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ASSESSMENT OF GENETIC DIVERSITY IN ADVANCED BREEDING LINES OF RICE (*ORYZA SATIVA* L.) FOR ENHANCED PRODUCTIVITY

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ABSTRACT

Rice (*Oryza sativa* L.), a vital global food source, faces increased demand due to population growth and evolving dietary habits, necessitating enhanced productivity on limited cultivable land. This study investigates the genetic diversity of 44 genotypes through Mahalanobis's D^2 statistics, focusing on critical traits such as panicle number, filled grains, and 100-grain weight. This experiment was carried out at Wetland Farm of S.V. Agricultural College, Tirupati, during the *Kharif* of 2020. The data was recorded for 15 yield and yield-related traits. The diversity analysis resulted in the formation of nine distinct clusters with varied inter-cluster and intra-cluster distances. Cluster I is the largest, comprising 14 genotypes, followed by Clusters III and V. Significant inter-cluster distances indicate diverse genotypic pedigrees. Cluster IX stands out with high mean values for several traits, while Clusters III, V, and VIII exhibit desirable characteristics. Days to 50% flowering, grain length, and 100grain weight contribute significantly to genetic divergence. The study provides insights for breeding programs to develop high-yielding and resilient rice varieties, contributing to sustainable intensification and global food security.

***Keywords*: ** Rice, Genetic diversity, Advanced breeding lines, Mahalanobis's D^2 statistics, Cluster analysis, Intra-cluster and inter-cluster distances

Introduction

Rice (*Oryza sativa* L.) stands as a cornerstone of global food security. It serves as a staple for more than half of the world's population, particularly in Asian and African countries. With an escalating demand for rice due to population growth and changing dietary habits, the need to enhance rice productivity becomes increasingly urgent. However, the expansion of cultivation areas is constrained, necessitating innovative approaches to boost yield. Genetic diversity studies in rice offer a promising avenue for addressing this challenge. Currently, rice is cultivated across 165.03 million hectares globally, yielding approximately 776 million tonnes of grains (Food and Agriculture Organization, 2023). In India alone, where rice holds paramount importance, the crop covers 46.40 million hectares, contributing 196.24 million tonnes to global production (Food and Agriculture Organization, 2023).

Advanced breeding lines, comprising homozygous genotypes derived from diverse breeding programs, represent a promising frontier in rice research. These lines encapsulate valuable gene combinations with the potential to address the challenges posed by limited cultivable land. Genetic diversity analysis of these advanced breeding lines is essential to unravel their potential and contribute to the global effort of developing high-yielding and resilient rice varieties. This research aims to delve into the genetic diversity of advanced breeding lines, employing robust statistical tools such as Mahalanobis's D^2 statistics. By understanding the genetic variations associated with key traits such as the number of panicles plant^{-1}, the number of filled grains panicle^{-1}, and 100grain weight, this study seeks to identify elite genotypes that can serve as the bedrock for future breeding programs. In doing so, the research endeavours to contribute substantively to the sustainable intensification of rice production and the global pursuit of food security.

Materials and Methods

The study utilized 44 rice genotypes, employing a randomized block design with three replications during the kharif season of 2020 at Wetland Farm, S.V. Agricultural College, Tirupati. Each genotype was cultivated in two rows, each spanning 3 meters in length, with a spacing of 20 cm between rows and 15 cm between individual plants within the row. Across the experimental setup, observations were made on five randomly chosen plants per genotype per replication, focusing on various traits such as plant height (cm), panicle length (cm), number of panicles plant^{-1}, biological yield plant^{-1} (g), harvest index (%), 100grain weight (g), grain length (L) (mm), grain breadth (B) (mm), grain size (LB ratio), number of filled grains panicle^{-1}, number of unfilled grains panicle^{-1}, spikelet fertility (%), and grain yield plant^{-1} (g). Notably, the parameters of days to 50% flowering and days to maturity were recorded at the plot level.

