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Genetic divergence and cluster analysis for yield and yield contributing traits in rice (*Oryza sativa* L.) genotypes

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Abstract
To increase crop production and productivity, genetic improvement is essential, which necessitates knowledge of genetic divergence and cluster distance between genotypes. The study was carried out during the primary cropping season of 2019. Incorporating augmented design was used to plan the experiment. 13 agronomic characters' worth of data were collected, and analysis of variance revealed significant variations between genotypes for every character. Eight divergent groups were identified by cluster analysis, with clusters II and V having the greatest inter-cluster distance (D^2=4.410) and clusters VI and VIII having the smallest inter-cluster distance (D^2=2.394). To obtain genotypes with high grain yield and early maturation, it is advised to cross genotypes from clusters II and V, V and VI. Parental material selection for upcoming breeding programmes that use hybridization should be done between clusters rather than within clusters.

Keywords: Cluster distance, genetic divergence, *Oryza sativa* (rice)

Introduction
One of the world's most important food crops is rice. Its genus and family are Poaceae and Oryza, respectively (Wang *et al*., 2014). In the genus Oryza, there are two domesticated species-Asian rice (*O. sativa*, 2n=24=AA) and African rice (*O. glaberrima*, 2n=24=AA]—as well as 22 wild species (2n=24, 48). (Singh *et al*., 2015) [2]. China's Yangtze and Mekon River valleys may be the primary source of *Oriza sativa's* ancestry (Zhao 2011; Gross and Zhao, 2014). Western tropical Africa is where *Oryza glaberrima* is grown; it is native to the upper valley of the Niger River (Ansari *et al.*, 2015) [2]. In comparison to *O. rufipogon*, rice grown for human consumption (*O. sativa*) mostly self-pollinates and has a lower capacity for out crossing. Cross-pollination rates of *O. sativa* are less than 1%, according to Messeguer *et al*. (2001) [11]. However, the estimated out-crossing rates in populations of wild rice range from 4.3% to 55.9%. (Oka, 1988) [12].

More than half of the world's population relies on rice as a staple food, making it the second most produced cereal after wheat (Luz *et al*., 2016). Asia is where rice is primarily grown and consumed worldwide (Chakravarthi and Naravaneni, 2006) [5]. Although Asia is the primary region for rice cultivation, rice is also grown in Latin America, Europe, the United States, and some regions of Africa (Zibaee, 2013). Asia produces the most of the total amount produced, totaling about 144.25 million tonnes, while Africa produces about 11.58 million tonnes (FAO, 2015) [6].

Rice is a well-established crop in many nations, and cultivars that are well-suited to regional conditions and the local market have been chosen. There are thought to be 120,000 different types of rice in existence worldwide (Sassaki and Moore, 1997).

A universal characteristic of all species in nature, the level of genetic variability in the population determines how much genetic improvement is possible (Dutta and Burua, 2013). The primary factor to be taken into consideration when making selections is the variability in genotypes for yield and yield component traits (Haydar *et al*., 2007) [8]. The character yield can be seen as the culmination of many other traits and reflects the performance of all plant parts. In other words, every plant has an innate physiological production capacity that uses the energy necessary for normal plant function. Not every genotype has the same physiological capacity to produce naturally (Welsh, 1981).

The ability to identify parental lines for hybridization programmes is aided by knowledge of genetic diversity and distance among groups of genotypes. The goal of the current study is to use cluster analysis to assess the degree of genetic divergence among lowland rice genotypes.

Materials and Methods

Plant Material and Experimental Design

The material for the present investigation consists of 250 genotypes and 5 checks. These were grown in a Augmented design during wet season 2019 at CRS (Crop Research Station), Masodha, Achraya Narandra Deva University of Agriculture and Technology, Ayodhya, U.P., India. The entries were direct seeded with ten rows per entry having 25 hills per row with 20 x 15 cm spacing. The nursery was sown on 01 July, 2019 on uniform raised beds applied with a fertilizer dose of N:P:K, 80:40:40. 25-30 days old seedlings were transplanted in main research plot with one seedling hill^{-1}.

