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Genetic Diversity Analysis by D^2 Analysis in Fine Scented Genotypes of Rice (*Oryza sativa* L.)

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A B S T R A C T

The present investigation on "Diversity analysis by D^2 analysis in fine scented genotypes of rice (*Oryza sativa* L.)" was used to investigate the diversity among 40 fine scented genotypes obtained from the Indira Gandhi Krishi Vishwavidyalaya in Raipur. The current studies was conducted at research cum instructional farm, IGKV, RMD Ambikapur, Chhattisgarh. The experiment was conducted in RBD with purpose to characterized 40 genotypes of rice along with 4 checks viz. CG Sugandhitbhog, CG Devbhog, Indira Sugandhit Dhan-1 and Dubrajsel 1 for diversity. Based on cluster analysis, the genotypes were grouped into 5 clusters in which cluster I was the largest consistin of 29 genotypes. While cluster IV & V were the smallest with only a single genotypes; each. Maximum intra cluster distance was found in the cluster II, Which comprises only 5 genotypes. The most divergent clusters observed were cluster III & V. The minimum cluster distance was recorded between cluster I & III.

Introduction

Rice is a world's most widely consumed crop. More people are fed directly from this crop than from any other. It is essentially a short day self-pollinated crop. On the global basis rice is grown in an area of 162.06 million hectare (Statista, 2021), with annual production in the world 503.17 million metric tonnes, (USDA 2020-21). India leading the world after china with an area of 43.79 million hectare (Statista, 2021). And the production of 177.65 million metric tonnes (Statista, 2021). It provides minerals, vitamins and fiber although all constituents excepts carbohydrates are reduced by milling. Rice accessions are a rice reservoir useful genes that rice breeder can harness for rice

improvement programme (Rashmi *et al.*, 2017). Genetic diversity is the pre-requisite for any crop improvement programme because it helps in the development of superior recombinants (Manonmani and Fazulullah Khan, 2003), through selection of parents having wider variability for different characters and ultimately for a rational use of genetic resources (Nayak *et al.*, 2004). D^2 statistics (Mahalanobis, 1936) have proven to be a powerful tool for assessing genetic variance. It enables one to discriminate between different cultivars according to the diversity presents in the genotypes under study.

Materials and Methods

Experimental material composed of 39 Fine scented genotypes of rice. The list of fine scented rice genotypes is accorded in table 1. These genotypes were sown in RBD with 3 replications at IGKV, RMD CARS, Research and Instructional Farm, Ambikapur during *Kharif* 2020-21. Every genotype was sown as R to R and P to P distance of 20 cm &15 cm, respectively. Standard agronomical package of practices was adapted for raising the rice crop.

The observations on quantitative and qualitative traits was recorded based on five randomly taken plants from each genotype for some observations and for other observations was recorded on whole plot basis. The field trials were carried out under irrigated transplanted condition. The plant material was sown in a raised bed nursery, and the seedlings were transferred into the field in a Randomized Block Design(RBD) after twenty-five days. Three replications of the experimental material were planted. Each entry was transplanted in six lines with 20 cm of spacing among row to row and 15 cm between plants to plant. Fertilizer dose @ of 80N: 50P: 30K Kg per ha and 1.7 kg IFFCO + potash per strip was applied.

Statistical Methods

Genetic divergence by Mahalanobis' D^2statistics

The estimated inter genetic distances between genotypes are used to determine genetic divergence between them. Mahalanobis'(1928) D^2 statistics is one of the most successful approaches for determining the genetic distance between genotypes using allelic frequencies at a sample of loci. The extent of gene similarity among genotypes is characterized as genetic similarity, which is the inverse of genetic divergence.

Testing difference in the Population

When the null hypothesis of no treatment changes fails in individual characters, a dispersion table was created from the variance analysis. A simultaneous test of differences between mean values of a number of correlated variables was done using the V statistic, which intern uses Wilks criterion (Δ). (Rao, 1952).

$$\text{Wilk's criterion } (\Delta) = \frac{\text{Determinants of error variance and covariance matrix (E)}}{\text{Determinants of genotypic + error variance and covariance matrix}}$$

$$V \text{ statistic} = -m \log_e \Lambda$$

$$= -\left\{n - \frac{p+q+1}{2}\right\} \log \Lambda$$

$$m = n - \frac{p+q+1}{2}$$

Where,

P = Number of variables or characters

q = Degrees of freedom for population (i.e., number of genotypes-1)

n = Degrees of freedom for error + genotypes

$\log_e \Lambda = 2.3026 \log_{10} \Lambda$

'V' statistic is distributed as χ^2 with q degrees of freedom. The test of significance of 'V' statistic showed that the differences between the means in respect of the pooled effect of p characters between different populations were significant. Hence further analyses were made to estimate D^2 -values.

