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Genetic divergence analysis of rice genotypes over environments

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Abstract
The present investigations were conducted in randomized block design with three replications in the net house of the department of Plant Molecular Biology and Genetic engineering, N.D.U.A.T, Kumarganj, Ayodhya to estimate the genetic divergence under normal and salinity conditions involving 30 rice genotypes during *Kharif* 2016, based on the relative magnitude of D^2 values, the clustering pattern of 30 rice genotypes under normal and saline conditions were grouped into six non-overlapped clusters. Under normal condition, Cluster 1 and IV having highest 8 rice genotype in each. Cluster II having five genotypes. Cluster III, V and VI having three rice genotypes in each. Under saline condition, Cluster V having 8 rice genotypes. Cluster VI having seven rice genotypes. Cluster III having five rice genotypes. Cluster I having four rice genotypes. Cluster IV having four and cluster II having two rice genotypes. It means the overall genetic similarity was found in the germplasms were presented within the cluster and the pattern of distribution of genotypes in different clusters exhibited that geographical diversity was not related to genetic diversity as genotypes of same geographical region were grouped into different cluster and vice-versa. The highest inter cluster distance was recorded between cluster 1 and cluster 5 (1494.17) followed by between cluster 1 and cluster 6 (1255.42), cluster 2 and cluster 5 (1101.02), cluster 3 and cluster 5 (1030.71) and cluster 3 and cluster 6 (716.81) under normal condition. Under saline condition the highest inter cluster distance was recorded between cluster 3 and cluster 6 (1676.69) followed by between cluster 3 and cluster 5 (1552.04), cluster 1 and cluster 3 (1106.63), cluster 4 and cluster 6 (871.92) and cluster 4 and cluster 5 (753.88). Cluster VI and Cluster III had highest cluster mean for grain yield per plant under normal and saline conditions, respectively. The results showed wide variation from one cluster to another in respect of cluster means for all characters, which indicated that varieties having distinctly different mean performance for various characters were reported into different clusters.

Keywords: Rice, salinity, genetic divergence, cluster

Introduction
Rice (*Oryza sativa* L.) is considered as one of the most important plants from poaceae. Today, rice has special position as a source of food for over 75% of Asian population and more than three billion of world populations representing 50 to 80% of their daily calorie intake (Khush, 2005) [11]. Rice is an economically important food crop with nutritional diversification and helps in poverty alleviation. Rice is ranked as the world's number one human food crop. In India, rice is grown in an area of 43.97 million hectares with the production and productivity levels of 104.32 million tonnes and 2372 kg/ha, respectively, (Indiastat, 2017-2018). In Andhra Pradesh, rice is grown in an area of 3.49 million hectares with the production and productivity levels of 10.19 million tonnes and 3126 kg/ha, respectively, (Anonymous., 2017) [1]. Breakthrough is always required for increasing the productivity of new rice varieties and through enhanced biological efficiency. Varietal and cultural diversity in rice is enormous and its improvement is therefore a challenging task. Plant breeding programme with diverse genetic base could sustain a high level of crop yield. The narrow genetic base of semi-dwarf varieties is likely to make them vulnerable to different biotic and abiotic stresses. Therefore, to meet the ever-increasing demand of food grains, higher production emphasis should be given to the genetic improvement of the existing germplasm varieties of rice. Diversity in rice has been well utilized to breed high yielding varieties.

Land races of rice are being collected over past several decades to use them in breeding programme to develop high yielding, resistant to biotic and abiotic stresses with better adaptability. The success of any breeding programme depends on the exploitation of existing variability and therefore, it is desirable to collect, evaluate and utilize the available diversity for crop improvement to suit specific need of a given ecosystem. Genetic divergence among the parents is important because a cross involving genetically diverse parents is likely to produce high heterotic effect and also more variability could be expected in segregating generations. Therefore, a meaningful classification of genotypes will enable the breeder to identify the best parents with wide genetic divergence and to utilize some of the selected diverse parents in the hybridization programme.

