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Research Note

Genetic divergence for quantitative traits in rice germplasm

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Abstract
An investigation was carried out with 52 genotypes of rice to study the nature and magnitude of genetic divergence using D^2 statistics. Fifty two genotypes were grouped into 8 clusters based on Euclidean cluster analysis with cluster-IV containing maximum of 14 genotypes. Maximum intra-cluster distance was observed in cluster- IV (187.87) indicating greater genetic divergence among genotypes belonging to this cluster. Number of spikelets panicle^{-1}, plant height, grain panicle^{-1} and grain yield plant^{-1} contributed maximum towards genetic divergence. Maximum inter-cluster distance was observed between cluster-IV and VIII (1875.84) followed by cluster IV and cluster VII (1453.19), cluster II and cluster VIII (1275.66) and cluster II and cluster VII (1034.22) indicating wide genetic diversity between the clusters which may be used in rice hybridization programme(s) for improving grain yield through transgressive breeding. Hence, the crosses between BPT-5204 × IR 58025B, IET 20924 × IR 68897B and BPT-5204 × IR 68897B (cluster-IV × cluster-VIII), BPT-5204 × Khuta Dhan and IET 20924 × Khuta Dhan (cluster-IV × cluster-VII), Dantaswari × IR 58025B and IDR-763 × IR 58025B (cluster-II × cluster-VIII), Dantaswari × Khuta Dhan and IDR-763 × Khuta Dhan (cluster-II × cluster-VII) could be suggested for the exploitation of transgressive segregants for both yield and yield traits.

Keywords
Oryza sativa, clusters, genetic divergence, germplasm, yield

Rice is a cereal crop belonging to genus *Oryza* of family *Poaceae*. About half of the world's population depends on rice for their survival. Rice is being cultivated in around 113 countries of the world. The present world rice area, production and productivity is 158.93 mha, 465.03 mt and 4.36 t/ha, respectively. In India, it is being grown in 45.10 mha area with production of 103.60 mt and productivity of 3.51 t/ha and contributes 25% to agricultural GDP (Foreign Agriculture Services/USDA, Office of Global analysis, April 2013).To feed the ever growing population, the targeted rice production of the world, China and India for the year 2030 is envisaged as 771.02, 168.90 and 130.02 million tonnes respectively. Genetic variability is a prerequisite for plant improvement to develop high yielding varieties of crops. The study of genetic divergence in rice genotypes provides an idea of genetic variability among the available genotypes therefore, collection and evaluation of effective germplasm lines becomes the primary objective for any breeding programme(s). Genetic diversity plays an important role in plant breeding since progeny originating from diverse parents exhibit greater heterosis and provide broad spectrum of variability in segregating generations. Genetic diversity is pre requisite for any crop improvement program, as it helps in the development of superior recombinants (Naik *et al*., 2006). Diversity not only results in inducing genetic variation but also provides new recombination of genes in gene pool. The estimate of genetic divergence in the available germplasm is important for the selection of desirable donors for breeding programme. Several workers have emphasized the importance of genetic divergence for the selection of desirable parents (Sinha *et al.* 1991, Rahman *et al.* 1997, Ashok *et al*.2014). The use of Mahalanobis's D2 statistic for estimating genetic divergence has been emphasized by Sarawagi and Rita (2007). The present investigation was aimed to estimate the magnitude of genetic divergence present in the 52 rice genotypes and to identify the diverse genotypes for future breeding programme(s) by using Mahalanobis D^2 analysis.

The present investigation was carried out during *kharif* 2012 at Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi (U.P.). All the 52 genotypes (Table 1) were sown in the nursery on 12th June 2012, and 25 days old seedlings were used for transplanting in the field. All entries were grown in a randomized block design (RBD) in three replications with spacing of 20 × 15 cm between row to row and plant to plant respectively with a row length of 5.0 m. Field was ploughed and puddled thrice until fine tilth of soil was obtained. The crop was raised under irrigated conditions. Cultural practices like weeding, irrigation and recommended dose of fertilizer were applied to obtain a good crop growth. Five competitive plants were selected randomly from each row of each genotype in each replication. Observations were recorded for the following attributes viz ; days to

50% flowering (period taken from the date of seeding to 50% panicle emergence) and days to maturity (by counting the number of days from date of sowing to grain ripening) on individual plot basis, plant height (measured in cm from ground level to the tip of the main panicle excluding awns at maturity), number of tillers plant^{-1}, number of effective tillers plant^{-1}, panicle length (measured in cm from collar to the tip of the panicle), number of spikelets panicle^{-1}, number of grains panicle $^{-1}$ (counted at maturity), sterile spikelets/ panicle pollen fertility (%), spikelets fertility (%), grain weight panicle^{-1} (g), 1000 grain weight (g), grain yield plant^{-1} (g), kernel length (mm), kernel breadth (mm) and kernel L/B ratio. Panicle and grain characters were recorded on five panicles of selected plants. The experimental data were compiled by taking mean value over randomly selected plants from all the replications and subjected to the following statistical analysis viz; analysis of variance (Panse and Sukhatme 1961) and genetic divergence analysis (Mahalanobis's 1936, Tocher's method as described by Rao 1952).