Mahalanobis D^2 –statistics

The current study used the "Mahalanobis D^2" generalized distance to estimate genetic divergence as described by Rao (1952).The fundamental condensation method of inversion matrices was used to convert original variable means to uncorrelated variables.

The sum of squares of differences in the values of the associated transformed variables was used to calculate the D^2 values between genotypes.

The mean deviation is calculated for each pair of combinations.

i.e. $di = Yi^1 – Yi^2$,

where,

The transformed variables Yi(where i =1,2,3,4, 5,.....p) were calculated, and the D^2 was determined as the sum of the squares of those deviations.$D^2 = \Sigma (Yi^1 – Yi^2)^2$

Where,

p is the Number of Characters.

D^2 -values were assessed for significance using chi-square ($\chi 2$) at p degrees of freedom, where p is the number of characters considered.

Tocher's method of genotype grouping

The genotypes were sorted into a number of clusters using Tocher's approach, introduced by Rao (1952), after arranging the D^2-values of all combinations of one genotype with the others in ascending order of magnitudes. This method's criterion was that any two kinds belonging to the same cluster had a lower D^2 -value on average than those belonging to two distinct clusters. The inter- and intra-cluster distances were then determined, and their relationships were shown graphically.

Individual character's contributions to divergence

Each character was ranked using di= Yi j – Y I k values over all genotype combinations. The biggest mean difference received rank 1 and the lowest mean difference received rank p, with p representing the total amount of characters. The percent contribution was computed by taking the total number of combinations and multiplying it by 100.

Results and Discussion

The 40 rice genotypes of rice, were analyzed for genetic divergence and the results revealed that significantly differed for all the 11 characters. Geographical separation or genetic barriers to crossability leads % arises the genetic diversity. Many geneticists used genetic distance analysis among the cultivars of a crop under the assumptions that cultivars within the group are genetically related whereas diverse cultivars are classified into the different clusters. This technique analyze the forces of differentiation at two levels viz., Intracluster and inter cluster. In the present investigate on it was measured by Mahalonobis D^2 statistics and Tocher's

method. The genetic distance was measured for each pair of genotypes in all possible combinations.

Clustering Pattern

Based on the D^2 value, for the 39 genotypes of rice were grouped into 5 clusters. Among these clusters, clusters I consist maximum 29 genotypes followed by cluster II 5 genotypes, cluster III 4genotypes, while Cluster IV and V consist (each 1 genotypes). The grouping pattern constellation proved the presence of significant amount of variability. The genotype clustering pattern revealed that the clustering did not follow any specific patterned clustering in relation to the origin.

Similar findings also reported by Sarawgi and Bisne (2008).

Intra and inter cluster distance

The average cluster distance (intra and inter) was measured for each clusters and each pair of clusters in all possible combinations (to find out the D^2 value) respectively in terms of D^2 value.

The results revealed that the highest intra cluster distance was observed on cluster II (47.25) including 5 genotypes, while the lowest intra cluster distance was recorded on cluster IV and cluster V (each 0.00)] cluster III (32.41) including 4 genotypes.

The highest inter cluster distance was observed between cluster V& cluster IV (265.64) and cluster V & III (221.81) while the lowest was observed between cluster III & cluster I (73.31). Genotypes consist in the same cluster indicate that they were closely related as compared to other clusters genotypes. Therefore, it may be expected that grouping of genotypes in one cluster less divergent than those genotypes present in different cluster.

The hybridization between the most diverse genotypes(higher inter cluster distance) may results desirable segregants with the accumulation of favorable genes in the segregating generation, which help in hybridization.

Cluster mean

The mean values of different clusters for 11 traits here been presents in table 4.

A considerable difference in cluster mean values were apparent for all the traits.

The cluster mean value for Days to 50% flowering was maximum 119.00 days for cluster IV and the minimum value was 103.60 days for cluster II. Cluster mean for days to 50% maturity was highest 152.33 days for cluster IV while the lowest was 141.67 days for cluster II. The highest mean value for plant height was recorded on cluster V(141.40) and the lowest was recorded on cluster IV (94.53). The mean value for panicle length was maximum 24.73 for cluster IV and the minimum was 22.70 for Cluster III. The highest mean value for the number of tillers per sq.mt. was 7.13 for Cluster V while the lowest was 6.40 for cluster I.

