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Genetic divergence assessment in aromatic rice (*Oryza* sativa L.)

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Abstract

The genetic divergence study was conducted to estimate the nature and magnitude of diversity in thirty aromatic rice accessions including fifteen improved varieties during *kharif* season, 2019 & 2020. The divergence analysis including Tocher's, canonical (vector) and Euclidian methods indicated the presence of appreciable amount of genetic diversity in the material. The thirty aromatic rice genotypes were grouped into six clusters by both Tocher's and Euclidian methods of divergence study. But the clusters of both methods were different on the basis of the genotypes and their numbers present in the cluster. The genetic parameters of all the characters have also been studied. The result obtained from different quantitative and qualitative characters have been drawn from the all three methods of divergence study. The most diverse parent combination for the component characters of grain yield have been identified on the basis of 3D diagram of PCA scores and Euclidian distance matrix, which were JGL 15336 x Tarori Basmati for effective tillers per plant, Badsabhog x Rajendra Kasturi for filled grain per panicle, JGL 15336 x Birsamati for grain weight per panicle and Gandhasala x Pusa Sugandha-3 for Leaf area index. These genotypes were found most suitable genotypes for the respective characters and can be used as potential donor for future breeding programmes.

Keywords: Aromatic rice, genetic divergence, cluster analysis

Introduction

India in well known for its native wealth of rice genetic resources and among these the large number of aromatic varieties cultivated in different agro-climatic regions of country. As per the growing demand of aromatic rice, the emphasis should be given to the development of fine grain aromatic rice for their outstanding quality traits like aroma, kernel elongation after cooking, fluffiness and taste. Genetic diversity is pre requisite for any crop improvement programme, as it helps in the development of superior recombinants (Naik, et.al. 2006) ^[11]. Genetic divergences among the genotypes play an important role in selection of parents having wider variability for different characters. Statistical analysis quantifies the genetically distance among the selected genotype and reflects the relative contribution of specific traits towards the total divergence. The crosses between parents with suitable genetic divergence are generally the most responsive for yielding the most promising segregants. The present study was, therefore, undertaken to assess the extent of genetic diversity in 30 aromatic rice genotypes which will help to select prospective parents to develop transgressive segregants.

Material and Methods

The present investigation was carried out in *kharif* 2019 and *kharif* 2020 in the Rice research field of Dr Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Geographically Pusa is located at 25.29° N latitude and 85.40° E longitude and 51.8 meters above the mean sea level. The relative humidity during crop season ranged from 85 to 94 per cent. The investigation was carried out in Randomized block design (RBD) with three replication and plot size of 1.80 m^2 (0.6 m x 3m) with spacing of 20cm and 15cm between rows and between plants, respectively. The experimental materials consist of thirty aromatic rice genotypes obtained from different parts of the country. i.e. form S.K. University of Agricultural Science and Technology, Jammu, Central Rice institute, Cuttack, Directorate of Rice Research , Hyderabad, and Rajendra Agricultural University, Pusa Samastipur. The observations were recorded for yield and yield attributing characters and quality characters of aromatic rices under study.

To study the genetic divergence among the genotype used in the present investigation and to know the fluctuation in clustering pattern of those genotypes, the D² values were calculated by using the method described by Mahalanobis (1936) ^[10]. Genetic divergence analysis using canonical (Vector) method is a sort of multivariate analysis where canonical vectors and roots representing different axes of differentiation and the amount of variation accounted for by each of such axes, respectively, were derived (Rao 1952) ^[14]. Nonhierarchical Euclidean cluster analysis (Beale, 1969, Katyal *et al.*, 1985) ^[3, 8] was conducted using computer package (Windostat version 8.5).

