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Genetic Diversity and Trait Contribution in Rice Genotypes Using Mahalanobis D² Statistic

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ABSTRACT

Rice (Oryza sativa L.) is a staple food for over half of the global population, Salinity is a major abiotic stress limiting rice productivity, especially in coastal and irrigated regions. Excess salts disrupt plant metabolism, reduce water uptake, and impair growth. Rice, being salt-sensitive at seedling and reproductive stages, requires genetic improvement and adaptive strategies to ensure stable yields under saline conditions. This study, conducted during the Kharif 2024 season at the Centre of Excellence for Rice, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya, evaluated 121 rice genotypes along with four checks (CSR-10, CSR-36, Sarjoo-52, and MTU-7029) under saline conditions. The experimental design followed an Augmented Block Design with a spacing of 20 cm between rows and 15 cm between plants. Genetic divergence was assessed using Tocher method D² statistics across 13 yield-related traits. The analysis grouped the genotypes into nine clusters using Tocher's method, revealing significant genetic diversity. Intra-cluster distances ranged from 0.00 (Clusters III, IV, VI, IX) to 4750.90 (Cluster VIII), while the maximum inter-cluster distance was 64.863 between Clusters V and IX. Traits contributing most to genetic divergence included the total number of spikelets per panicle (48.39%), biological yield per plant (14.50%), filled spikelets per panicle (13.83%), flag leaf area (11.54%), and plant height (7.02%). These findings underscore the importance of utilizing genetically diverse parents in hybridization programs to develop high-yielding, protein-rich rice varieties, thereby addressing malnutrition and enhancing food security.

INTRODUCTION

Rice is the staple food for over half of the global population, with Asia contributing nearly 90% of its production and consumption. However, its cultivation is increasingly threatened by soil salinity, particularly in coastal and irrigated regions. Salinity stress adversely affects plant growth, yield, and grain quality. This is a serious concern for food security and nutrition in countries like India, where rice is a primary energy and protein source. Developing salt-tolerant rice varieties is crucial to sustain productivity and improve the nutritional profile, especially in salt-affected areas. (Mall et, al., 2011).

Rice protein is considered superior among cereals due to its balanced amino acid profile and high digestibility, making it suitable for infant and clinical nutrition. However, under salinity stress, both yield and protein content can be adversely affected. Breeding high-protein, salt-tolerant rice varieties requires a comprehensive understanding of genetic diversity. D² analysis is a powerful multivariate tool for assessing genetic divergence among rice genotypes under saline conditions, aiding in the identification of genetically diverse and stress-resilient parents for targeted hybridization programs (Manasa et, al., 2023). Studies have demonstrated significant genetic variability among rice landraces, with traits like plant height, days to flowering, and grain yield contributing notably to diversity. In India, where rice is a dietary cornerstone and malnutrition remains a pressing issue, developing high-yielding, protein-rich rice varieties is imperative. Utilizing genetic diversity analyses can accelerate breeding programs aimed at enhancing both the nutritional quality and yield of rice, thereby contributing to improved food security and public health (Singh et, al., 2015).

1. Material and method:

1.1 Plant material:

The research was conducted in kharif-2024 season and material under study constituted 121 Genotypes with 4 checks of rice obtained from Center of excellence for rice Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya 224229. The list of genotypes is mentioned in table 1.

Table-1. A total of 121 rice genotypes were used in the study which include 4 checks.