Data collection

Observations on quantitative traits like days to 50% flowering, seedling vigor, plant height, flag leaf area, panicle bearing tillers plant^{-1}, panicle length, no. of spikelet panicle^{-1}, no. of grains panicle^{-1}, spikelet fertility, biological yield plant^{-1}, harvest index, test weight, grain yield plant^{-1} were recorded on five randomly selected plants excluding the border rows from each entry while days to 50% flowering and plot yield were recorded on plot basis. The recommended agronomic practices were followed to raise a good and healthy crop.

Statistical Analysis

A measure of a group distance based on multiple characters was given by generalized Mahalanobis D^2 statistics (Mahalanobis, 1936) [10] for 13 quantitative characters and was analyzed using the procedure Procdiscrim of SAS Software. Squared distance (D^2) for each pair of genotype combinations was computed using the following formula:

$D^2p = ((Xi – Xj)\ S\ –1(Xi- Xj)$ Where, D^2p= the squared distance between any two genotypes i and j; Xi and Xj = the p mean vectors of genotypes i and j, respectively. S-1 = the inverse of the pooled covariance matrix.

Testing the significance of the squared distance values obtained for a pair of clusters was taken as the calculated value of $\chi 2$(chi-square) and tested against the tabulated $\chi 2$ values at p-1 degree of freedom at 5% and 1% probability level, where p = number of traits used for clustering the genotypes.

The average inter cluster distances were calculated by the formula given by Singh and Chaudhary (2005).

Square of the inter cluster distance = $\Sigma D2\ i/ninj$ Where, $\Sigma D2\ i$ is the sum of distances between all possible combinations (ninj) of the genotypes included in the clusters under study. ni is number of genotypes in cluster i and nj is number of genotypes in cluster j.

Cluster analysis based on Average's method was performed using MINITAB 17 statistical packages (Minitab, 1998) to cluster the genotypes based on their agronomic traits.

Table 1: List of rice genotypes used for this study

Code No.	Genotypes	Code No.	Genotypes	Code No.	Genotypes
1	IR18A1533	86	IR15F1706	171	IR 117677-319-1-2-1
2	IR18A1362	87	IR18A1185	172	IR17A3032
3	IR18A1838	88	IR18A1070	173	IR18A1703
4	IR18A2000	89	IR15F1745	174	IR17A3040
5	IR18A1653	90	IR18A1126	175	IR18A1789
6	IR17A2891	91	IR18A1482	176	IR17A3038
7	IR18A1115	92	IR18A1963	177	IR18A1358
8	IR18A1989	93	FR 13 A	178	BPT 5204
9	IR17A2772	94	IR18A2043	179	IR18A1347
10	IR18A2044	95	IR 126952-28-55-9-3-19-1-5	180	IR18A1058
11	IR18A1145	96	SAHBAGIDHAN	181	IR18A1809
12	IR18A1381	97	IR18A1040	182	IR18L1163
13	IR18T1172	98	IR18A1027	183	IR18A1317
14	IR17A3016	99	IR18A1122	184	IR18A1269
15	IR18A1973	100	IR17A2796	185	IR15F1754
16	IR18A1944	101	IR17A3012	186	IR18A1768
17	IR17A3019	102	IR17A3030	187	IR18A1150
18	IR 126952-29-65-16-2-2-B	103	IR 126952-28-55-332-2-18-8-B	188	IR15F1886
19	IR18T1135	104	IR18T1340	189	IR18A1287
20	IR18A2040	105	IR18A1918	190	IR18A1156
21	IR18A1243	106	IR18A1468	191	IR17A3065
22	IR18A2129	107	IR17A3086	192	IR18A1325
23	IR18A1442	108	IR 64	193	IR18A1786
24	IR17A2854	109	IR17A2792	194	IR18A1896
25	IR17A3028	110	IR18A1558	195	IR18A2038
26	IR18A1650	111	IR18A1914	196	IR18L1140
27	IR18A1935	112	IR18A2139	197	IR18A1658
28	IR18A2109	113	IR17A3033	198	IR15F1710
29	IR18A1427	114	IR18A2134	199	IR18A1565
30	IR18A1491	115	IR17A2771	200	IR15T1330
31	IR18A1876	116	IR17A2969	201	IR18A1480
32	IR18A1082	117	IR17A2832	202	IR18L1171
33	IR18A2011	118	IR17A3083	203	IR18A1971