Result and Discussion

Knowledge of genetic divergence in any crop is essential for selecting the useful parent for hybridization, so, that maximum heterosis could be utilized and useful segregants could be selected from transgressive, breeding programme. More is the parental diversity greater will be the success expected from any hybridization programme (Joshi and Dhawan 1966; Ananda and Murty 1968) ^[7, 1]. In crop like aromatic rice D² study were conducted by Pradhan and Mani (2005)^[13], Singh *et al.* (1996) ^[19], Zaman *et. al.* (2005) ^[20], Sharma *et. al.* (2002) ^[18], Sharma *at. el.* (2008) ^[17], Chaudhary and Sarawgi (2002)^[4], Naik et. al. (2006)^[11], Roy et. al. (2002) ^[16], Nayak et. al. (2004) ^[12], Gupta et. al. (1999) ^[5], Ravindra et .al. (2006) [15], Awasthi et al. (2005) [2]. On the basis of results obtained from the present study high degree of genetic divergence was observed. It is evident as more number of cluster (six) formed by the 30 aromatic rice genotypes and high rang of values of inter and intra cluster distance. The more diversity of parents, the greater chance of obtaining high heterosis (Zaman et. al. 2005)^[20]. The pattern of distribution of genotypes within various clusters was random and independent of geographical isolation (Sharma et. al. 2002) ^[18]. So there is no association between the geographical distribution and genetic diversity (Sharma et. al. 2008) ^[17]. Amongst six clusters formed by Tocher's method (Fig. 1), cluster I was largest (with 15 genotypes) and cluster III, IV, V and VI were smallest with one genotype only (Table 1). The maximum inter cluster distance was observed between cluster II and cluster III. Due to maximum inter cluster distance between them (Table 2), exhibited high degree of genetic diversity and thus may be utilized under inter varietal hybridization programme.

The result obtained from different methods of divergence study was slightly different from each other. As per the Tocher's method (Table 3) highest values for tall plant height (155.06), Spiklets/panicle (215.75), filled grain/panicle, grain wt./panicle, days to 50% flowering, days to maturity, head rice recovery and elongation ratio was observed for cluster II. The highest cluster mean value for panicle length, grain length, kernel L/B ratio, alkalie digestion value and test weight of seed was observed for cluster IV. The highest cluster mean value for effective tillers/plant, kernel length and kernel length after cooking was observed for cluster III. The highest cluster mean value for leaf area index, harvest index, grain yield/plot, and grain yield per plant was observed for cluster V. Whereas, the highest cluster mean of specific leaf weight was observed for cluster VI.

Although D2 statistics using Tocher method for classifying the genotypes is useful in general but non-hierarchical Euclidian cluster analysis (based on Wards minimum variance dendrogram) Fig. 1 more critically identifies sub clusters of the major groups at different levels and offers additional opportunity than that of Tocher method to plant breeders in planning of hybridization programme aimed at crop improvement.

The 30 aromatic rice genotypes were subjected to nonhierarchical Euclidian cluster analysis using the computer software (Windostat version 8.5) divided the genotypes in six clusters (Fig. 1). Thus overcoming the limitations of Tocher method. Maximum number of genotypes i.e. 11 grouped in cluster D and minimum i.e. 2 genotypes in each cluster B and C. whereas cluster E has only one genotype. The clusters D, A, F, B, C and E comprised of 11, 9, 5, 2, 2 and 1 genotypes, respectively (Table 1). The relative association among the different genotypes is presented in the form of Wards Minimum Variance Dendrogram which was prepared using the rescaled distance. The resemblance coefficient between two genotypes is the value at which their branches join. The dendrogram elaborate the relative magnitude of resemblance among the genotypes as well as the clusters. It is clear from the perusal of wards minimum variance dendrogram that "fence sitter" single genotype, grouped by Tocher method in cluster III (Tarori Basmati) and cluster IV (RAU 3055) were precisely accommodated in cluster F exhibiting more similarity (less variance) between Tarori Basmati and RAU 3055 similarly, single genotype, grouped by Tocher method in cluster V (Birsamati) was accommodated in cluster B with NDR 9542 exhibited similarity between them.

Geographical and genetic diversity exhibited no correspondence between them as genotypes from one and different geographic reason are grouped together, which might be due to free exchange of genetic material from different regions. On the basis of Euclidian method the highest inter cluster distance was recorded between C and F with 2 and 5 genotypes, respectively, whereas minimum between D and F with 11and 5 genotypes, respectively. The genotypes with high order of divergence were found in clusters C and F followed by C and E, C and D, B and E, A and F, B and C, B and F, E and F, A and E.

On the basis of cluster mean values, maximum divergence for plant height was exhibited by cluster A; earliness for days to 50% flowering and days to maturity in cluster D; effective tillers/plant, leaf area index, specific leaf weight in cluster E; grain weight/panicle, harvest index, alkalie digestion value, grain yield per plot in cluster B; spikelets/panicle, filled grain /panicle, grain yield/plant, head rice recovery, elongation ratio in cluster C; and maximum divergence for six characters namely panicle length, grain length, kernel L/B ratio, test weight of seed, kernel length and kernel length after cooking were exhibited by genotypes in cluster F (Table 3).