The data collected on different yield and its component characters was analyzed by using D^2 statistics given by Mahalanobis (1936) to determine genetic divergence among the genotypes. Variances were calculated for 15 characters, and a test of significance was performed. Analysis of covariance was estimated for the character pairs based on mean values (Panse and Sukhatme, 1961). After testing the difference between genotypes for each of the characters, a simultaneous test of significance for differences between the mean values of number of correlated variables with regard to the pooled effect of 15 characters was carried out using 'V' statistic, which utilized Wilk's 'Λ' criterion (Wilks, 1932). The grouping of genotypes into different clusters was performed by using Tocher's method, as described by Rao (1952). The criterion was that the two varieties belonging to the same cluster, at least on 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 their magnitude in a tabular form as described by Singh and Chaudhary (1977).

Results and Discussions

Wilks 'Λ' (statistic) criterion was used to know about the significant differences among the genotypes based on the pooled effects of all the characters. The significance of 'Λ' statistic value was tested by χ^2 at 645 degrees of freedom. The calculated value of the 'Λ' statistic was highly significant (more than the tabulated χ^2 value), indicating that genotypes differed significantly when all the characters studied were considered simultaneously together (Table 1).

Table 1 : Analysis of variance for dispersion in 44 rice genotypes

Source of variation	**Degrees of freedom**	**Mean sum of squares**
Genotypes	43	$2.37 \times 10^{14**}$
Error	85	1.45×10^{-1}
Total	128	7.97×10^{13}

**: Significant at 1% level

Grouping of Genotypes into Clusters:

In accordance with Torcher's method (Rao, 1952), all 44 rice genotypes were classified into nine distinct clusters. These clusters were discerned based on the premise that genotypes within the same cluster exhibit smaller Mahalanobis's D^2 values compared to those belonging to different clusters. The distribution of genotypes among these clusters is detailed in Table 2 and illustrated in Fig. 1.

Among the nine clusters, Cluster I emerged as the largest, comprising 14 genotypes, followed by Cluster III with 11 genotypes and Cluster V with seven genotypes. Clusters II and VII contained five and three genotypes, respectively, while Clusters IV, VI, VIII, and IX each consisted of a solitary genotype. Notably, genotypes within these unique clusters possess distinctive characteristics, rendering them particularly valuable for breeding endeavors. A noteworthy observation is the alignment between clustering patterns and genotypic pedigree, suggesting a parallelism between genetic divergence and parental lineage.

Intra and Inter-Cluster Average Distance

The average intra-cluster and inter-cluster Mahalanobis's D^2 and D values are presented in Table 3 and Fig. 2. Intra-cluster distances ranged from 0.00 to 68.88, with Cluster VII exhibiting the maximum intra-cluster distance, followed by Clusters III, V, II, and I. Conversely, Clusters IV, VI, VIII, and IX maintained an intra-cluster distance of 0.00, indicating their monogenic nature.

Inter-cluster D^2 values ranged from 57.44 to 506.51. The most substantial inter-cluster distance (506.51) was observed between Clusters V and IX, followed by Clusters III and IX, Clusters VIII and IX, Clusters IV and IX, and Clusters VII and IX. Conversely, the minimum inter-cluster distance of 57.44 was recorded between Clusters I and IV, suggesting a closer genetic affinity. Overall, inter-cluster distances exceeded intra-cluster distances, underscoring the wider genetic diversity present between clusters rather than within clusters.

Cluster Means for Yield and Yield Attributes

Cluster means for the 15 yield and its component traits are detailed in Table 4. Considerable variation among cluster means for all characters underscores the divergent nature of the formed clusters.