Materials and Method

Rice genotypes

A total of 30 traditional and improved rice genotypes were used in the study *viz*., IR91167-31-3-1-33, IR91167-99-1-1-1-3, IR91167-133-1-1-2-3, IR91171-66-3-2-1-3, IR91175-27-1-3-1-3, IR91158-85-3-2-3-3, IR92953-49-1-3, IR92960-75-1-3, IR92966-95-1-3, IR92971-70-3-3, IR68144-2B-2-2-3-1-166, IR82475-110-2-2-1-2, IR83294-66-2-2-3-2, IR83668-35-2-2-2, IR84722-82-2-3-3-3, RP-BIO-5478-185M, RP4993-55-14-3-5-1, R-RHZ-2, R-RHZSM-4, R-RHZIH-7, IR68144-2B-2-2-3-1-120, IR68144-2B-2-2-3-1-127, TARAMON, SWARNA, IR-64, NUD-3, NDR-359, AYAAR, SAMBHA MANSSURI and MTU-1010.

Method

The experiments were conducted in Randomized Complete Block Design with three replications under normal and saline conditions in the net house of the department of Plant Molecular Biology and Genetic engineering N.D.U.A.T, Kumarganj, Ayodhya during *Kharif* 2016.

The data were recorded on days to 50% flowering, plant height, panicle bearing tillers/plants, panicle length (cm), spikelets/panicle, grains/panicle, spikelet fertility (%), test weight (g), biological yield (g), harvest index (%), grain yield (g) Na^+ content, P^+ content (mg g^{-1}) and Na^+/P^+ ratio.

Observations were recorded on randomly selected five plants from each variety in each replication at maturity except for days to 50% flowering which were recorded on the plot basis at flowering stage.

The pH and EC were maintained at 5 and 12 dS m^{-1}.

Estimation of genetic divergence (D^2)

The genetic divergence of 30 genotypes of rice was worked out using Mahalanobis (1936) [12] D^2 statistics (Rao, 1952) [18].

The calculation of D^2 values involved following steps

1. A set of uncorrelated linear combinations (y's) was obtained by pivotal condensation of the common dispersion matrix (Rao, 1952) [18] of a set of correlated variables (x's). The common dispersion matrix was arranged with the help of error mean sum of squares and mean sum of products.
2. Using the relationship between y's and x's the mean values of different genotypes for different characters (X_1 to X_{10}) were transformed into the mean values of a set of uncorrelated linear combinations (Y_1 to Y_{10}).
3. The D^2 values between i^{th} and j^{th} genotypes for k^{th} characters is calculated as:

$D^2_{ij} = K (Y_{it}-Y_{jt})^2$

Where, t = 1

The K components were calculated separately and added to get D^2_{ij}.

1. The 'K' components of 'D^2_{ij}' for each combination were ranked in descending order of magnitude.
2. These ranks were added up for each component D^2_{ij} over all combinations of i and j^{th} rank totals were obtained.

Group constellation

The D^2 values were arranged in an increasing order of magnitude. The grouping of the strains into different clusters was done using Tocher's method (Rao, 1952) [18]. The two most closely associated groups were chosen and third groups were found which had the smaller average D^2 value from the first two. Similarly, the fourth was chosen to have the smallest average D^2 from the first three and so on. The D^2 value did not fit in with the former group and was, therefore, taken as another cluster.

Intra and inter-cluster distance

The inter-cluster D^2 was calculated as the sum of n (n-1)/2 genotypes within a cluster divided by total number of combinations. All possible D^2 values between the groups of two clusters were added and then divided by $n_1 \times n_2$ for computing inter-cluster distance.

Where, n_1 and n_2 = the number of genotypes in two clusters.

Cluster mean

The cluster mean for the particular character is the summation of mean values of the strains included in a cluster divided by number of strain in the cluster.