Principal factors were carried out using principal component (PC) method for factor extraction. Differentiation among populations occurs in stages, or in other words in different axes of differentiation which accounts for total divergence. Theoretically as many as axes of differentiation can be envisaged as there are characters contributing to total variation, but it is not absolutely. It is possible that most of the variation is accounted for by the first two or more axes of differentiation. In the present investigation only the first three principal components showed eigen values more than one and cumulatively they explained 64.233% variability (Table 4). The first principal component explained 37.881 per cent of the total variation and the second and third principal

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components explained 14.646 per cent and 11.706 per cent variation, respectively. The first principal component (λ 1) absorbed and accounted for maximum (37.881%) proportion of variability and remaining once accounted for progressively lesser and lesser amount of variation (14.646, 11.706, 7.649, 6.991, 5.403 and 4.337) for $\lambda 2$, $\lambda 3$, $\lambda 4$, $\lambda 5$, $\lambda 6$ and $\lambda 7$, respectively. The study through canonical analysis revealed that on the basis of two axes the cumulative percentage of variation absorbed by $\lambda 1 + \lambda 2$ was 52.527% where as there are three effective axes Z1, Z2 and Z3 where $\lambda 1 + \lambda 2 + \lambda 3 =$ 64.233%. In Z1 grain length with element value -0.326; in Z2 days to maturity and leaf area index with element value 0.399 and -0.394 respectively and in Z3 filled grain per panicle with element value -0.385, contributed maximum to the total divergence at primary, secondary and tertiary axes of differentiation based on canonical vectors Z1, Z2 and Z3, respectively. Jagadev et.al. (1991) ^[6] reported that the character contributing maximum to the divergence should be given greater emphasis for deciding the type of cluster for purpose of further selection and the choice of parents for hybridization.

Genetic divergence between genotypes is measured in terms of spatial distance and resulted in formation of three dimensional (3D) based on three PCA scores (λ 1, λ 2 and λ 3 graphs) as depicted in Fig.2. Three principal factors scores were used to plot all the 30 aromatic rice genotypes using PCA1, PCA2 and PCA3 i.e. 3D plot which accounted for most important component traits namely grain length, days to maturity and filled grain per panicle. Similar type of study was also carried out by Khalequzzaman *et. al.* (2005)^[9], who reported 2D diagram using two PCA scores reflecting the relative position of the genotypes and distributed the genotypes in six clusters.

Amongst 30 aromatic rice genotypes, studied in the present study, exhibited great extent of genetic diversity on the basis of 3D diagram based on PCA scores and Euclidian distance matrix, which reflected highest diversity between RAU 3055 and Badsabhog, while minimum genetic diversity between HUR-ASG-GN and Jeerakasala.



Fig 1: Dendrogram showing clusters formed by Tocher's method and Euclidian method.



Table 1: Clustering pattern of 30 genotypes of aromatic rices on the basis of D2 statistics (Tocher's Method and Euclidian method).

		Tocher's Method	Euclidian method				
Cluster	No. of genotypes	Name of genotypes	No. of genotypes	Name of genotypes			
ΙA	15	Basmati 385(9), Ranvir Basmati(10), Basmati 370(8), Kasturi(2), Sanwal Basmati(11), Rajendra Suwasni(25), RR 564(12), RAU 3036(23), KJT 4-4-36(20), NDR 8018(16), Jasmaine(4), CR 2603(18), Pusa Sugandha-3(3), PAU 3025-50(29) and Narendra Lalmati(14)	9	HUR-ASG-GN, RNR 2465-1, Jeerakasala, Gandhasala, Narendra Lalmati, NDR 6242, NDR 6235, Kalanamak, HUR-ASG-KN			
II B	11	Badsabhog(27), JGL 15336(30), RNR 2465-1(21), HUR-ASG-KN(1), Gandhasala(7), Jeerakasala(6), HUR-ASG-GN(19), NDR 6235(15), NDR 6242(17), NDR 9542(28) and Kalanamak(26)	2	Birsamati(, NDR 9542			
III C	1	Tarori Basmati(13)	2	Badsabhog, JGL 15336			
IV D	1	RAU 3055(22)	11	Kasturi, Rajendra Suwasni, RR 564, Sanwal Basmati, NDR 8018, KJT 4-4- 36, CR 2603, Basmati 385, Ranvir Basmati, Basmati 370, RAU 3036			
V E	1	Birsamati(5)	1	Rajendra Kasturi			
VI F	1	Rajendra Kasturi(24)	5	Jasmaine, PAU 3025-50, Pusa Sugandha-3, Tarori Basmati.			