For instance, early flowering was observed in Cluster III (88.91 days), while late flowering was noted in Cluster IX (145.67 days). Days to maturity ranged from 116.55 days in Cluster III to 173.67 days in Cluster IX. Plant height varied from 96.87 cm in Cluster IV to 125.53 cm in Cluster IX. Panicle length ranged from 21.65 cm (Cluster IV) to 28.33 cm (Cluster IX). Cluster VI recorded the highest number of panicles plant^{-1} (14.00), while Cluster VII had the lowest (6.73). Biological yield plant^{-1} ranged from 44.09 g in Cluster IV to 96.47 g in Cluster IX. Harvest index varied from 29.18% in Cluster VII to 61.47% in Cluster IX.

Cluster means for 100 grain weight ranged from 1.22 g in Cluster VI to 2.43 g in Cluster V. The number of filled grains panicle^{-1} varied from 113.93 (Cluster IV) to 320.53 (Cluster VII). The number of chaffy grains panicle^{-1} ranged from 13.82 (Cluster V) to 49.60 (Cluster IX).

Spikelet fertility ranged from 82.60% (Cluster VII) to 95.07% (Cluster VIII). Grain length varied from 7.03 mm (Cluster IX) to 8.76 mm (Cluster V). Grain breadth was highest in Cluster IV (2.74 mm) and lowest in Cluster VI (2.15 mm). Grain size ranged from 2.73 (Cluster IX) to 3.52 (Cluster VIII). Grain yield plant^{-1} ranged from 16.76 g (Cluster IV) to 61.60 g (Cluster IX).

Cluster Mean Analysis

Cluster means were scored across clusters for all 15 characters, with the highest cluster mean securing the first rank. Clusters with similar means were assigned the same rank. Cluster V and Cluster VIII shared the first rank with an overall score of 52. Cluster III secured the third rank, followed by Cluster VI and Cluster IX in the fourth and fifth positions, respectively. These rankings indicate the presence of desirable genotypes in these clusters, rendering them suitable for further crop improvement programs. Clusters II, VII, and IV obtained the lowest rankings. The values and rankings for the above are given in Table. 4

In breeding programs, utilizing cluster means helps in selecting clusters with diverse traits. For instance, Cluster III showcases favourable means for days to 50% flowering and days to maturity, while Cluster IX demonstrates notably higher mean values for panicle length, biological yield plant$_{-1}$, harvest index, filled grains panicle^{-1}, and grain yield plant^{-1}. These clusters present the second-highest average inter-cluster distance, signifying substantial divergence.

Cluster VIII excels in spikelet fertility and grain size, presenting a contrast with Cluster IX. Meanwhile, Cluster V, characterized by high grain weight, grain length, and a low number of chaffy grains panicle^{-1}, complements Cluster IX, further aided by the highest inter-cluster distance. By strategically selecting clusters with divergent traits, breeding programs can effectively harness a broad range of characteristics to develop rice varieties with enhanced performance.

Relative Contribution of Each Character Towards Diversity

The number of times each of the 15 characters appeared in the first rank and their respective percentage contributions towards genetic divergence is detailed in Table 5 and Fig. 3. Among all characters studied, days to 50% flowering contributed the most towards genetic divergence (32.98%), followed by grain length (27.91%), 100-grain weight (11.42%), and grain breadth (7.61%). Conversely, number of panicles plant^{-1} (0.42%), spikelet fertility (0.63%), and harvest index (0.42%) contributed the least towards genetic divergence.

In the present study, days to 50% flowering, grain length and 100-grain weight were found to be the best discriminatory characters for better selection of diverse genotypes and contributing maximum towards divergence in rice genotypes, so these traits could be exploited maximum to get varieties with a higher yield. The results obtained for the relative contribution of each character towards diversity were supported by earlier findings of Chandramohan *et al.*(2016) and Mishra *et al.*(2020) for days to 50% flowering and

100-grain weight; Chamundeswari *et al.*(2016) and Ranjith *et al.*(2018) for grain yield plant^{-1}.