Result and Discussion

The selection of suitable diverse parents for hybridization is an important feature of any crop breeding programmes because parental diversity in optimum magnitude is required to obtain superior genotypes in segregating generations (Moll *et al.*, 1962) [15]. The importance of genetic divergence in crop improvement has been emphasized by several scientists (Griffing and Lindstrom, 1954; Moll *et al.*, 1962 [7, 15]; Arunachalam (1981) [2] and Hawkas (1981). Mahalanobis D^2 statistic has been utilized by several workers for the assessment of genetic divergence in different crops (Malhotra and Singh, 1971) [13].

In the present study, the thirty genotypes of rice were grouped into six different non-overlapping clusters under normal field and saline conditions (Table 1a and b), suggesting considerable amount of genetic diversity in the materials. In control condition (Table 1a) cluster I and cluster IV having highest number of genotypes (8) namely, IR-68144-2B-2-2-3-1-120, IR-68144-2B-2-2-3-1-127, IR-84722-82-2-3-3-3, Sambha mansuri, IR-91171-70-3-3, IR-91167-99-1-1-1-3, Ayaar and R-RHZ-14-7 in cluster I and NDR-359, IR-91175-27-1-3-1-3, IR-91167-31-3-1-33, IR-92953-49-1-3, R-RHZSM-4, Swarna, IR-91158-85-3-2-33 and IR-83668-35-2-2-2 in cluster IV. Cluster II having 5 genotypes *i.e.* IR-64, IR-68144-2B-2-2-3-166, RP-4993-55-14-3-5-1, MTU-1010 and Taramon. Cluster III, V and VI having three rice genotypes in each, these were IR-91167-133-1-1-2-3, IR-92960-75-1-3 and NUD-3 in cluster III; RP-BIO-5478-185M, IR-82475-110-2-2-1-2 and IR-92966-95-1-3 in cluster V and IR-83294-66-2-2-3-2, R-RHZ-2 and IR-91171-66-3-2-1-3 in cluster VI. While

in saline condition (Table 1b), Cluster V having highest number (8) of rice genotypes namely, NDR-359, IR-92953-49-1-3, RP-BIO-5478-185M, IR-68144-2B-2-2-3-1-127, IR-91171-70-3-3, IR-91167-99-1-1-1-3, Ayaar and IR-92966-95-1-3. Cluster VI having 7 rice genotypes i.e., R-91175-27-1-3-1-3, IR-91167-31-3-1-33, IR-83294-66-2-2-3-2, R-RHZSM-4, Swarna, IR-91158-85-3-2-33 and IR-83668-35-2-2-2. Cluster III having five rice genotypes these were IR-91167-133-1-1-2-3, IR-92960-75-1-3, NUD-3, R-RHZ-2 and IR-91171-66-3-2-1-3. Cluster I and Cluster IV having four rice genotype in each namely, IR-68144-2B-2-2-3-166, RP-4993-55-14-3-5-1, IR-68144-2B-2-2-3-1-120 and MTU-1010 in cluster I and IR-84722-82-2-3-3-3, Sambha mansuri, R-RHZ-14-7 and IR-82475-110-2-2-1-2 in cluster IV. The cluster II having two genotypes *i.e.* IR-64 and Taramon. It means the overall genetic similarity was found in the germplasms were presented within the cluster and the pattern of distribution of genotypes in different clusters exhibited that geographical diversity was not related to genetic diversity as genotypes of same geographical region were grouped into different cluster and vice-versa, as supported by earlier finding of Devi Shashi and Dwivedi, (2016) [6]; Cheema *et al.* (2004) [4]; Devi *et al.* (2006) [5]; Hosan *et al.* (2010) [9]; Ismail *et al.* (2010) [10]; Mall *et al.* (2011) [14] and Ovung *et al.* (2012) [17].

Table 1a: Clustering pattern of 30 rice genotype on the basis on D^2 analysis for 14 characters in normal condition.

