowering	0.12140	0.85937	0.24062
2	Plant height (cm)	0.02266	0.22340	0.37378
3	No. of effective tillers/m ²	-0.91796	0.11374	0.11220
4	Panicle length (cm)	-0.11969	0.20012	0.15780
5	No. of filled grains/panicle	0.23592	0.06160	0.10120
6	Test weight (g)	-0.19838	0.00391	0.24828
7	Grain filling percentage (%)	-0.10454	0.02774	-0.33046
8	Germination (%)	-0.14395	0.54140	0.25485
9	Root length (cm)	-0.64514	-0.48105	-0.25161
10	Shoot length (cm)	-0.24790	-0.03060	0.45559
11	Seedling length (cm)	-0.38888	-0.02100	-0.11792
12	Seed Vigour Index	-0.11402	-0.18927	-0.02874
13	Grain yield (kg/ha)	0.09398	-0.34378	0.76354

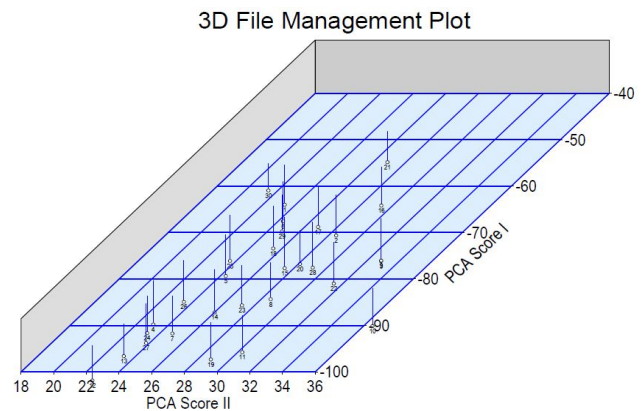


Fig. 1: Principal component analysis diagram for rice genotypes

Table 3 : Clustering pattern of rice genotypes

Cluster	No. of genotypes	Name of the genotype
I	8	WGL-14, JGL-24423, JGL-18047, KPS-3754, RNR-11718, BPT-5204, MTU-3626, MTU-1271
II	13	WGL-915, WGL-962, KNM-1638, KPS-2874, KPS-6262, Indira Aerobic-1, MTU-1061, MTU-1010, RNR-28361, KNM-7037, JGL-21078, WGL-739, WGL-20471
III	4	RNR-29325, JGL-27356, KNM-733, RNR-15048
IV	1	WGL-44
V	1	KNM-118
VI	1	DRR DHAN-42
VII	1	JGL-28545
VIII	1	KPS-6251