This study facilitated the differentiation of genotypes into nine clusters based on genetic distances. Clusters V and IX, Cluster III and IX, and Clusters VIII and IX were identified as having high inter-cluster distances, making them potential candidates for parent selection in crop improvement programs aimed at developing promising varieties.

Conclusion

The divergence study utilizing D^2 statistics, an in-depth examination of rice genotypes, showcased significant diversity, leading to their classification into nine distinct clusters with varying inter-cluster distances. Cluster I emerged as the largest, comprising 14 genotypes, followed by Cluster III with 11 and Cluster V with seven genotypes. Clusters II and VII contained five and three genotypes, respectively, while Clusters IV, VI, VIII, and IX stood as solitary entities, highlighting a considerable degree of heterogeneity within these clusters. Each of these solitary clusters has a single genotype distinguished by unique characteristics.

The examination of inter-cluster distances unveiled considerable dissimilarity, with the highest inter-cluster distance recorded between Cluster V and IX (506.51). Conversely, lower inter-cluster distances were noted between Cluster I and IV (57.44). The maximum intra-cluster distance was observed in Cluster VII (68.48), succeeded by Cluster III (52.70) and Cluster V (46.71).

Among the nine clusters, Cluster IX showcased elevated mean values for panicle length, biological yield plant^{-1}, harvest index, number of filled grains panicle^{-1}, and grain yield plant^{-1}. Similarly, other clusters exhibited desirable mean values for specific traits, such as Cluster III for days to 50% flowering and days to maturity, Cluster V for grain length, 100-grain weight, and a lower number of chaffy grains panicle^{-1}, and Cluster VIII for grain size and spikelet fertility. Genotypes from divergent clusters demonstrating high mean performance for specific traits hold promise for selection in breeding programs, aligning with the objectives of improvement.

Out of 15 traits studied, the character days to 50% flowering emerged as the major contributor to genetic divergence, recording 32.98%, followed by grain length (27.91%), 100-grain weight (11.42%), grain breadth (7.61%), grain yield plant^{-1} (5.50%), number of filled grains panicle^{-1} (4.44%), plant height (4.12%), number of chaffy grains panicle^{-1} (2.43%), and grain size (2.11%). This emphasizes the importance of prioritizing these characters in selection procedures for effective crop improvement. The findings of this study provide valuable insights for the development of diverse and high-performing rice varieties through targeted breeding strategies.

Table 2 : Clustering composition of 44 rice genotypes based on Tocher's method

Cluster	Genotypes	Number of genotypes
I	SA3, SA5, SM211, LRG5, SM227, LG5, SA2, SA7, G3R, G5R, GG, MM11, DA4, SM201	14
II	SA1, SA8, SA6, SM120, BPT5204	5
III	NN3, NLR3354, NLR34449, MM43, NN1, NN2, DA2, DA5, MM73, DA3, DA1	11
IV	GGR	1
V	DA6, MM151, MM10, DA7, MM129, MM164, DA8	7
VI	NN4	1
VII	LGG, LG3, SA4	3
VIII	MM152	1
IX	NLR33892	1
	Total	**44**

Table 3 : Average intra and inter-cluster distances (D^2 values) for nine clusters of 44 genotypes in rice

Cluster	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**
I	**35.66 (5.97)**	80.57 (8.98)	68.61 (8.28)	57.44 (7.58)	122.66 (11.08)	67.64 (8.22)	93.27 (9.66)	135.15 (11.63)	269.96 (16.42)
II		**37.91 (6.16)**	160.71 (12.68)	135.37 (11.6)	269.52 (16.42)	59.91 (7.74)	77.29 (8.79)	278.12 (16.68)	230.25 (15.17)
III			**49.11 (7.01)**	83.99 (9.16)	99.96 (9.99)	90.74 (9.53)	140.35 (11.85)	106.49 (10.32)	439.82 (20.97)
IV				**0.00 (0.00)**	81.95 (9.05)	144.71 (12.03)	92.84 (9.64)	171.44 (13.09)	361.65 (19.02)
V					**45.65 (6.76)**	225.37 (15.01)	195.16 (13.97)	117.87 (10.86)	506.51 (22.51)
VI						**0.00 (0.00)**	86.70 (9.31)	194.36 (13.94)	307.80 (17.54)
VII							**68.88 (8.30)**	262.82 (16.21)	341.96 (18.49)
VIII								**0.00 (0.00)**	398.28 (19.96)
IX									**0.00 (0.00)**

