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"CANONICAL ROOT ANALYSIS AND CLUSTERING FOR CHARACTERIZATION AND EVALUATION OF AROMATIC RICE GERMPLASM BASED ON MORPHOLOGICAL CHARACTERS

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ABSTRACT
The present study was conducted to estimate the magnitude of genetic diversity among the genotypes and to identify the major morphological traits contributing for the observed variations. A total of 50 aromatic rice genotypes were evaluated in Randomized Block Design with three replications during -2018-2019 kharif season to study genetic diversity using Mahalanobis D^2 statistics for seed yield and its components. Cluster analysis classified the fifty genotypes into 12 groups. Maximum inter cluster distance was exhibited between clusters VIII and IX (227.03) followed by clusters I and IX (199.86), clusters VII and VIII (190.65), clusters V and IX (187.34) and cluster VII and IX (180.52). The lowest inter cluster divergence was observed between clusters IV and VII (11.01). The intra-cluster distance (D) ranged from 10.23 (cluster-I) to 25.36 (cluster-XII). The attributes, *viz*., 1000 grain weight (37.63%), Plant height (25.71%) and Seed yield per plant (18.61%) contributed much to the total genetic divergence. PCA revealed three most informative principal components with Eigen values of 6.222, 4.577 and 1.900, respectively, which together accounted 83.323 % of the total variance. The result ensures the existence of high genetic divergence among the studied genotypes and can be used by breeders to develop high yielding rice varieties and have immense applications in rice improvement.

INTRODUCTION

Rice is the staple food across Asia where around half of the world's poorest people live. It is most important human food crop in the world, directly feeding more people than any other crop. Although most rice is consumed in the countries where it is produced, a growing demand in some areas is feeding the international rice trade. Generally, non-aromatic rice varieties are high yielding, demonstrating good agronomic performance, and are highly adapted to the environmental condition and produced in all the rice-growing countries. Conversely, most of the aromatic rice varieties are low yielding, demonstrating inferior agronomic performance, highly prone to the environmental condition and are produced in few countries (Prodhan *et al*., 2017). Despite inferior performance, the aromatic rice is highly regarded for their excellent aroma and superior grain quality (Wakte *et al*., 2017). Aromatic rice fetch high price in international trade. So there is a need to develop aromatic rice varieties which are resistant to biotic and abiotic stresses and also high yielding. The development of varieties is a continuous process and the success of the plant breeding programme depends upon the selection of suitable genotypes to be utilized in breeding programme. The effectiveness of selection depends primarily upon the magnitude of genetic diversity in the breeding material at hand. Appropriate and most efficient approach should be used for germplasm evaluation and characterization.

Mahalanobis's D^2 statistics has been used in several crops for identifying diverse parents for hybridization programmes. It is a powerful tool used to quantify the genetic divergence between the accessions and to relate clustering pattern with the geographical origin. Principal component score strategy has been employed for the identifying the plant characters that categorize the distinctiveness among the promising genotypes. PCA helps to eradicate redundancy in data sets due to regular variation occurring regularly in the crop species (Maji and Saibu, 2012) and (Ramakrishnan *et al*., 2016). Hence, the importance of PCA is considered and has been successfully used in the germplasm evaluation of crops for understanding the relationship and correlation among the variables studied (Zafar *et al*., 2008). Therefore this study was organized to estimate the magnitude of genetic distance and to identify the major traits contributing for the observed variations among the studied genotypes.