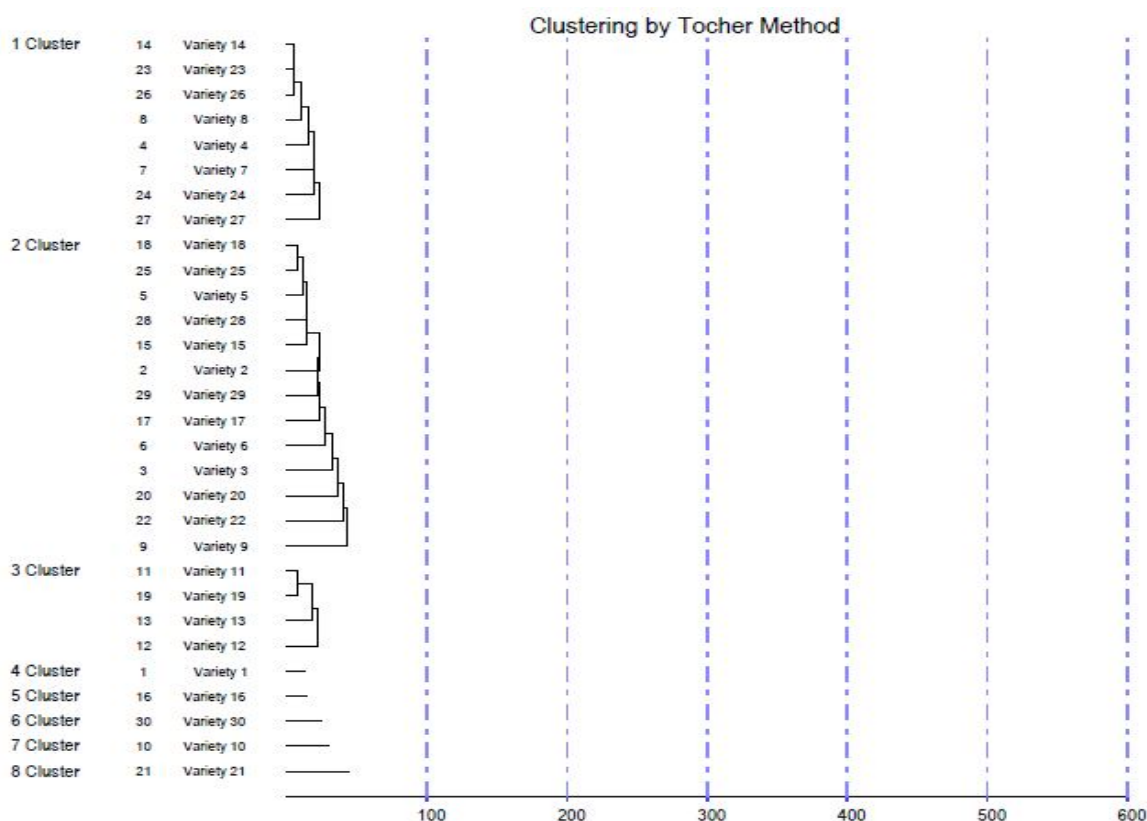


Fig. 2: Dendrogram showing clustering of rice genotypes

Cluster distances and cluster means

The genetic divergence among the rice genotypes as indicated by intra and inter cluster distances for eight different clusters are presented in Table 3 (Fig 3). Highest intra cluster distance of 91.27 was recorded for cluster II followed by cluster III (66.56), cluster I (54.94), thus suggesting that different genotypes included in these clusters might have different genetic architecture. The clusters with lowest intra cluster distance indicated that the genotypes resembled one another genetically and appeared to have evolved from a common gene pool (Singh *et al.*, 2022) the inter cluster distance ranged from a minimum of 65.40 (between cluster IV and VI) to a maximum of 1822.85 (between cluster III and VIII). The values of other inter cluster distances which are on the higher side are 1306.84 (between cluster III and cluster VI), 1265.79 (between cluster III and cluster IV) 1262.23 (between cluster VI and cluster VIII), 1230.64 (between cluster III and cluster V), 1094.83 (between cluster I and cluster VIII), 976.69 (between cluster VI and cluster VII) and 895.10 (between cluster IV and VII). Clusters IV, V, VI, VII and VIII are solitary clusters with intra cluster distance of 0.00. The perusal of mean in table 3 revealed that inter-cluster distances were greater than intra-cluster distances revealing considerable amount of genetic diversity among the genotypes studied

(Singh *et al.*, 2022 and Shafina *et al.*, 2014). Genotypes belonging to clusters with maximum intra-cluster distance are genetically more divergent and hybridization between divergent clusters is likely to produce wide variability with desirable segregants. The maximum amount of heterosis is expected from the crosses with parents belonging to the most divergent clusters i.e., between cluster III and VIII followed by parents in clusters of III and cluster VI, from parents in clusters III and cluster IV, from parents in clusters III and cluster VI and from parents in clusters VII and cluster VIII and so on . These results are in agreement with earlier reports of Madhu Kumar *et al.*, 2026, Chandraker *et al.*, 2024 and Singh *et al.*, 2022. The progenies derived from such crosses are expected to show wide variability, providing greater scope for isolating transgressive segregants in the advanced generations which can be used for selecting desirable genotypes for seed yield improvement in rice. Conversely, the smallest distances were observed between clusters such as Cluster IV and Cluster V, Cluster IV and Cluster VI suggesting close similarity and minimal chances of producing wide variability through crossing. Several researchers, including Madhu Kumar *et al.* (2026), Chandraker *et al.* (2024) and Sar and Kole (2023) have reported the utility of Mahalanobis distance in identifying promising and

genetically distant parents for rice improvement. The patterns observed in the present analysis strongly align with these findings