Figures in parenthesis indicate D values

Table 4 : Cluster means for grain yield and its component traits in rice

Cluster No.	**Characters**															**Total score**	**Final rank**
	DFF	**DM**	**PH**	**PL**	**NPP**	**BY**	**HI**	**HGW**	**FGP**	**CGP**	**SF**	**GL**	**GB**	**GS**	**GY**		
Cluster 1	104.49 (6)	132.29 (6)	113.53 (5)	24.70 (6)	9.20 (7)	64.84 (4)	44.20 (5)	1.85 (5)	161.20 (4)	16.45 (4)	91.07 (3)	7.46 (6)	2.52 (4)	2.99 (7)	28.14 (6)	**78**	**6**
Cluster 2	118.33 (8)	147.80 (8)	124.20 (8)	25.16 (5)	9.99 (3)	64.28 (5)	35.01 (8)	1.41 (8)	161.03 (5)	20.21 (6)	89.86 (5)	7.10 (8)	2.27 (8)	3.15 (6)	22.27 (7)	**98**	**9**
Cluster 3	**88.91 (1)**	**116.55 (1)**	97.64 (2)	23.67 (7)	9.53 (6)	48.79 (8)	54.38 (3)	1.78 (6)	159.61 (6)	13.83 (2)	91.94 (2)	7.74 (5)	2.46 (5)	3.17 (5)	26.54 (5)	**64**	**3**
Cluster 4	103.67 (5)	130.00 (5)	**96.87 (1)**	21.65 (9)	7.13 (8)	44.09 (9)	37.30 (7)	2.28 (2)	113.93 (9)	17.53 (5)	86.76 (7)	7.87 (3)	**2.74 (1)**	2.85 (8)	16.76 (9)	**88**	**7**
Cluster 5	91.57 (2)	119.57 (2)	121.01 (7)	25.27 (3)	9.87 (5)	63.34 (6)	47.06 (4)	**2.43 (1)**	127.60 (7)	**13.82 (1)**	90.17 (4)	**8.76 (1)**	2.63 (2)	3.33 (3)	30.17 (4)	**52**	**1**
Cluster 6	102.67 (4)	129.33 (4)	104.53 (3)	25.17 (4)	**14.00 (1)**	78.47 (2)	42.84 (6)	1.22 (9)	183.60 (3)	25.73 (7)	87.95 (6)	7.11 (7)	2.15 (9)	3.31 (4)	33.11 (3)	**72**	**4**
Cluster 7	112.11 (7)	141.00 (7)	119.18 (6)	25.63 (2)	9.84 (4)	62.38 (7)	29.18 (9)	1.50 (7)	126.56 (8)	26.04 (8)	82.60 (9)	7.82 (4)	2.29 (7)	3.43 (2)	18.65 (8)	**95**	**8**
Cluster 8	92.33 (3)	120.00 (3)	104.80 (4)	22.03 (8)	6.73 (9)	71.91 (3)	60.39 (2)	2.14 (3)	306.47 (2)	15.87 (3)	**95.07 (1)**	8.39 (2)	2.39 (6)	**3.52 (1)**	43.52 (2)	**52**	**1**
Cluster 9	145.67 (9)	173.67 (9)	125.53 (9)	**28.33 (1)**	10.53 (2)	**96.47 (1)**	**61.47 (1)**	1.90 (4)	**320.53 (1)**	49.60 (9)	85.25 (8)	7.03 (9)	2.58 (3)	2.73 (9)	**61.60 (1)**	**76**	**5**
Cluster mean	106.62	134.47	111.92	24.62	9.65	66.06	45.76	1.83	184.50	22.12	88.96	7.70	2.45	3.16	31.18		

Note: Numbers in the parenthesis indicate the ranks based on cluster mean. The total score is the summation of rank numbers for all characters, based on which final rank is indicated. Bold numbers indicated the highest mean values for each character.