Cluster No.	No. of genotypes	Genotypes
I	8	IR-68144-2B-2-2-3-1-120, IR-68144-2B-2-2-3-1-127, IR-84722-82-2-3-3-3, Sambha mansuri, IR-91171-70-3-3, IR-91167-99-1-1-1-3, Ayaar, R-RHZ-14-7
II	5	IR-64, IR-68144-2B-2-2-3-166, RP-4993-55-14-3-5-1, MTU-1010, Taramon
III	3	IR-91167-133-1-1-2-3, IR-92960-75-1-3, NUD-3
IV	8	NDR-359, IR-91175-27-1-3-1-3, IR-91167-31-3-1-33, IR-92953-49-1-3, R-RHZSM-4, Swarna, IR-91158-85-3-2-33, IR-83668-35-2-2-2
V	3	RP-BIO-5478-185M, IR-82475-110-2-2-1-2, IR-92966-95-1-3
VI	3	IR-83294-66-2-2-3-2, R-RHZ-2, IR-91171-66-3-2-1-3

Table 1b: Clustering pattern of 30 rice genotype on the basis on D^2 analysis for 14 characters in saline condition

Cluster No.	No. of genotypes	Genotypes
I	4	IR-68144-2B-2-2-3-166, RP-4993-55-14-3-5-1, IR-68144-2B-2-2-3-1-120, MTU-1010
II	2	IR-64, Taramon
III	5	IR-91167-133-1-1-2-3, IR-92960-75-1-3, NUD-3, R-RHZ-2, IR-91171-66-3-2-1-3
IV	4	IR-84722-82-2-3-3-3, Sambha mansuri, R-RHZ-14-7, IR-82475-110-2-2-1-2
V	8	NDR-359, IR-92953-49-1-3, RP-BIO-5478-185M, IR-68144-2B-2-2-3-1-127, IR-91171-70-3-3, IR-91167-99-1-1-1-3, Ayaar, IR-92966-95-1-3
VI	7	IR-91175-27-1-3-1-3, IR-91167-31-3-1-33, IR-83294-66-2-2-3-2, R-RHZSM-4, Swarna, IR-91158-85-3-2-33, IR-83668-35-2-2-2

The estimates of average intra and inter cluster distances for presented in table 2a and table 2b revealed that the maximum intra cluster distance was exhibited by the genotypes of cluster 6 followed by cluster 4, cluster 5, cluster 1 cluster 3 and cluster 2. The highest inter cluster distance was recorded between cluster 1 and cluster 5 (1494.17) followed by between cluster 1 and cluster 6 (1255.42), cluster 2 and cluster 5 (1101.02), cluster 3 and cluster 5 (1030.71) and cluster 3 and cluster 6 (716.81), under normal condition while, under saline condition the highest intra cluster distance was recorded by cluster 3 followed by cluster 2, cluster 4 and cluster 1. The highest inter cluster distance was recorded between cluster 3 and cluster 6 (1676.69) followed by between cluster 3 and cluster 5 (1552.04), cluster 1 and cluster 3 (1106.63), cluster 4 and cluster 6 (871.92) and cluster 4 and cluster 5 (753.88) suggesting wide diversity between them and germplasm in these clusters could be used as parents in hybridization programme to develop desirable type because crosses between genetically divergent lines will generate heterotic segregants. As heterosis can be best exploited and chances of getting transgressive segregants are maximum when generating diverse lines are crossed (Zaman *et al.*, 2005 and Saxesena *et al.*, 2013) [20, 19]. Many workers in different crops have also reported that selection of parents for hybridization should be done from two clusters having wider inter-cluster distance to get maximum variability in segregating generations. Heterosis is generally attributed to genetic divergence among the parental lines involved in the cross. Nevertheless, the genetic divergence for the maximum expression of the heterotic effects has a limit Moll *et al.*, (1965) [16] and Arunachalam and Bandyopadhyay, (1984) [3].