Table 4 : Average Intra (in bold) and Inter-cluster D² values of rice genotypes

Cluster	I	II	III	IV	V	VI	VII	VIII
I	54.94	277.53	166.38	644.58	639.25	717.41	169.18	1094.83
II		91.27	679.64	176.87	163.03	260.71	392.82	417.57
III			66.56	1265.79	1230.64	1306.84	200.52	1822.85
IV				0.00	67.68	65.40	895.10	147.11
V					0.00	119.66	746.25	120.60
VI						0.00	976.69	116.56
VII							0.00	1262.23
VIII								0.00

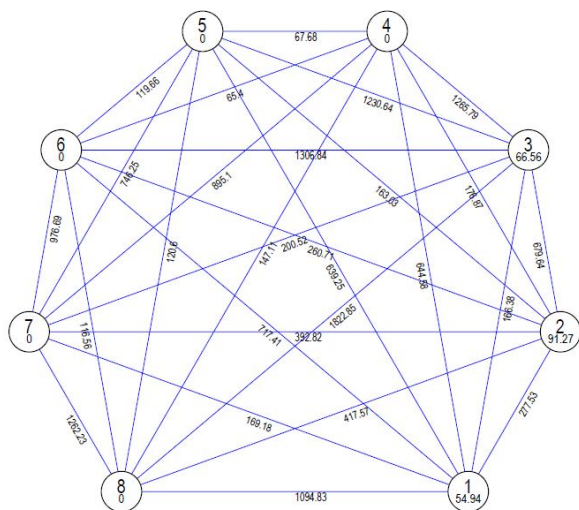


Fig. 3: Mahalanobis Euclidean distances for rice genotypes

Table 5 : Cluster means among the rice genotypes

Cluster	DFF	PH	NET	PL	NFG	TW	GFP	G	RL	ShL	SL	SVI	GY
I	87.13	93.50	515.88	21.89	107.63	21.30	85.14	98.64	16.57	10.89	27.50	2717.22	5603.38
II	93.50	103.73	524.69	24.35	116.62	18.90	87.38	93.48	12.65	10.45	23.08	2156.15	5426.54
III	81.88	93.75	583.38	23.81	121.13	22.46	82.68	98.12	19.53	11.13	30.68	2993.66	6294.13
IV	101.50	101.00	443.50	21.80	149.00	14.28	88.98	82.25	11.07	9.63	20.87	1721.44	5718.00
V	96.00	92.00	546.50	19.00	166.00	14.80	74.50	92.47	10.54	8.61	19.14	1839.15	5550.00
VI	93.00	102.00	450.00	23.00	148.00	16.60	82.40	72.01	12.54	7.33	19.92	1455.75	4307.50
VII	87.50	105.00	713.50	25.05	108.00	29.70	93.30	100.46	16.05	11.09	26.60	2759.76	7593.00
VIII	83.00	79.00	495.00	20.45	133.00	19.30	91.20	73.61	9.51	7.89	17.40	1290.93	5557.50

DFF: Days to 50% flowering, PH: Plant Height (cm), NFG: No. of effective tillers/m², PL: Panicle length (cm), NFG: No. Of filled grains/panicle, TW: Test weight (g), GFP: Grain filling percentage, G: Germination (%),RL: Root length (cm), ShL: Shoot length (cm), Sd.L Seedling length (cm), SVI: Seed Vigour Index, GY: Grain yield (kg/ha)

Percent contribution towards genetic divergence

The relative contribution of different quantitative traits toward total genetic divergence revealed substantial variability among the studied characters is shown in Table 5 (Fig.4). Grain yield (24.14%) contributed the maximum towards total divergence, followed by Seed Vigour Index (22.99%) and shoot length (20.69%), indicating their predominant role in differentiating the genotypes under dry direct-seeded conditions. The number of effective tillers per m² also

The cluster means for thirteen traits included in the present study are shown in Table 4. The lowest mean value for days to first flowering was recorded by cluster III and highest value by cluster IV. Cluster VII exhibited highest mean value for plant height and cluster VIII recorded lowest value. The highest mean value for number of effective tillers/m² was found in cluster VII and lowest in cluster IV. Cluster VII recorded highest mean value for panicle length and cluster V recorded lowest value. Higher and lower number of filled grains per panicle of 166 and 107.63 was recorded in cluster V and cluster I respectively. Higher cluster mean value for test weight was obtained in the cluster VII and lowest value recorded by the cluster IV.