DFF: Days to 50% flowering
DM: Days to maturity
PH: Plant height (cm)
PL: Panicle length (cm)
NPP: Number of panicles plant^{-1}
BY: Biological yield plant^{-1} (g)
HI: Harvest index (%)
HGW: 100-grain weight (g)
FGP: Number of filled grains panicle^{-1}
CGP: Number of chaffy grains panicle^{-1}
SF: Spikelet fertility (%)
GL: Grain length (L) (mm)
GB: Grain breadth (B) (mm)
GS: Grain size (LB ratio)
GY: Grain yield plant^{-1} (g)

Table 5 : Per cent contribution of grain yield and its component traits towards genetic diversity

S. No.	Character	Number of times ranked first	% contribution
1	Days to 50% flowering	312	32.98
2	Days to maturity	0	0.00
3	Plant height (cm)	39	4.12
4	Panicle length (cm)	0	0.00
5	Number of panicles plant^{-1}	4	0.42
6	Biological yield plant^{-1} (g)	0	0.00
7	Harvest index (%)	4	0.42
8	100-grain weight (g)	108	11.42
9	Number of filled grains panicle^{-1}	42	4.44
10	Number of chaffy grains panicle^{-1}	23	2.43
11	Spikelet fertility (%)	6	0.63
12	Grain length (L) (mm)	264	27.91
13	Grain breadth (B) (mm)	72	7.61
14	Grain size (LB ratio)	20	2.11
15	Grain yield plant^{-1} (g)	52	5.50