The data collected for all the characters were subjected to statistical analysis as mentioned earlier. The ANOVA for all the characters was found to be highly significant, thus indicating a wide variation for all the traits considered. The studies on genetic divergence based on 17 yield and yield contributing traits among 52 genotypes of rice under three different replications was done by adopting Mahalanobis's D2 statistic analysis. Wilk's 'V' (statistic) criterion was used to test the significant differences between the groups based on the pooled effects of all the characters. The 'V' statistic value was highly significant indicating that genotypes differed significantly when all the characters were considered simultaneously. The significance of genotypes clearly indicated the significant pooled effect of all the characters between different genotypes. Hence, further analysis was made to estimate D2 values.Genetic divergence has been considered as an important factor in discriminating the genetically diverse parents for efficient and successful hybridization programme in order to get potential transgressive segregants. Multivariate analysis using Mahalanobis's D2 statistic has been found to be a potential biometrical tool in quantifying the degree of divergence in germplasm collections of crop plants. Genetic divergence analysis was conducted to identify suitable parents out of 52 rice genotypes to initiate a breeding programme for development of varieties for seventeen yield and yield contributing characters using Mahalanobis's D2 analysis. The significance of Wilk's 'V' (statistic) value indicated that the genotypes differed significantly among themselves when all the characters were considered simultaneously. This indicated that there is a considerable diversity in the material. Tocher's method of grouping is the most widely used procedure of clustering.

The analysis of variance showed significant difference among the 52 genotypes for all the seventeen characters indicating the existence of high genetic variability among the genotypes for all the traits (Table 2). The D^2 values of inter-cluster the genotypes ranged from 0.00 to 1875.84 indicating that the material was quite diverse. Based on the genetic distance, All 52 two genotypes were grouped into eight clusters by using Tocher's method (Singh and Choudhary 1977). Maximum 14 genotypes were grouped in cluster IV followed by cluster III with 13 genotypes and Cluster I with nine genotypes. Cluster II, Cluster V and cluster VIII had four genotypes. Cluster VI and cluster VII had two genotypes (Table 3). The clustering pattern revealed that the genotypes from different sources clustered together indication that there was no association between eco geographical distribution of genotypes and genetic divergence. The possible reason for grouping of genotypes of different states in one cluster could be the free exchange of germplasm among the breeders of different regions, or unidirectional selection practiced by breeder in tailoring the promising cultivars for different regions. Similar findings were reported by Chaturvedi and Maurya (2005). This indicated that, in general, selection has been towards the same goal in the different centers of origin of these genotypes and yet, there is sufficient genetic variability, which distinctly differentiates them into 8 clusters.

The intra and inter-cluster D^2 values among eight clusters revealed that intra-cluster average D^2 values ranged from 0 to 187.87 and inter-cluster average D^2 values ranged from 0 to 1875.84 (Table 4, Fig 3). The relative divergence of each from other cluster i.e., inter-cluster distance, indicated greater divergence between cluster IV and cluster VIII (1875.84) followed by cluster IV and cluster VII (1453.19); cluster II and cluster VIII (1275.66) and cluster II and cluster VII (1034.22) (Table 4) suggesting highest genetic divergence existing between the genotypes of these clusters.. Parental lines selected from these clusters may be used in a hybridization programme, since hybridization between divergent parents is likely to produce wide variability and transgressive segragations with high heterotic effects (Rama, 1992). Bose and Pradhan (2005) have also reported that selection of parents for hybridization should be done from two clusters having wider inter-cluster distances to get maximum variability in the segregating generations. The inter-cluster distance was minimum between cluster I and cluster V (105.94) followed by cluster I and III (148.46). The lines