The Pharma Innovation Journal

 Table 2: Mean inter and intra cluster distance among six clusters in aromatic rice on the basis of D2 statistics (Tocher's Method and Euclidian method).

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI
	Cluster A	Cluster B	Cluster C	Cluster D	Cluster E	Cluster F
Cluster I	265.58	1312.57	746.07	792.15	815.15	1093.91
Cluster A	1332.87	2980.52	2781.18	2513.37	4253.48	5777.23
Cluster II		577.90	3012.44	2992.60	1434.30	1680.18
Cluster B		1643.68	4863.78	2751.15	5811.05	4778.41
Cluster III			0.00	504.52	1402.08	1364.47
Cluster C			297.54	6973.65	7242.19	11965.76
Cluster IV				0.00	872.90	2567.38
Cluster D				556.77	3142.13	1542.84
Cluster V					0.00	2086.49
Cluster E					0.00	4755.19
Cluster VI						0.00
Cluster F						971.88

 Table 4: Canonical vectors which supply best linear function of variates, value of canonical roots and percentage of variation absorbed by respective roots.

	1 Vector	2 Vector	3 Vector	4 Vector	5 Vector	6 Vector	7 Vector
Eigene Value (Root)	7.955	3.076	2.458	1.606	1.468	1.135	0.911
% Var. Exp.	37.881	14.646	11.706	7.649	6.991	5.403	4.337
Cum. Var. Exp.	37.881	52.527	64.233	71.882	78.872	84.275	88.612
Plant Height cm	0.147	0.140	0.351	0.313	0.103	0.218	0.411
Panicle Length cm	-0.209	0.059	0.124	0.199	-0.422	0.104	-0.026
Effective Tillers/ plant	0.045	0.102	-0.349	0.330	0.238	0.454	0.113
Spiklets/ Panicle	0.311	0.013	0.048	-0.241	-0.034	0.072	0.297
Filled Grain/ Panicle	0.134	-0.101	-0.385	-0.226	-0.419	-0.227	-0.039
Grain Weight/ Panicle	0.201	-0.258	-0.094	0.359	-0.316	-0.017	0.045
Leaf Area Index	-0.051	-0.394	-0.113	-0.291	0.003	0.494	0.017
Specific leaf weight	-0.041	-0.325	0.196	0.114	0.362	-0.482	0.059
Grain Length	-0.326	-0.106	-0.096	0.130	-0.101	0.053	0.041
Kernal L/B ratio	-0.233	0.107	-0.112	-0.248	-0.139	-0.225	0.466
Harvest Index	0.234	-0.343	0.020	0.072	0.036	0.081	-0.123
Alkali digestion value	-0.070	0.244	0.321	-0.138	-0.274	0.168	-0.450
grain yield / plot gm	-0.300	0.055	0.001	0.166	-0.263	-0.013	0.222
grain yield/plant gm	0.276	-0.005	-0.192	0.250	-0.175	-0.239	0.127
100 seeds weight gm	-0.323	-0.029	0.104	0.170	-0.132	0.046	0.092
Days to 50% flower	-0.218	-0.163	0.290	-0.293	0.057	0.069	0.276
Days to maturity	-0.121	0.399	-0.048	0.045	0.227	-0.200	-0.158
Head rice recovery %	0.269	0.315	0.128	0.094	-0.090	-0.007	-0.016
Kernal length	-0.277	0.025	-0.260	0.175	0.090	-0.016	0.073
Cooked kernal length	-0.227	0.024	-0.354	0.007	0.226	0.023	-0.212
Elongation ratio	0.139	0.372	-0.246	-0.263	0.038	0.100	0.246



Fig 2: Three dimensional representation of genotypes using 3 principal component based on canonical variates. \sim 4548 \sim