S.No.	Genotypes	S.No.	Genotypes	S.No.	Genotypes
1	NPBR-1	41	NPBR-58	81	NPBR-121
2	NPBR-2	42	NPBR-59	82	NPBR-122
3	NPBR-4	43	NPBR-60	83	NPBR-123
4	NPBR-5	44	NPBR-62	84	NPBR-124
5	NPBR-6	45	NPBR-65	85	NPBR-125
6	NPBR-7	46	NPBR-66	86	NPBR-126
7	NPBR-10	47	NPBR-67	87	NPBR-127
8	NPBR-11	48	NPBR-69	88	NPBR-128
9	NPBR-12	49	NPBR-71	89	NPBR-132
10	NPBF-13	50	NPBR-72	90	NPBR-133
11	NPBR-14	51	NPBR-73	91	NPBR-134
12	NPBR-15	52	NPBR-74	92	NPBR-135
13	NPBR-16	53	NPBR-75	93	NPBR-136
14	NPBR-17	54	NPBR-76	94	NPBR-137
15	NPBR-19	55	NPBR-77	95	NPBR-140
16	NPBR-20	56	NPBR-79	96	NPBR-145
17	NPBR-21	57	NPBR-80	97	NPBR-146
18	NPBR-23	58	NPBR-81	98	NPBR-147
19	NPBR-25	59	NPBR-82	99	NPBR-148
20	NPBR-26	60	NPBR-86	100	NPBR-149
21	NPBR-27	61	NPBR-87	101	NPBR150
22	NPBR-28	62	NPBR-90	102	NPBR-152
23	NPBR-29	63	NPBR-91	103	NPBR-153
24	NPBR-32	64	NPBR-92	104	NPBR-156
25	NPBR-33	65	NPBR-96	105	NPBR-161
26	NPBR-36	66	NPBR-97	106	NPBR-162
27	NPBR-41	67	NPBR-98	107	NPBR-163
28	NPBR-42	68	NPBR-100	108	NPBR-165
29	NPBR-43	69	NPBR-102	109	NPBR-166
30	NPBR-44	70	NPBR-104	110	NPBR-168
31	NPBR-45	71	NPBR-106	111	NPBR-169
32	NPBR-46	72	NPBR-109	112	NPBR-173
33	NPBR-47	73	NPBR-110	113	NPBR-174
34	NPBR-48	74	NPBR-112	114	NPBR-175
35	NPBR-49	75	NPBR-113	115	NPBR-179
36	NPBR-50	76	NPBR-115	116	NPBR-180
37	NPBR-54	77	NPBR-116	117	NPBR-182
38	NPBR-55	78	NPBR-117	118	CSR-10 Tolrent
39	NPBR-56	79	NPBR-118	119	CSR-36 Tolrent
40	NPBR-57	80	NPBR-119	120	Sarjoo-52 Suseptible
				121	MTU 7029 Suseptible

2.2. Experimental layout

The 121 genotypes and 4 checks (CSR- 10, CSR-36, SARJOO-52 and MUTU-7029) were sown in Augmented Randomized Complete Block Design. The spacing of 20 cm between rows and 15 cm between plants was followed.

2.3. Estimation of genetic divergence (D^2)

The genetic divergence of 121 genotypes of rice was worked out using Mahalanobis (1936) D^2 statistics (Rao, 1952). The thirteen characters under saline condition in rice were included for this analysis.

Experimental Design: Augmented Design

The augmented design was chosen due to the large number of unreplicated test entries and limited field space. The design included:

- **Blocks**: The experiment was divided into [9] blocks.
- **Test entries**: Each test genotype was grown once in a block.
- **Checks**: [4] check varieties were randomly distributed and replicated in each block.

Data on agronomic and yield-related traits (e.g., plant height, days to flowering, grain yield, etc.) were recorded using standard

protocols.

Data Adjustment and Standardization

The raw data were adjusted using the performance of check varieties across blocks to account for environmental variation. Standardization (mean = 0, standard deviation = 1) was applied to the trait data to ensure uniform contribution of all traits to the genetic distance.

Genetic Divergence and Clustering: Tocher's Method

1. **Distance Matrix**: Mahalanobis D^2 statistics were computed for all possible genotype pairs using the standardized trait data.
2. **Clustering**: Tocher's method was applied to the D^2 matrix to group genotypes based on genetic divergence:

- The first cluster was formed using the pair with the smallest D^2 value.
- Additional genotypes were added to this cluster if the average intra-cluster distance was less than the inter-cluster distance.
- If this condition was not met, a new cluster was initiated.
- This process continued until all genotypes were grouped.