belonging to these clusters were relatively closer to each other, in comparison to lines grouped in other clusters. Such analysis was meant to avoid selection of parents from genetically homogenous clusters, and to maintain a relatively broad genetic base. The minimum intra-cluster distance was recorded for cluster-I (59.6), while it was zero for cluster-VI, VII and VIII. The largest intra-cluster distance was recorded for cluster IV (187.87) followed by clusters VI (176.69) and cluster III (140.61) which showed that the lines included in clusters IV, VI and III were relatively more diverse than those in the other clusters. Fifty two genotypes were grouped into eight clusters and each consisted of genotypes from different geographical regions indicating that there was no association between geographical distribution and genetic diversity in corroboration with the reports of earlier workers viz Vivekanandan and Subramanian (1993), Kaw *et al*. (1995), Senapati and Sarkar (2005), Kumar (2008), Sabesan *et al.* (2009), Ashok *et al.*(2014). The genetic diversity among the genotypes may be due to factors like history of selection, heterogeneity, selection under diverse environments and genetic drift. Therefore, for hybridization, the selection of parents should be based on genetic diversity besides *per se* performance and empirical knowledge of the breeder. The intra-cluster distance was found minimum for cluster-I (59.6) and maximum for cluster-IV (187.87), while it was zero for cluster-VI, VII and VIII (Table 4). The inter-cluster distance was minimum between cluster I and cluster V (105.94) indicating close relationship and similarity for most of the characters of genotypes falling in these clusters. Such analysis was meant to avoid selection of parents from genetically homogenous clusters and to maintain a relatively broad genetic base.

The cluster means (Table 5) for days to 50% flowering varied from 83.56 (Cluster-IV) to 119.75 (Cluster-VI). Days to maturity ranged from 96.92 (Cluster-I) to 170.36 (Cluster-VII). For plant height, cluster means extended from 7.10 (Cluster-VII) to 13.79 (Cluster-III). The mean values for number of tillers plant^{-1} varied from 5.09 (Cluster-VII) to 11.89 (Cluster-III) and for number of effective tillers plant^{-1} extended from 22.40 (Cluster-V) to 34.85(Cluster-VII). Similarly, panicle length varied from 112.61(Cluster-IV) to 147.16 (Cluster-VI), whereas the number of spikelets panicle^{-1} ranged from 134.50 (Cluster-IV) to 469.06 (Cluster-VIII) and number of grains panicle^{-1} ranged from 112.66 (Cluster-IV) to 436.00 (Cluster-VIII). The cluster mean for Sterile Spikelets/ Panicle extended from17.20 (Cluster-I) to 40.91 (Cluster-VI), The cluster mean for Pollen Fertility (%) extended from 85.34 (Cluster-VI) to 93.83(Cluster-VIII). The cluster mean for spikelets fertility % extended from 84.48 (Cluster-VI) to 90.57 (Cluster-VIII). The cluster mean for grain weight panicle^{-1} ranged from 2.24 (Cluster-IV) to 8.66 (Cluster-VII) and for grain yield plant^{-1} ranged from 15.86 (Cluster-V) to 34.68 (Cluster-VII). The 1000-grain weight varied from 12.04 (Cluster-V) to 24.77 (Cluster-VII). The Kernel Length varied from 4.56 (Cluster-VI) to 7.30 (Cluster-II). The Kernel Breadth varied from 1.82(Cluster-VIII) to 2.17 (Cluster-V) and Kernel L/B Ratio varied from 2.39 (Cluster-V) to 3.63 (Cluster-IV)

In all combinations of inter-cluster distances, each character is ranked on the basis of inter-cluster distances. The relative contribution of different plant characters to the total genetic divergence estimated by D2 analysis indicated that maximum contribution (Table 6) spikelets panicle^{-1} (37.48%) followed by plant height (29.03%), grains panicle^{-1} (16.29%) and grain yield Plant^{-1} (6.56%) were the important traits contributing maximum towards divergence in rice. Similar result reported by Kanwal *et al.* (1983), Bansal *et al.* (1999) and Prasad *et al.* (2009) for grain yield plant^{-1} and plant height Singh *et al.* (2006). Other characters viz; days to 50% flowering, grain weight panicle^{-1}, number of effective tillers plant^{-1}, 1000 grain weight, panicle length, kernel length and kernel breadth contributed 4.75, 1.51, 1.44, 1.43, 0.75, 0.53 and 0.15%, respectively to genetic divergence in decreasing order. On the contrary, number of tillers plant^{-1}, days to maturity, sterile spikelets panicle^{-1}, pollen fertility and kernel L/B ratio contributed none towards genetic divergence

The above study recommends the crosses between BPT-5204 × IR 58025B, IET 20924 × IR 68897B, BPT-5204 × IR 68897B, IET 20924 × IR 58025B and RPBIO-226 × IR 58025B (cluster-IV × cluster-VIII), BPT-5204 × Khuta Dhan and IET 20924 × Khuta Dhan (cluster-IV × cluster-VII), Dantaswari × IR 58025B, IDR-763 × IR 58025B and Dantaswari × IR 68897B (cluster-II × cluster-VIII), Dantaswari × Khuta Dhan and IDR-763 × Khuta Dhan (cluster-II × cluster-VII) could be suggested for the exploitation of transgressive segregants for both yield and yield traits.