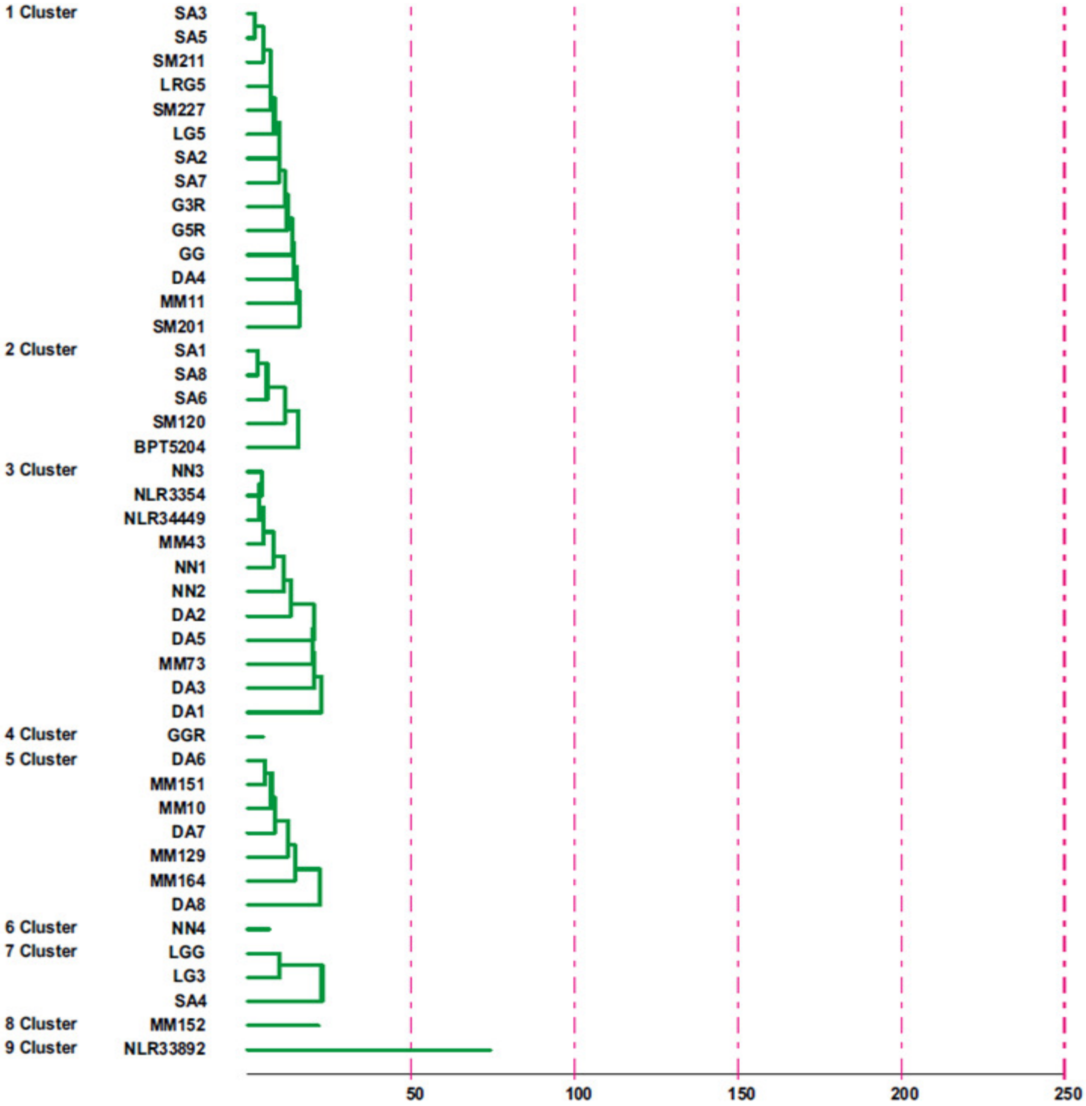

Fig. 1 : Dendrogram depicting clustering of 44 rice genotypes through Tocher's method.