Clustering and distance analysis were performed using [software used, e.g., custom Excel templates].

2.4. Intra and inter-cluster distance

The inter-cluster D^2 was calculated as the sum of n (n-1)/2 genotypes within a cluster divided by total number of combinations. All possible D^2 values between the groups of two clusters were added and then divided by n_1 X n_2 for computing inter-cluster distance.

2.5. 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

3.1. Phenotypic based diversity analysis

The selection of appropriate various oldsters for sexual union is a vital feature of any crop breeding programmes as a result of parental diversity in optimum magnitude is needed to get superior genotypes in segregating generations (Moll et al. 1965). The importance of genetic divergence in crop improvement has been stressed by many scientists Griffing and Lindstrom, 1954, felon et al., 1962 and Arunachalam 1981. D^2 analysis has been used by many employees for the assessment of genetic divergence in many crops Malhotra and Singh (1971).

In a study involving 121 rice genotypes and four checks, 13 yield-related traits were analyzed. The genotypes were grouped into nine clusters using F-test statistics, revealing significant genetic diversity. Intra-cluster distances ranged from 0.00 (Clusters III, IV, VI, IX) to 4750.90 (Cluster VIII), while the maximum inter-cluster distance was 64.863 between Clusters V and IX. These distances suggest substantial genetic variability among clusters, which is crucial for selecting genetically diverse parents in hybridization programs to enhance desirable trait combinations in progeny. The findings underscore the importance of statistical tools like D^2 analysis in identifying diverse genotypes for breeding strategies. To identify genetically diverse and agronomically superior parents for hybridization, the use of inter-cluster distances and mean performance of genotypes across traits is crucial (Ray et al., 2022; Sadhana et al., 2022; Sruthi et al., 2023; Manasa et al., 2023).

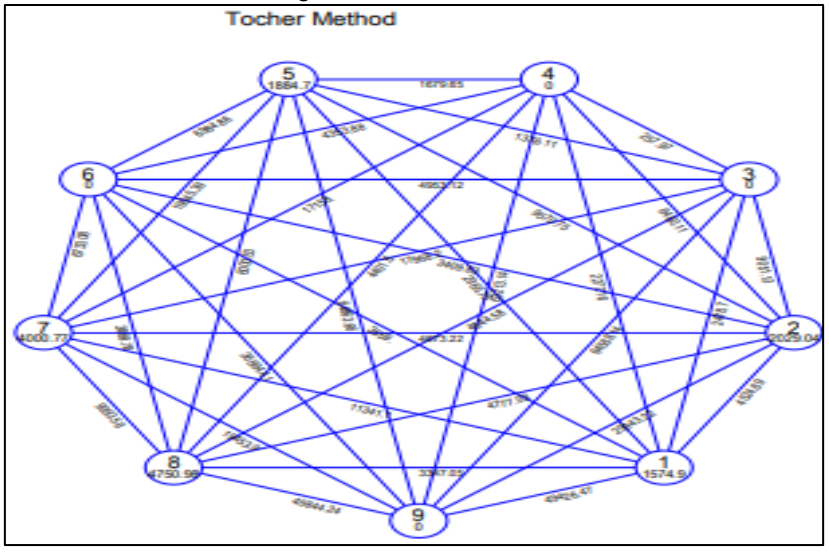

Fig.1. Distance matrix based on Tocher Method

Table 2. Inter and intra cluster distances of 121 rice genotypes

Cluster	I	II	III	IV	V	VI	VII	VIII	IX
I	**1.574**								
II	4.524	**2.029**							
III	2.478	9.051	**0**						
IV	2.277	8.460	2.579	**0**					
V	2.965	9.570	1.376	1.679	**1.884**				
VI	3.559	3.409	4.953	4.353	6.984	**0**			
VII	11.341	4.673	17.968	17.158	19.045	6.733	**4.000**		
VIII	3.347	4.777	4.844	4.401	6.000	3.868	9.993	**4.750**	

IX	49.426	29.943	64.588	63.213	64.863	36.884	19.453	45.844	0

3.2. Cluster contribution

Cluster contribution the maximum percent contribution in genetic divergence in rice genotypes was observed by total no. of spikelets per panicle (48.39%) followed by biological yield per plant (14.50 %), filled spikelets per panicle (13.83%), flag leaf area(cm^2) (11.54%) and plant height (7.02%). Singh et al., (2015) and Devi et al., (2020) showed similar result.