MATERIALS AND METHODS

Experimental material consists of 50 selected aromatic rice genotypes on the basis of diverse grain morphological characteristics. The experiment was conducted at Rice Research Centre, Agricultural Research Institute, Professor Jayashankar Telangana State Agricultural University, Rajendranagar during Kharif 2018. Randomized Block Design

(RBD), was adopted to conduct the experiment with three replications. Healthy nursery was raised and thirty one days old seedlings were transplanted in well prepared main field. Two rows for each entry with spacing of 20 x 15 cm and each row consists of 25 plants per entry. First weeding was carried out 20 days after transplanting and second weeding 30 days after first weeding. Fertilizer application was carried at recommended dosage. Necessary crop protection measures were taken up based on need of crop. Observations were recorded for quantitative traits on randomly selected five plants for each entry in three replications. The replication wise mean values were used for statistical analysis. Differences among genotypes were tested by Wilk's criterion (Rao, 1952) for the pooled effect of all the 8 characters. The D^2 values for all the possible pairs of varieties were grouped into a number of clusters according to Tocher's method as suggested by Rao (1952). The characters were ranked on the basis of their contribution to D^2 values, in all the combinations. A measure of group distance based on multiple characters was given by Mahalanobis (1936) using D^2 statistic. With the help of this, genetic divergence between genotypes was estimated.

RESULTS AND DISCUSSION

The analysis of variance for individual characters revealed significant differences among genotypes. The value of V-statistic (1860.88) with 343 degrees of freedom showed highly significant differences among the genotypes for aggregate of 8 characters. Thus, one can proceed for further diversity analysis. Grouping of the genotypes was carried-out by following the Tocher's method (Rao, 1952) and were grouped into tweleve clusters, with 50 genotypes. The distribution of genotypes into various clusters is shown in (Table 1). The composition of different clusters varied from 1 to 22 genotypes. Cluster II was largest comprising of twenty two genotypes followed by cluster III with eight genotypes, cluster I with six genotypes, cluster IX with 4 genotypes and cluster XII with 3 genotypes. The clusters IV, V, VI, VII, VIII, X and XI were represented by single genotype exhibiting high degree of heterogeneity among the genotypes. The discrimination of lines into so many discrete clusters suggested presence of high degree of genetic diversity in the material evaluated. Similarly 75 diverse genotypes of soft rice were grouped into seven clusters by pragnya *et al*. (2018). 216 genotypes were grouped into 15 clusters by Praveen *et al*. (2019). Twenty six cultivars of rice were grouped into six clusters by Shivani *et al*. (2018). The clustering pattern of genotypes from different eco-geographical regions into these clusters was apparently random genotypes of different origins were clubbed in one cluster, whereas the genotypes belonging to same state or origin were grouped in different clusters thereby indicating non-relationship between geographical and genetic diversity. Singh *et al*. (2007) also reported that there was no parallelism between geographic distribution and genetic diversity. Pattern of distribution of genotypes among various clusters reflected the considerable genetic diversity present in the genotypes under study. The choice of suitable diverse parents based on genetic divergence analysis would be more fruitful than the choice made on the basis of geographic distances in accordance with Raut *et al*. (2009).

Number of times that each of the yield and its component characters appeared in first rank and its respective percent contribution towards genetic divergence is presented in (Table 4). The results recorded that the contribution of 1000-grain weight towards genetic divergence was highest (37.63 %) by taking 461 times ranking first similar with the results of Reddy *et al*. (2013), followed by plant height (25.71) by taking 315 times first, seed yield per plant (18.61) by taking 228 times first, number of grains per panicle (11.75) by first ranking 144 times, days to fifty percent flowering (5.87) ranking 72 times first, no. of effective tillers per plant (0.4%) by ranking 5 times first. Three characters out of the seven characters studied, namely 1000-grain weight, plant height, seed yield per plant contributed (81.95 %) to the total genetic divergence. The results obtained in the present study are in accordance to the findings of Tripathi *et al*. (2017), Ahamed *et al*. (2018).