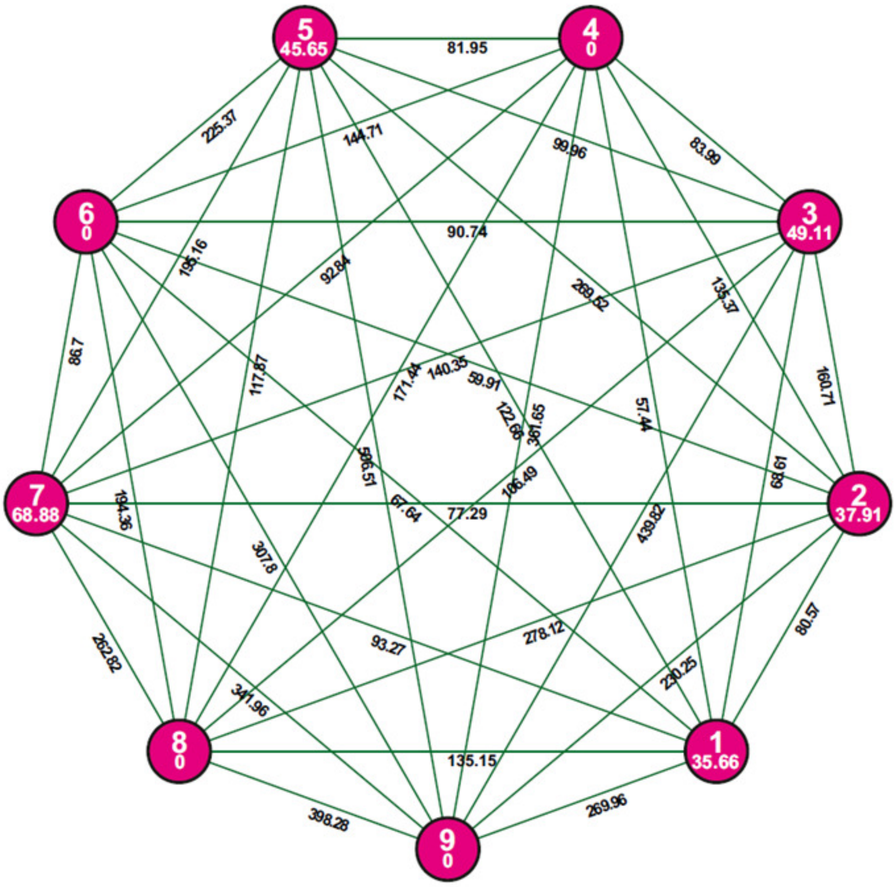

Fig. 2 : Intra-cluster and inter-cluster distances (D^2) among nine clusters of rice.

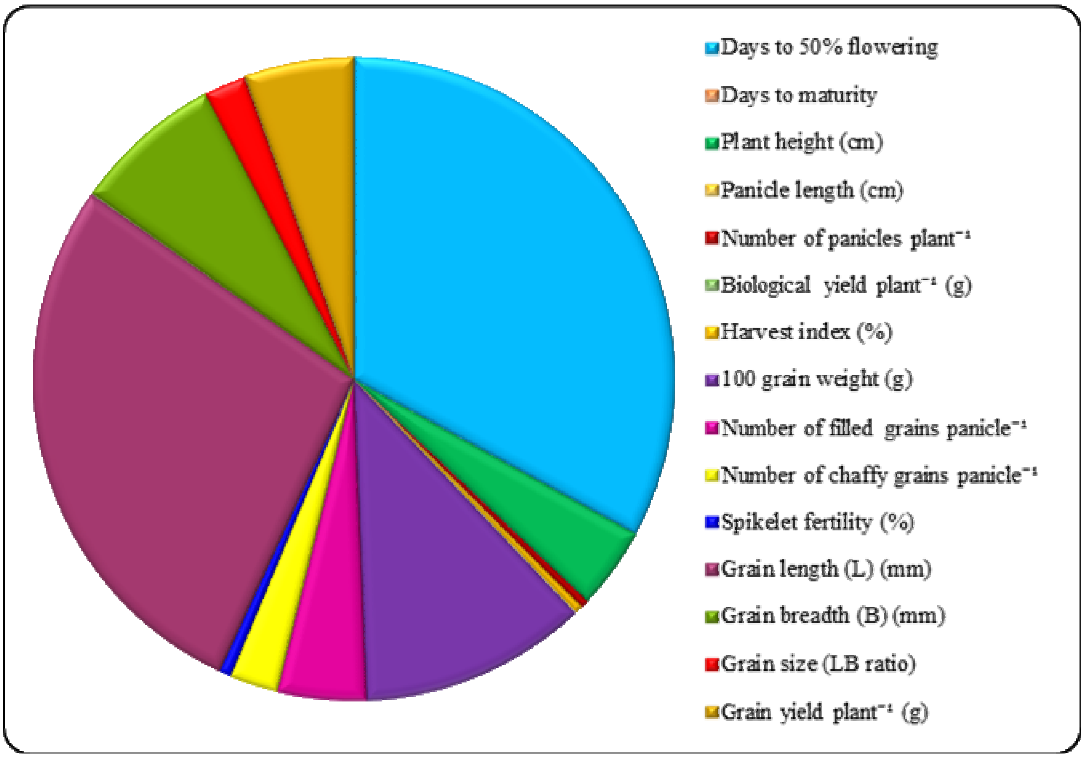

Fig. 3 : Per cent contribution of 15 characters to total genetic diversity in rice.

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