34	IR18A1925	119	IR15F1907	204	IR16F1065
35	IR18A1195	120	IRRI 123	205	IR18A1776
36	IRRI 154	121	IR18A1807	206	IR18A1791
37	IR17A2952	122	IR18A1430	207	IR17A2942
38	IR18A1355	123	IR18A1926	208	IR16F1201
39	IR18A1911	124	IR17A2913	209	IR18A1281
40	IR18A1293	125	IR16A4261	210	IR17A3041
41	IR 64	126	IR17A2949	211	Sarjoo-52
42	IR 87959-6-2-3-1-2-BAY B-CMU 1	127	IR17A3075	212	IR18A1022
43	IR18A1231	128	IR17A3101	213	IR17A2799
44	IR18A1057	129	IR14F690	214	IR18A1742
45	IR18A2022	130	IR18A1986	215	IR16F1097
46	IRRI 104	131	IR17A3044	216	IR17A3047
47	IR17A3123	132	IR18A1042	217	IR18A1051
48	IR18A1329	133	IR17A2845	218	IR17A2921
49	IR18A1651	134	IR18A1611	219	IRRI 156
50	IR18A1711	135	IR17A2769	220	IR18A1451
51	IR18A1335	136	IR18A1715	221	IR18A2005
52	IR15F1709	137	IR18A2066	222	IR17A2947
53	IR18A2058	138	IR17A2977	223	IR17A2855
54	IR18A1411	139	IR18A1564	224	IR18A1133
55	IR18A1423	140	IR18A1212	225	IR18A2037
56	IR 126952:173-AC 16-1-B	141	IR18A1967	226	IR18A1072
57	IR18T1192	142	IR18A2041	227	IR17A2906
58	IR18A1559	143	IR18T1248	228	IR17A3003
59	IR18A1877	144	IR18A1026	229	IR17A3050
60	IR18A1020	145	IR18A1771	230	IR17A2808
61	IR18A1069	146	IR17A2831	231	IR18A1671
62	IR18A2130	147	IR17A2839	232	IR18A1440
63	IR17A3093	148	IR18A1371	233	IR18A1726
64	IR17A3046	149	IR16F1243	234	IR15F1764
65	IR18T1275	150	IR18A1732	235	IR17A3036
66	IR14T156	151	IR15F1869	236	IR18A1507
67	IR17A3137	152	IR16F1021	237	IR18L1127
68	IR18A1135	153	IR17A3091	238	IR18A2036
69	IR06M139	154	IR17A2943	239	IR18A1190
70	IR18A1068	155	IR18A1665	240	IR18A1256
71	IR 96321-315-294-B-1-1-1-8-B	156	IR18A1076	241	IR18A1090
72	IR18A1884	157	IR18A1474	242	IR 126952-29-27-265-1-53-B
73	IR18A1130	158	IR18A1690	243	IR18A1607
74	IR18A1197	159	IR18A1987	244	IR17A2985
75	IR18A1155	160	IR18A1517	245	IR18A1252
76	IRRI 119	161	IR18A1383	246	IR18A1955
77	IR17A2990	162	IR18A1579	247	IR18A2001
78	IR17A2907	163	IR14T136	248	IR18A1794
79	IR 121151-307-1-1-1-1	164	IR18A1866	249	IR18A1567
80	IR18A1745	165	IR18A1322	250	IR18A1073
81	IR18A1061	166	IR17A2923	251	IRRI 168
82	IR18A1777	167	IR18A2013	252	IR 42
83	IR18A1016	168	IR18A1811	253	IRRI 148
84	IR17A2801	169	IR18A1110	254	IRRI 174
85	IR17A3037	170	IR17A3105	255	NDR 2065

Results and Discussion

Genetic Divergence Analysis

Clustering of genotypes

The 255 rice genotypes exhibited significant differences for 13 characters. The presence of significant differences among genotypes for all the characters justified further calculation of D2 (Sharma, 1998). The D^2 values were based on the mean of genotypes; cluster VII was the largest cluster which consisted of 42 genotypes (16.47%) followed by Cluster I and II which comprised of 35 genotypes (13.72%), cluster III had 33 (12.94%) genotypes and cluster IV and V had 28 genotypes (10.98), while Cluster VI and VIII had the lowest number of genotypes that comprises only 27 genotypes (10.58%).