Cluster means of yield and its component traits were presented in (Table 3). Cluster means data shows that days to 50% flowering was highest in cluster X (117.67) and lowest in cluster VI (100.33), plant height recorded highest in cluster IX (174.11) and lowest in cluster VII (101.67), no. of effective tillers per plant was observed highest in cluster VII (19.53) and lowest in

Table 1: Clustering pattern among 50 aromatic rice genotypes for yield and its component traits by Tocher method

Cluster number	No, of Geno types	Genotypes
I	6	RNR 15462-2, RNR 15459-1, RNR 15460-1, RNR-15462-4, RNR-15453-2, Sugandh Samba
II	22	RNR-29420, RNR-29421, RNR-29422, RNR-29423, NVSR 406, RNR-28408, RNR-28405, RNR-26058, RNR-15488-2, RNR-28404, RNR-2600, RNR-28403, RNR-29301, NVSR 407, RNR-26009, RNR-26010, RNR-29296, Vasumathi, RNR-29296, RNR-26020, RNR-28402, RNR 17500.
III	8	RNR 28410, Ketekijoha, CR 3663-261-8-4, RNR17501, RNR 28410, CR 3715-119-18-9-2, CR 2981-16-2-6, CR 2982-14-6-3
IV	1	RNR-29305
V	1	PUSA-1121
VI	1	Sumathi
VII	1	RNR-29306
VIII	1	RNR-15459-6
IX	4	OR (CZ)-70, BM 4, Dubraj, JDP-K-37
X	1	CR 3715-119-18-9-2
XI	1	Chittimutyalu
XII	3	NWGR 9081, Pusa Basmathi, Shobini

Table 2: Intra (diagonal) and inter cluster distances of D^2 values of 50 genotypes yield and its component characters

Clusters	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	Cluster 7	Cluster 8	Cluster9	Cluster10	Cluster11	Cluster12
Cluster 1	10.23	57.54	55.61	122.42	108.95	43.38	123.45	21.28	199.86	117.28	27.21	120.72
Cluster 2		18.67	41.78	29.45	40.28	29.39	43.49	81.17	156.08	34.47	56.42	53.08
Cluster 3			22.16	66.09	68.44	42.27	75.94	73.64	115.84	43.59	68.6	54.14
Cluster 4				0	50.52	70.58	11.01	166.92	174.4	16.12	113.13	41.36
Cluster 5					0	22.52	98.39	95.84	187.34	32.62	120.33	78.42
Cluster 6						0	105.64	34.09	156.54	58.95	57.56	74.01
Cluster 7							0	190.65	180.52	36.44	105.22	58.77
Cluster 8								0	227.03	140.54	53.72	154.81
Cluster 9									22.2	152.24	138.44	161.87
Cluster 10										0	121.86	42.26
Cluster 11											0	136.38
Cluster 12												25.36

Table 3: Cluster means of 50 rice genotypes for yield and its component characters by Tocher method

Characters	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Days to 50% flowering	107.67	107.15	114.67	109.33	104.67	100.33	114.33	105.67	112.67	117.67	108.67	105.11
Effective tillers per plant	19.09	17.71	18.59	18.67	18.83	19.2	19.53	17.78	18.9	17.47	17.87	12.79
Plant height	102.57	105.96	112.6	102.27	106.73	111.43	101.67	103.78	174.11	103.8	125.09	103.78
Panicle length	21.91	24.82	24.23	24.33	25.83	26.63	22.53	22.31	27.8	22.43	25.25	26.3
Panicle weight	3.26	7.56	6.52	5.52	4.31	3.56	5.26	7.14	6.54	5.54	5.23	5.63
Grains per panicle	168.22	155.67	173.46	169.67	89.33	119.33	204.67	122	190.83	147.67	173.33	196.56
1000 grain weight	8.44	17.77	15.3	23.94	21.06	14.94	22.56	7.13	15.63	22.99	8.81	21.42
Seed yield per plant	23.82	27.38	16.11	29.13	23.74	22.4	31.3	20.2	18.21	21.55	32.07	17.59

Table 4: Relative contribution of yield and yield contributing characters to genetic diversity

S. No	Characters	Times ranked first	contribution (%)
1	Das to 50% flowering	72	5.87%
2	Plant height (cm)	315	25.71%
3	No. of effective tillers per plant	5	0.40%
4	Panicle length (cm)	0	0.02%
5	Panicle weight (g)	0	0.01%
6	1000 grain weight (g)	461	37.63%
7	No. of grains per panicle	144	11.75%
8	Seed yield per plant (g)	228	18.61%

Table 5: Eigen values, contribution of variability and factor loading for the principal component axis .