The mean value for total number of grains per panicle was highest 423.73 for cluster V and the lowest was 165.56 for cluster II. The maximum value for filled grain per panicle was 372.07 in cluster V and the maximum value was 117.92 for cluster II. Cluster mean for unfilled grain per panicle was highest 98.27 for cluster IV while the lowest was 44.09 for cluster I.

Table.1 List of 39 fine scented genotypes of rice used in the present study

S.N.	Entry	Parentage
1	R 1656-2151-1-412-1	Swarna x Jira Shankar
2	R 1919-537-1-160-1	Shymala x G 93-02
3	R 2054-685-1-205-1	R 1033-2559-1-1 x Gopal bhog
4	R1624-61-1-59-1	Surekha X Madhuri
5	R1624-61-2-60-1	Surekha X Madhuri
6	R1624-61-3-61-1	Surekha X Madhuri
7	R1896-82-1-60-1	Suresha X Madhuri
8	R1915-115-1-88-1	CN1269-7-21 X Madhuri
9	R2054-147-1-103-1	R1033-2559-1-1 X Gopal Bhog
10	R2054-147-2-104-1	R1033-2559-1-1 X Gopal Bhog
11	R2054-147-3-105-1	R1033-2559-1-1 X Gopal Bhog
12	R2281-308-1-185-1	R1238-258-3-86-1 X I S D-1
13	R2282-552-1-309-1	R1244-1246-1-605-1 X I S D-1
14	R2032-87-1-23-1	R979-1528-2-1 X BPH-12
15	CG SugandhitBhog (c)	
16	CG Devbhog (c)	
17	Indira Sugandhit Dhan-1 (c)	
18	DubrajSel 1(c)	
19	NagriDubraj Mutant-1	Mutant of NagriDubraj
20	Samundrachini 5-50	Mutant of Samundchini
21	Jhilli Mutant 13-5	Mutant of JhilliDhan
22	Jeeraphool Mutant 5	Mutant of Jeeraphool
23	Vishnubhog Mutant V-74-6	Mutant of Vishnubhog
24	R2369-481-1-258-1	SAWRNA X KALA NAMAK
25	R2369-475-2-252-1	SAWRNA X KALA NAMAK
26	R2369-483-1-259-1	SAWRNA X KALA NAMAK
27	R2369-478-1-255-1	SAWRNA X KALA NAMAK
28	R2369-475-1-251-1	SAWRNA X KALA NAMAK
29	R2369-479-1-256-1	SAWRNA X KALA NAMAK
30	R2400-562-1-339-1	RNR2354 X KUBERI MOHAR
31	Ker ghul	Selection
32	R2369-480-1-257-1	SAWRNA X KALA NAMAK
33	Banspatri	Selection
34	Maharaji	Selection
35	Kasturi	Selection
36	R 2400-562-2-340-1	RNR2354X KUBRI MOHAR
37	RL 910 (LAYCHA)	Sel. from local germplasm
38	RM 504 (Mahraji)	Sel. from local germplasm
39	JDP-2520-2-4-1	Germplasm Sel. from Jagdalpur
40	R-FS-2019-1	Karma Mahsuri x CG Devbhog
41	R-FS-2019-2	Karma Mahsuri x CG Devbhog

Sources:- IGKV,Raipur (C.G.)

Table.2 Grouping of 40 rice genotypes into 5 clusters (by tocher's method)

Clusters	Number of genotypes	Name of genotypes
I	29	CG Sugandhitbhog (C), R2369-475-1-251-1, R2369-475-2-252-1, R2369-481-1-258-1, R2369-480-1-257-1, R2369-479-1-256-1, Banspatri, R2369-478-1-255-1, R2369-483-1-259-1, JDP-2520-2-4-1, Nagridubraj mutant-1, Indira sugandhit dhan-1(C), R2400-562-1-339-1, CG Devbhog (C), R2054-147-1-103-1, R2054-147-3-105-1, R2054-147-2-104-1, R1915-115-1-88-1, R2032-87-1-23-1, R2282-552-1-309-1, R2281-308-1-185-1, Kasturi, R1896-82-1-60-1, Ker ghul, R1919-537-1-160-1, Dubraj (C), jhilli mutant 13-5, R2400-562-2-340-1, Samundracharini 5-50,
II	5	Maharaji, RM 504(Maharaji), RL910(LAYCHA) Jeeraphool Mutant 5, R-FS-2019-2,
III	4	R1624-61-1-59-1, R1624-61-3-61-1, R1624-61-2-60-1, R2054-685-1-205-1
IV	1	Vishnubhog Mutant V-74-6,
V	1	R 1656-2151-1-412-1