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Table 1. List of 52 genotypes of rice and their Source/origin

S. No.	Genotypes	Source/origin	S. No.	Genotypes	Source/origin
1.	IET 20924	B.H.U., Varanasi	27.	Pant Sughand Dhan- 17	GBPUAT, Pantnagar
2.	IET 21519	B.H.U., Varanasi	28.	Khuta Dhan	B.H.U., Varanasi
3.	IET 22218	B.H.U., Varanasi	29.	Nagina-22	B.H.U., Varanasi
4.	IET 22251	B.H.U., Varanasi	30.	GR-32	B.H.U., Varanasi
5.	IET 20935	B.H.U., Varanasi	31.	Karhani	B.H.U., Varanasi
6.	IET 20556	B.H.U., Varanasi	32.	Sona Choor	B.H.U., Varanasi
7.	IET 22228	B.H.U., Varanasi	33.	HUR-8-1	B.H.U., Varanasi
8.	IET 22225	B.H.U., Varanasi	34.	BPT 5204	B.H.U., Varanasi
9.	IET 22202	B.H.U., Varanasi	35.	RPBIO-226	B.H.U., Varanasi
10.	IET 21528	B.H.U., Varanasi	36.	Adam Chini	B.H.U., Varanasi
11.	IET 22237	B.H.U., Varanasi	37.	HUR-5-2	B.H.U., Varanasi
12.	IET 20524	B.H.U., Varanasi	38.	Anjali	B.H.U., Varanasi
13.	IET 21542	B.H.U., Varanasi	39.	IDR-763	B.H.U., Varanasi
14.	Vardhan	B.H.U., Varanasi	40.	Type-3	B.H.U., Varanasi
15.	Akshaya Dhan	B.H.U., Varanasi	41.	Kanak Jeer	B.H.U., Varanasi
16.	HUR 3022	B.H.U., Varanasi	42.	Improved GR-32	B.H.U., Varanasi
17.	HUR 105	B.H.U., Varanasi	43.	MTU-7029	B.H.U., Varanasi
18.	HUBR 2-1	B.H.U., Varanasi	44.	Kala Namak	B.H.U., Varanasi
19.	Rajendra Kasturi	B.H.U., Varanasi	45.	Vandana	B.H.U., Varanasi
20.	Sarjoo - 52	NDUAT Faizabad	46.	Dantaswari	B.H.U., Varanasi
21.	NDR 359	NDUAT Faizabad	47.	CR-2496	B.H.U., Varanasi
22.	NDR 97	NDUAT Faizabad	48.	IR 79156B	DRR, Hyderabad
23.	Pusa Basmati-1	IARI, New Delhi	49.	Pusa 6B	IARI, New Delhi
24.	Improved PB-1460	IARI, New Delhi	50.	IR 80555B	DRR, Hyderabad
25.	Pant Dhan-4	GBPUAT, Pantnagar	51.	IR 58025B	B.H.U., Varanasi
26.	Pant Dhan-12	GBPUAT, Pantnagar	52.	IR 68897B	B.H.U., Varanasi

Table 2.ANOVA for Dispersion

Source of Variations	df	Mean Squares
Varieties	51	1.0111***
Error	101	6.2231
Total	152	3.3925

Table 3. Cluster composition of 52 rice genotypes (Tocher's method)

Clusters	Number of genotypes	Name of genotypes
I	9	IET 20935, IET 20556, IET 22202, IET 22237, Akshaya Dhan, Pusa Basmati-1, Karhani, Type-3, Vandana
II	4	Nagina-22, HUR-5-2, IDR-763, Dantaswari
III	13	IET 22218, IET 20524, HUR 3022, HUR 105, HUBR 2-1, Sarju 52, NDR 359, NDR 97 ,Improved PB 1460 ,Pant Dhan-12, HUR-8-1, Anjali, IR 79156B
IV	14	IET 20924, IET 21519, IET 22228, IET 22225, IET 21528, IET 21542 ,Vardhan, Rajendra Kasturi , Pant Dhan-4, Pant Sughand Dhan -17, BPT 5204, RPBIO-226, MTU-7029, CR-2496
V	4	GR-32, Sona Choor, Adam Chini, Kanak Jeer
VI	2	Improved GR-32, Kala Namak
VII	2	IET 22251, Khuta Dhan
VIII	4	Pusa 6B, IR 80555B, IR 58025B, IR 68897B