Table 3: Mean value of six clusters for 21 characters in aromatic rice based on Tocher's	and Euclidian cluster analysis.
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	Plant Height cm	Panicle Length cm	Effective Tillers/ plant	Spiklets/ Panicle	Filled Grain/ Panicle	Grain Weight/ Panicle	Leaf Area Index	Specific leaf weight	Grain Length	Kernal L/B ratio	Harvest Index	Alkali digestion value	Grain yield / plot gm	Grain yield/ plant gm	100 seeds weight gm	Days to 50% flowering	Days to maturity	Head rice recovery %	Kernal length mm	Cooked kernal length mm	Elongation ratio
Cluster I	126.556	26.373	15.556	150.556	83.889	1.415	4.467	0.007	10.332	4.341	22.752	2.933	494.525	19.316	1.800	109.911	131.400	58.781	7.741	9.228	1.189
Cluster A	157.222	26.285	13.074	198.111	127.889	1.940	3.798	0.009	7.794	3.043	23.956	3.333	539.774	23.817	1.581	117.407	139.815	62.485	5.879	7.559	1.291
Cluster II	155.061	24.939	12.576	215.758	142.818	1.964	5.258	0.008	7.226	2.850	25.850	3.576	521.700	24.067	1.445	120.212	142.636	62.869	5.479	7.350	1.354
Cluster B	109.333	24.600	15.333	185.000	107.167	2.046	11.630	0.005	9.025	3.407	32.013	6.000	643.000	28.872	1.703	118.333	140.833	60.437	6.463	7.900	1.223
Cluster III	144.667	25.833	26.333	105.000	56.667	0.918	10.209	0.005	11.250	4.863	22.303	2.667	458.287	19.503	1.781	114.333	137.667	55.643	9.647	10.643	1.100
Cluster C	153.333	20.183	16.000	289.833	227.333	1.790	8.038	0.006	5.652	2.408	28.312	2.500	409.702	29.900	1.013	121.167	142.333	63.235	4.270	6.782	1.582
Cluster iv	116.667	26.933	6.667	132.667	75.667	1.615	9.267	0.008	13.297	5.893	20.933	6.333	377.333	10.000	2.005	110.667	130.333	52.620	8.657	9.640	1.107
Cluster D	127.242	26.161	14.061	153.000	82.576	1.417	4.394	0.007	10.026	4.265	22.853	2.939	473.764	16.905	1.745	110.242	131.727	59.288	7.596	9.270	1.215
Cluster V	98.000	24.867	15.000	191.333	110.667	1.947	12.986	0.004	10.450	4.260	30.367	5.333	636.333	27.387	1.800	113.667	136.667	58.067	7.050	8.423	1.193
Cluster E	112.667	20.633	17.667	213.333	133.667	1.190	2.830	0.012	5.940	2.503	27.907	2.667	470.667	22.160	0.818	116.000	140.667	62.350	8.480	9.400	1.103
Cluster vi	112.667	20.633	17.667	213.333	133.667	1.190	2.830	0.012	5.940	2.503	27.907	2.667	470.667	22.160	0.818	116.000	140.667	62.350	8.480	9.400	1.103
Cluster F	124.667	26.733	16.933	129.000	68.800	1.317	7.092	0.006	11.934	5.109	22.319	3.667	490.752	18.703	1.926	112.267	134.200	55.545	8.763	9.725	1.106

Conclusion

Superior diverse genotypes were selected based on three methods of divergence study and mean performance for important component characters of yield. These are JGL 15336, Jasmaine, Pusa Sugandha-3, Sanwal Basmati, N. Lalmati, NDR 8018 and Tarori Basmati for Effective tillers per plant; Badsabhog, NDR 6235, JGL 15336, Rajendra Kasturi and RAU 3036 for Filled grain per panicle; JGL 15336, Birsamati, NDR6235, KJT 4-4-36 and CR 2603 for grain weight per panicle; RNR 2465-1, Birsamati, S. Basmati, Rajendra Suwasani, Pusa Sugandha-3, Gandhasala, Narendra Lalmati, NDR 6242 and Kalanamak for leaf area index; and JGL 15336, NDR 6235, Birsamati and Sanwal Basmati for harvest Index.

Based on 3D diagram of PCA scores and Euclidian distance matrix cross combinations were formed for important component characters of yield involving suitable parents which were also found diverse for the particular characters. Those were JGL 15336 X Tarori Basmati for Effective tillers per plant, Badsabhog X Rajendra Kasturi for Filled Grain per Panicle, JGL 15336 X Birsamati for Grain Weight per Panicle (gm), Gandhasala X Pusa Sugandha-3 for Leaf Area Index (cm²) and JGL 15336 X Sanwal Basmati for Harvest Index (%). These cross combinations can be recommended for making further research strategies and breeding programmes.

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