Table.3 Average of intra and inter cluster distance in among rice genotypes

	Cluster Distances				
	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5
Cluster 1	38.59	77.56	73.31	93.31	209.15
Cluster 2		47.25	187.38	81.82	276.72
Cluster 3			32.41	149.27	221.81
Cluster 4				0.00	265.64
Cluster 5					0.00

Table.4 Mean values of 11 quantitative characters in 5 clusters of 40 rice genotypes

S.no.	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 6
Days to 50% flowering	106.35	103.60	111.50	119.00	112.00
Days to 50% maturity	145.95	141.67	148.50	152.33	151.00
Plant height	108.67	127.47	99.04	94.53	141.40
Panicle length	23.78	24.63	22.70	24.73	23.80
No. of effective tillers/sq m	6.40	6.45	6.60	6.47	7.13
Total no. of grains/panicle	169.94	165.56	200.53	257.47	423.73
Filled grains/panicle	126.09	117.92	135.80	159.20	372.07
Unfilled grains/panicles	44.09	47.64	62.97	98.27	51.67
1000 grains wt.	24.72	22.23	29.92	20.17	23.33
Spikelet fertility%	74.27	71.27	69.26	63.06	87.80
Grain yield	25.50	8.57	40.74	15.05	30.35

Table.5 Contribution of each characters towards genetic divergence

S.No.	Source	Contribution %
1	**Days to 50% flowering**	2.00
2	**Days to 50% maturity**	3.00
3	**Plant height**	0.77
4	**Panicle length**	2.00
5	**Number of effective tillers per sq.mt.**	2.95
6	**Total number of grains per panicle**	19.23
7	**Filled grains per panicle**	4.49
8	**Unfilled grains per panicle**	0.38
9	**1000 grain weight in grams**	22.44
10	**Spikelet fertility %**	1.54
11	**Grain yieldqtls/ha**	48.21

The mean value for 1000 grains weight was maximum 29.92 for cluster II and the minimum was 20.17 for cluster IV. The highest mean value for spikelet fertility was 87.80 for cluster V while the lowest was 63.06 for cluster IV. The mean value for grain yield was maximum 40.74 for cluster III while the minimum was 8.57 for cluster II. The high mean value genotypes may be use directly for adaptation or in breeding work.

Percent contribution of each character

Percent contribution of 11 traits for genetic divergence is presented in table 5. The selection and choice of parents mainly depends upon the contribution of trait towards genetic divergence (Babu *et al.,* 2003). In the present study the highest contribution in expansion of genetic divergence was exhibited by the traits.

On the basis of genetic diversity analysis, the maximum percent contribution (table 5) towards genetic divergence was from grain yield qtls/ha. i.e. 48.21% followed by 1000 grains weight (22.44%), total number of grains per panicle (19.23), filled grains per panicle (4.49%), days to 50% maturity (3%) number of effective tillers per sq.mt. (2.95%) days to 50% flowering (2%), panicle length (2%), spikelet fertility % (1.54%), plant height (0.77%) and unfilled grains per panicle (0.38%).

Using Mahalonobis D^2 statistics the genotypes of rice were grouped into 5 clusters. The highest intra cluster distance was recorded on cluster II and the lowest was on cluster III. The highest inter cluster distance was recorded between cluster V and cluster II and the lowest was recorded between cluster III and I.

The present study revealed that cluster V had highest mean value for the characters plant height(141.49), number of effective tillers per sq. meter (7.13), total number of grains per panicle (423.73), filled grains per panicle (372.07) and spikelet fertility percent (87.80).

The cluster IV had highest mean value for the characters Days to 50% flowering (119.00), Days to 50% maturity (152.33) panicle length (23.73) and unfilled grains per panicle (98.27). The cluster III had highest mean value for the characters 1000 grains weight (29.92) and grain yield quintals per ha(40.74). The percent contribution towards genetic diversity was

highest for grain yield qtls/ha (48.21%) followed by total number of grains per panicle (19.23) and filled grains per panicle (4.49%) and the lowest contribution sown by unfilled grain per panicle (0.38%).

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