Table 2: Clustering pattern of 255 genotypes (including 5 Checks) on the basis of Mahalanobis D^2 statistics of rice germplasm

Clusters	No of genotypes	Genotypes
I	35	7, 11, 17, 35, 36, 37, 38, 40, 45, 47, 51, 65, 67, 70, 73, 100, 102, 108, 117, 120, 127, 128, 133, 138, 141, 147, 150, 169, 176, 179, 189, 203, 216, 217, 223
II	35	2 3 5 8 12 13 14 16 19 24 25 27 31 32 68 74 78 80 83 88 92 94 95 109 157 158 165 168 172 175 181 206 229 252 253
III	33	10 22 29 30 33 41 42 44 46 59 60 61 63 81 84 101 103 113 118 135 146 153 155 167 170 180 214 220 226 235 241 246 251
IV	28	4 21 57 75 87 114 140 143 156 160 174 177 184 196 207 208 210 213 219 221 231 232 233 236 239 242 250 254
V	28	48 49 52 54 56 62 66 71 90 106 111 115 116 119 121 122 123 124 131 132 136 144 145 149 151 154 205 228
VI	27	1 9 15 20 26 53 58 72 76 79 82 86 89 91 93 96 97 98 161 164 186 190 191 195 204 237 248
VII	42	6 28 39 43 55 64 104 105 107 110 112 125 126 134 137 142 152 159 163 173 183 185 188 192 194 197 198 199 201 202 209 211 212 215 222 224 225 234 240 245 247 255
VIII	27	18 23 34 50 69 77 85 99 129 130 139 148 162 166 171 178 182 187 193 200 218 227 230 238 243 244 249

According to various authors, there is genetic diversity among rice genotypes, which can be grouped into a variety of distinct clusters. Twenty irrigated lowland rice genotypes with 11 morphological characters were grouped into four clusters by Baloch *et al*. (2016) [3], who also demonstrated significant genetic diversity among the tested genotypes. Worede *et al*. (2014) [15] divided 24 upland rice genotypes into two clusters based on 17 morpho-agronomic traits. 39 genotypes of rice grown under irrigation were divided into six separate clusters by Chakma *et al.* (2012) [4]. Thirty-two early maturing rice genotypes were divided into three clusters by Sarker *et al.* (2013). 24 rice accessions grown under irrigation were divided into five clusters by Ravi Kumar *et al*. (2015). Alamir (2018) [1] demonstrated the existence of genetic diversity among the tested genotypes by grouping 36 low land rice genotypes with 12 morphological characters into seven clusters.

Cluster mean analysis

The mean value of genotypes in each cluster was computed and cluster means are presented in Table 3. There was considerable difference among the clusters for different characters.

For days to 50% flowering the cluster mean ranged from 95.57 (cluster VII) to 114.63 days (cluster VIII). Maximum mean for days to 50% flowering was recorded for cluster VIII (114.63 days) followed by cluster IV (113.04 days), cluster VI (110.52 days), cluster V (104.96 days) while minimum mean was recorded for cluster VII (95.57 days), cluster I (100.43 days), cluster III (101.13 days) and cluster II (103.07 days).

For Seedling Vigor the cluster mean ranged from 26.9 (cluster V) to 38.25 (cluster VII). Maximum mean for days to seedling vigor was recorded for cluster VII (38.7) followed by cluster VIII (37.12), cluster III (34.83), cluster IV (34.04) while minimum mean was recorded for cluster V (26.9), cluster VI (27.63), cluster I (27.93) and cluster II (28.45).

For days to Plant Height the cluster mean ranged from 102.18 (cluster III) to 131.48 (cluster VI). Maximum mean for Plant Height was recorded for cluster VI (131.48) followed by cluster VII (128.27), cluster V (124.96), cluster VIII (122.19) while minimum mean was recorded for cluster III (102.18), cluster I (110.51), cluster IV (113.91) and cluster II (116.5).