Table-3. Cluster contribution in genetic divergence

S.No.	Source	Contribution %	Times ranked 1st
1.	Days to 50% flowering	1.75%	127
2.	Chlorophyl content	0.52%	38
3.	Flag leaf area	11.54%	838
4.	Plant Height	7.02%	510
5.	Panicle Length	0.03%	2
6.	Number of tiller's	0.30.%	35
7.	Total No. of grain per panicle	48.39%	3513
8.	Filled grain per plant	13.83%	1004
9.	Spiklet fertility %	0.26%	19
10.	Test Weight (g)	0.06%	4
11.	Biological Yield (g)	14.5%	1053
12.	Harvest index	1.87%	136
13.	Grain yield per plant (g)	0.22%	16

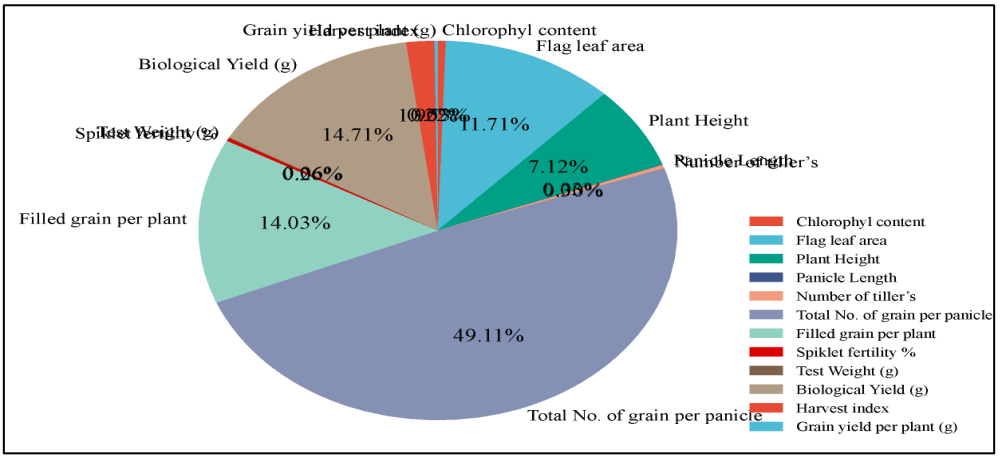

Figure-2. Pie chart of contributing traits

CONCLUSION

In conclusion, the comprehensive analysis of 121 rice genotypes using Mahalanobis D^2 statistics and non-hierarchical Euclidean cluster analysis revealed substantial genetic diversity across 13 yield-related traits. The intra-cluster distances ranged from 0.00 (Clusters III, IV, VI, IX) to 4750.90 (Cluster VIII), and the maximum inter-cluster distance was observed between Clusters V and IX (64.863), underscoring the presence of significant genetic variability among the clusters. Trait-wise contribution to genetic divergence highlighted the total number of spikelets per panicle as the most influential factor (48.39%), followed by biological yield per plant (14.50%), filled spikelets per panicle (13.83%), flag leaf area (11.54%), and plant height (7.02%). These findings align with previous studies emphasizing the importance of these traits in rice breeding programs. The significant genetic variability and the identification of key contributing traits provide valuable insights for rice breeding strategies. Selecting genetically diverse parents from highly divergent clusters can enhance the probability of obtaining superior progeny with desirable trait combinations. This approach is crucial for developing high-yielding, protein-rich rice varieties, thereby addressing nutritional deficiencies and improving food security.

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