Table 4. Inter-cluster and Intra-cluster (diagonal) average of D^2 and D values (parenthesis) of 52 rice genotypes (Tocher's method)

Clusters	I	II	III	IV	V	VI	VII	VIII
I	59.601 (7.720)	156.305 (12.502)	148.464 (12.184)	409.821 (20.244)	105.936 (10.292)	337.118 (18.360)	721.577 (26.862)	753.607 (27.451)
II		86.176 (9.283)	149.846 (12.241)	193.978 (13.927)	265.766 (16.302)	391.879 (19.795)	1034.218 (32.159)	1275.658 (35.716)
III			140.609 (11.857)	315.166 (17.752)	229.805 (15.159)	340.776 (18.460)	846.994 (29.103)	1016.507 (31.882)
IV				187.874 (13.706)	552.461 (23.504)	587.900 (24.446)	1453.190 (38.120)	1875.844 (43.311)
V					0.000 (0)	252.146 (15.879)	613.963 (24.778)	525.965 (22.933)
VI						176.691 (13.292)	487.603 (22.081)	847.234 (29.107)
VII							0.000 (0)	370.540 (19.249)
VIII								0.000 (0)

Table 5.Cluster means for seventeen characters in 52 genotypes of rice (Tocher's Method)

Clusters	Days to 50% Flowering	Days to Maturity	Plant Height(cm)	Tillers Plant^{-1}	Effective Tillers Plant^{-1}	Panicle Length (cm)	Spikelets Panicle^{-1}	Grains Panicle^{-1}	Sterile Spikelets Panicle^{-1}	Pollen Fertility (%)	Spikelet Fertility (%)	Grain Weight Panicle^{-1} (g)	1000 Grain Weight	Grain Yield Plant^{-1} (g)	Kernel Length (mm)	Kernel Breadth (mm)	Kernel L/B Ratio
I	105.792	96.923	12.185	9.684	25.582	135.208	243.033	225.787	17.208	92.159	90.333	4.432	20.371	29.603	6.737	2.132	3.211
II	94.768	103.574	11.659	8.582	26.889	122.319	189.359	165.577	24.661	90.818	86.134	3.659	23.769	25.980	7.308	2.052	3.566
III	96.917	108.525	13.792	11.898	26.084	125.208	228.417	194.958	33.042	87.758	84.752	3.115	21.298	30.526	6.556	1.847	3.587
IV	83.556	113.622	12.003	9.711	25.383	112.611	134.500	112.667	21.722	88.302	84.492	2.242	22.732	20.790	6.682	1.880	3.632
V	105.000	105.633	11.333	7.607	22.400	134.000	297.000	276.333	20.667	91.300	85.800	2.997	12.040	15.867	5.177	2.173	2.390
VI	119.750	154.733	9.483	7.591	28.400	147.167	287.833	247.583	40.917	85.343	84.480	3.369	14.507	19.518	4.568	1.915	2.461
VII	107.000	170.367	7.100	5.090	34.850	134.333	391.667	361.667	30.000	92.887	90.367	8.660	24.773	34.687	5.753	1.843	3.113
VIII	107.000	111.700	10.567	7.753	25.400	132.667	469.067	436.000	33.067	93.833	90.560	6.717	14.317	26.397	6.883	1.823	3.283

Table 6. Contribution of seventeen characters in 52 genotypes of rice

Sources	Times Ranked 1st	Contribution %
1 Days to 50% Flowering	63	4.75
2 Plant Height (cm)	385	29.03
3 Tillers plant^{-1}	0	0.00
4 Effective Tillers plant^{-1}	19	1.43
5 Panicle length(cm)	10	0.75
6 Days to maturity	0	0.00
7 Spikelets panicle^{-1}	497	37.48
8 Grains panicle^{-1}	216	16.29
9 St. spikelets panicle^{-1}	0	0.00
10 Pollen fertility %	0	0.00
11 Spikelet fertility %	1	0.08
12 Grain weight panicle^{-1} (g)	20	1.51
13 1000 Grain weight (g)	19	1.43
14 Grain Yield Plant^{-1}	87	6.56
15 Kernel length (mm)	7	0.53
16 Kernel Breadth (mm)	2	0.15
17 Kernel L/B Ratio	0	0.00