For days to Flag Leaf Area the cluster mean ranged from 23.92 (cluster VII) to 17.08 (cluster III). Maximum mean for Flag Leaf Area was recorded for cluster III (27.08) followed by cluster IV (26.49), cluster II (26.34), cluster V (25.87) while minimum mean was recorded for cluster VII (23.92), cluster I (24.05), cluster VIII (24.24) and cluster VI (24.99).

For days to Panicles bearing tillers per plant the cluster mean ranged from 7.24 (cluster VIII) to 9.63 (cluster I). Maximum mean for Panicles bearing tillers per plant was recorded for cluster I (9.63) followed by cluster II (9.62), cluster VII (9.57), cluster VI (9.49) while minimum mean was recorded for cluster VIII (7.24), cluster IV (8.47), cluster V (8.76) and cluster III (9.35).

For Panicle Length the cluster mean ranged from 23.41 (cluster III) to 28.83 (cluster VI). Maximum mean for Panicle Length was recorded for cluster VI (28.83) followed by cluster VIII (28.15), cluster VII (27.51), cluster V (26.99) while minimum mean was recorded for cluster III (23.41), cluster I (24.96), cluster IV (25.06) and cluster II (25.42).

For days to No of spikelets per Panicles the cluster mean ranged from 72.75 (cluster V) to 141.13 days (cluster VI). Maximum mean for No of spikelets per Panicles was recorded for cluster VI (141.13) followed by cluster II (127.89), cluster I (122.55), cluster VIII (118.01) while minimum mean was recorded for cluster V (72.75), cluster III (104.23), cluster VII (105.21) and cluster IV (114.27).

For No of grains per Panicle the cluster mean ranged from 79.24 (cluster IV) to 91.95 (cluster III). Maximum mean for No. of grains per Panicle was recorded for cluster III (91.95) followed by cluster VI (91.22), cluster V (87.36), cluster VIII (82.37) while minimum mean was recorded for cluster IV (79.24), cluster I (79.66), cluster VII (79.9) and cluster II (82.24).

For spikelets fertility the cluster mean ranged from 65.42 (cluster II) to 123.8 (cluster V). Maximum mean for spikelets fertility was recorded for cluster V (123.8) followed by cluster III (90.04), cluster VII (77.78), cluster IV (71.82) while minimum mean was recorded for cluster II (65.42), cluster VI (65.88), cluster I (67.02) and cluster VIII (71.32).

For Biological yield per plant the cluster mean ranged from 19.5 (cluster I) to 38.44 (cluster II). Maximum mean for Biological yield per plant was recorded for cluster II (38.44) followed by cluster IV (33.4), cluster VI (31.63), cluster VII (30.48) while minimum mean was recorded for cluster I (19.5), cluster V (20.2), cluster III (28.23) and cluster VIII (29.83).

For Harvest index the cluster mean ranged from 26.23 (cluster IV) to 48.85 (cluster I). Maximum mean for Harvest index was recorded for cluster I (48.85) followed by cluster V (48.64), cluster VIII (44.09), cluster VI (43.33) while minimum mean was recorded for cluster IV (26.23), cluster VII (30.27), cluster III (36.77) and cluster II (38.93).

For Test Weight the cluster mean ranged from 21.78 (cluster VII) to 25.62 days (cluster II). Maximum mean for Test Weight was recorded for cluster II (25.62) followed by cluster V (25.08), cluster III (24.38), cluster VI (23.27) while minimum mean was recorded for cluster VII (21.78), cluster IV (22.03), cluster I (22.25) and cluster VIII (22.34).

For Grain yield per plant the cluster mean ranged from 8.51 (cluster IV) to 14.79 (cluster II). Maximum mean for Grain yield per plant was recorded for cluster II (14.79) followed by cluster VI (13.81), cluster VIII (12.89), cluster III (10.28) while minimum mean was recorded for cluster IV (8.51), cluster VII (9.01), cluster I (9.31) and cluster V (9.48).