Table 2a: Estimates of average inter cluster D^2 value under normal condition

Cluster No.	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6
Cluster 1	134.79	441.23	244.47	644.09	1494.17	1255.42
Cluster 2		70.21	222.18	416.34	1101.02	480.67
Cluster 3			120.44	357.3	1030.71	716.81
Cluster 4				205.18	426.61	429.78
Cluster 5					127.06	476.06
Cluster 6						234.27

Table 2b: Estimates of average inter cluster D^2 value under saline condition

Cluster No.	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6
Cluster 1	41.84	149.26	1106.63	460.27	80.71	127.60
Cluster 2		62.42	607.39	191.88	288.58	348.22
Cluster 3			89.890	232.57	1552.04	1676.69
Cluster 4				74.04	753.88	871.92
Cluster 5					0.00	63.96
Cluster 6						0.00

The comparison of cluster means revealed considerable differences among the clusters of different characters in normal and saline soil (Table 3a and 3b).

Under normal condition cluster mean for grain yield per plant ranged from 11.34 (cluster V) to 13.90g (cluster VI). Maximum cluster mean was recorded by the cluster VI (13.90g) followed by cluster II (13.41g) and cluster IV (13.39g). In saline condition cluster mean for grain yield per plant ranged from 8.05 (cluster VI) to 12.95g (cluster III). Maximum cluster mean was recorded by the cluster III (12.95) followed by cluster IV (12.06) and cluster I (11.23g). The results showed wide variation from one cluster to another in respect of cluster means for all characters, which indicated that varieties having distinctly different mean performance for various characters were reported into different clusters as supported by earlier finding of Devi Shashi and Dwivedi, (2016) [6]; Gaurav and Dwivedi, (2018) [8].

Table 3a: Cluster mean of 30 rice genotypes under normal condition for 14 characters

Characters	Days to 50 % flowering	Plant height (cm)	Panicle bearing tillers/plant	Panicle length (cm)	Spikelets/ panicle	Grain/ panicle	Spikelet fertility (%)	Test weight (g)	Biological yield/ plant (g)	Harvest index (%)	Grain yield/ plant	Na+	K+	Na+/K+
Cluster 1	104.06	74.75	9.57	21.74	78.07	71.95	92.10	19.21	27.81	44.14	12.01	3.63	25.73	0.14
Cluster 2	116.33	115.91	13.61	23.36	108.33	102.71	94.67	20.06	31.50	42.48	13.41	3.47	26.59	0.13
Cluster 3	111.85	78.55	12.67	23.79	106.34	96.19	90.39	22.67	30.37	42.88	12.59	3.24	23.71	0.14
Cluster 4	107.88	84.10	13.79	24.94	142.34	133.26	93.69	19.00	35.48	38.47	13.39	3.25	26.61	0.12
Cluster 5	102.78	89.78	9.29	27.56	187.11	177.78	95.08	18.66	30.76	37.20	11.34	3.57	23.54	0.15
Cluster 6	115.17	127.35	10.03	26.61	162.56	153.17	94.01	22.75	37.78	36.85	13.90	3.17	22.35	0.14

Table 3b: Cluster mean of 30 rice genotypes under saline condition 14 characters

Characters	Days to 50 % flowering	Plant height (cm)	Panicle bearing tillers/plant	Panicle length (cm)	Spikelets/ panicle	Grain/ panicle	Spikelet fertility (%)	Test weight (g)	Biological yield/ plant (g)	Harvest index (%)	Grain yield/ plant	Na+	K+	Na+/K+
Cluster 1	94.75	69.73	9.15	19.44	74.71	69.71	90.69	16.76	27.02	41.50	11.23	4.04	27.36	0.15
Cluster 2	105.52	78.18	10.97	21.09	102.43	95.72	93.40	20.33	26.81	39.89	10.64	3.90	25.52	0.16
Cluster 3	97.33	80.23	10.85	23.02	173.41	165.61	95.50	19.31	31.00	41.63	12.95	4.01	25.32	0.16
Cluster 4	101.50	80.65	11.30	22.91	132.33	125.00	94.47	17.01	32.77	37.21	12.06	3.88	28.07	0.14
Cluster 5	103.67	64.42	7.96	15.48	55.28	48.84	88.40	23.16	28.29	38.33	10.84	4.30	26.50	0.16
Cluster 6	101.67	71.35	8.32	20.10	56.20	49.05	87.33	20.59	17.72	45.43	8.05	3.27	29.46	0.11

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