The highest mean value for grain yield was recorded by cluster VII followed by Clusters III & IV. The higher mean values for seedling vigour index was recorded in cluster III followed by cluster VII and I and cluster VIII noted the lowest value. Higher mean values for root length and shoot length were recorded in cluster III. Highest value for germination percentage is recorded in cluster VII followed by cluster clusters I and III. Hence crossing between these genotypes can be better exploited for genetic introgression studies (Preeti Kumari *et al.*, 2023)

contributed considerably (14.48%), highlighting its importance in yield determination under DDSR systems. Moderate contributions were observed for seedling length (6.21%) and panicle length (3.22%), whereas plant height (2.30%) and root length (2.30%) showed relatively lower contributions. Traits such as days to 50% flowering (1.38%) and test weight (1.15%) had minor influence on genetic divergence. Very minimal contributions were recorded for grain filling percentage (0.69%), number of filled grains per panicle (0.23%), and germination percentage (0.23%),

suggesting comparatively lesser variability among genotypes for these characters. The predominance of yield and seedling vigor-related traits in contributing to total divergence emphasizes the significance of early growth vigor and productivity attributes under dry direct-seeded conditions. These findings suggest that selection of genotypes differing widely for grain yield, seed vigour index, shoot length, and effective tillers may enhance the efficiency of hybridization programs aimed at developing high-yielding DDSR-adapted cultivars. Maximum contribution of seed vigour index was reported by Ravikanth *et al.*, (2018), for grain yield, number of filled grains per panicle was reported by Alok Kumar Singh *et al.* (2026), Sneha *et al.*, 2024, Pallavi *et al.*, 2023, Singh *et al.*, 2022, Tandekar *et al.*, 2018, Singh *et al.*, 2018.

Table 6 : Relative contributions of yield and yield components to genetic diversity in rice

Character	Times ranked 1st	Contribution %
Days to 50% flowering	6	1.38
Plant height (cm)	10	2.30
No. of effective tillers/m ²	1	0.23
Panicle length (cm)	14	3.22
No. of filled grains/panicle	63	14.48
Test weight (g)	5	1.15
Grain filling percentage (%)	3	0.69
Germination (%)	1	0.23
Root length (cm)	10	2.30
Shoot length (cm)	90	20.69
Seedling length (cm)	27	6.21
Seed Vigour Index	100	22.99
Grain yield (kg/ha)	105	24.14

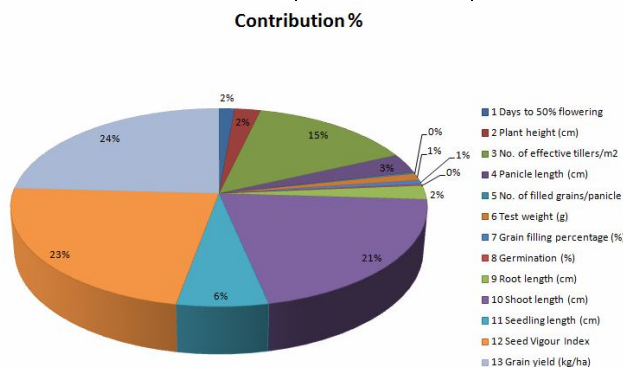


Fig. 4: Pie chart showing percentage contribution of 13 biometrical traits of rice genotypes towards genetic divergence

Conclusion

For the improvement of rice genotypes under dry direct seeded condition diverse parents could be selected based on genetic distance. Based on D² values, the 30 rice genotypes were grouped into eight clusters. Among these, Cluster II contained the highest number

of genotypes (13), followed by Cluster I (8) and Cluster III (4), while Clusters IV, V, VI, VII, and VIII each contained one genotype. The rice genotypes RNR-29325, JGL-27356, KNM-733, and RNR-15048 belonging to Cluster III, and KPS-6251 belonging to Cluster VIII, exhibited the highest inter-cluster distance, indicating that they are the most genetically diverse from each other. The genotypes present in these two clusters (III and VIII) provide a broad spectrum of variability in segregation and may be utilized as parents in future hybridization programs to develop desirable high-yielding rice genotypes. These crosses may also produce superior transgressive segregants. The characters grain yield per hectare, seed vigour index, shoot length, number of filled grains per panicle and seedling length contributed the maximum towards genetic divergence and should therefore be given due consideration during selection for crop improvement.

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