Table 3: Cluster group means for 13 characters among rice genotypes

Characters	I	II	III	IV	V	VI	VII	VIII
Days of 50% Flowering	100.43	103.07	101.13	113.04	104.96	110.52	95.57	114.63
Seedling Vigor	27.93	28.45	34.83	34.04	26.9	27.63	38.25	37.12
Plant Height	110.51	116.5	102.18	113.91	124.96	131.48	128.27	122.19
Flag Leaf Area	24.05	26.34	27.08	26.49	25.87	24.99	23.92	24.24
Panicles bearing tillers per plant	9.63	9.62	9.35	8.47	8.76	9.49	9.57	7.24
Panicle Length	24.96	25.42	23.41	25.06	26.99	28.83	27.51	28.15
No of spikelets per Panicles	122.55	127.89	104.23	114.27	72.75	141.13	105.21	118.01
No of grains per Panicle	79.66	82.24	91.95	79.24	87.36	91.22	79.9	82.37
spikelets fertility	67.02	65.42	90.04	71.82	123.8	65.88	77.78	71.32
Biological yield per plant	19.5	38.44	28.23	33.4	20.2	31.63	30.48	29.83
Harvest index	48.85	38.93	36.77	26.23	48.64	43.33	30.27	44.09
Test Weight	22.25	25.62	24.38	22.03	25.08	23.27	21.78	22.34
Grain yield per plant	9.31	14.79	10.28	8.51	9.48	13.81	9.01	12.89

Estimation of inter cluster square distances (D^2)

In order to treat the values calculated between pairs of clusters as chi-square values and test for significance using p-1 degrees of freedom, the distance between clusters was estimated using the Mahalanobis distance, where "p" denotes the number of characters used (Singh and Chaudhary, 1985) [14]. Table 4 displays the results of the distance measurements between clusters. As a result, the 2-test revealed a highly significant difference between the four clusters.

The maximum inter cluster distance was observed between the clusters II and V (4.410) which suggested that members of these two clusters are genetically very diverse to each other.

The inter cluster values between clusters V and VI (4.269), Clusters IV and V (4.182), Clusters V and VIII (3.847), Clusters V and VIII (3.724) were also very high. The minimum Inter cluster D^2 value (15.783) was recorded in case of Clusters VI and VIII (2.394) followed by Clusters II and VI (2.404). Compared to genotypes grouped in other clusters, those in these clusters were relatively close to one another. Rama (1992) asserts that crossing genotypes from those clusters may not result in a narrower range of variability in the segregating F2 population and a higher heterotic value in the F1 population. In order to maintain a relatively diverse genetic base, such analysis was intended to prevent choosing parents from genetically homogeneous clusters.

Therefore, it is well known that the genetic diversity between the genotypes would be greater the further apart the clusters are. Therefore, it is crucial for the rice breeding programme that highly divergent genotypes produce a wide range of variability in the following generation to enable further selection and improvement.

Table 4: Average inter-cluster squared distance (D^2) between clusters based on 13 characters of 255 rice genotypes

Clusters	I	II	III	IV	V	VI	VII	VIII
I	3.035							
II	3.071	2.772						
III	2.803	2.876	3.017					
IV	3.020	2.811	2.734	2.785				
V	3.680	4.410	3.279	4.182	3.122			
VI	3.302	2.404	3.703	3.403	4.269	2.782		
VII	2.860	3.069	3.103	2.460	3.724	3.259	2.859	
VIII	3.094	2.884	3.484	2.553	3.847	2.394	2.944	2.852

Conclusion

The goal of the current study was to determine the degree of genetic separation between recently introduced rice genotypes for use in the breeding programme. A total of 250 rice genotypes with 5 checks were assessed using augmented design to produce this data. For each of the 13 studied traits, the analysis of variance revealed highly significant differences among the tested genotypes, indicating that there is a significant genetic variance between the genotypes. While the minimum cluster distance was discovered between clusters six and eight, the maximum cluster distance was discovered between clusters two and four. Based on the findings of the current investigation, it can be concluded that the majority of quantitative characters evaluated have sufficient genetic variation, and that in order to increase grain yield, genotypes with high grain yield should be chosen from various clusters and crossed. The study also recommended the release of the top-performing genotype for possible commercialization and identified it for further testing.

Parental material selection for upcoming hybridization-based breeding programmes should be done between clusters rather than within clusters. To predict genotypic performance across seasons and locations and to help validate the current results, it is advised to repeat the study at more locations and seasons with more genotypes. To further support the results of the current study findings, molecular characterization should be added to rice research in the future.

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