	PC 1	PC 2	PC 3
Eigenvalue	6.222	4.577	1.9
Variability (%)	40.823	30.032	12.467
Cumulative %	40.823	70.856	83.323
Days to 50% flowering	0.114	0.092	0.144
Productive tillers	0.008	0.113	-0.028
Plant height (cm)	0.814	0.444	-0.326
Panicle length (cm)	-0.001	-0.083	-0.065
Panicle weight (gm)	0.042	-0.023	0.091
No of filled grains per panicle	0.178	0.117	0.209
1000 Seed weight	0.494	-0.86	0.025
Yield per plant	0.219	-0.139	0.907

cluster XII (12.79), panicle length was highest in cluster IX (27.80) and lowest in cluster I (21.91), 1000 grain weight recorded highest in cluster IV (23.94) and lowest in cluster VIII (7.13), no. of grains per panicle was found to be highest in cluster VII (204.67) and lowest in cluster V (89.33), seed yield per plant was recorded was highest in cluster XI (32.07) and lowest in cluster III (16.11).

The average intra and inter cluster D^2 values are presented in (Table 2). Intra cluster D^2 values are minimum (0.00) in clusters , IV and V, VI, VII, VIII, X, XI as these were monogenotypic clusters and genotypes in these clusters were more divergent and they could be utilized as parents for hybridization. Intra cluster distance observed in cluster I is (10.23), cluster II (18.67), cluster III (22.16), cluster IX (22.20), cluster XII (25.36) revealing that some genetic divergence still existed among the genotypes of the cluster. Similar results were seen in findings of Tripathi *et al*. (2017). Selection in these clusters might be executed based on maximum mean value for the desirable characters. The intra cluster distance was lower than inter cluster distance (Table 2), indicating the existence of genetic diversity among the genotypes under study.

Maximum inter cluster distance was exhibited between clusters VIII and IX (227.03) followed by clusters I and IX (199.86), clusters VII and VIII (190.65), clusters V and IX (187.34) and cluster VII and IX (180.52). The lowest inter cluster divergence was observed between clusters IV and VII (11.01) followed by clusters IV and X (16.12), clusters I and VIII (21.28) describing that the genotypes included in these clusters were closely related. The genotypes grouped into same cluster displayed the lowest degree of divergence as over genotypes from different clusters ,which indicates transgressive segregation is possible in the later case. Therefore, hybridization programmes should always be formulated in such a way that the parents belonging to different clusters with maximum divergence could be utilized. Ahamed *et al*. (2018) studied genetic divergence in 50 rice germplasm lines and found that the inter cluster distance in most cases was larger than intra cluster distance suggesting wider diversity among the germplasm of different groups.

The purpose of the PCA is to obtain a small number of factors which account for maximum variability out of the total variability. Eigen vector values, percentage of variance and the cumulative percentage are presented in (Table 5). In this case, PCA revealed three most informative principal components with Eigen values of 6.222, 4.577 and 1.900, respectively, which together accounted 83.323 % of the total variance for all the characters 3 components had Eigen values greater than 1.0. Percentage of variance for the three factors

was 40.823, 30.032, and 12.467 per cent. Together they accounted for 83.32 % of the variability of the genotypes used for the diversity analysis. According to principal component 1, characters such as plant height (0.814), 1000 seed weight (0.494) and seed yield per plant (0.219) had relatively higher contributions (40.823%) to the total morphological variability, while the second principal component accounted for 30.032% of the total variation with plant height (0.444) giving the highest contribution. Maji, A.T and Shaibu AA. (2012) carried out P.C.A with one hundred and twenty three rice germplasm in which first two components accounting for 78% of the total variation. The first three principal components contributed 74.20% of the total variation when 52 genotypes were analyzed with P.C.A by Enyew *et al*. (2019).

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