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Multivariate clustering utilizing R software analytics

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Abstract
Hierarchical clustering approach was used to group the Maize genotypes and the distance measure used was Euclidean distance. Cluster analysis using the R software grouped the 55 genotypes into distinct clusters using the Euclidean distances between the various genotypes. All the three types of dendrograms obtained indicate single, complete, and average linkage. Single linkage group the genotypes on the basis of the similarity. It was found that when the dendrogram for single linkage was cut at a distance of 4, it revealed two distinct clusters for the 55 genotypes. It clearly classified the genotypes, with cluster one containing the individual plants and cluster two containing crosses. Level plot was also obtained which indicated at least two distinct groups with large inter cluster distance.

Keywords: Cluster, D^2 statistics, genetic divergence, maize genotypes, multivariate analysis

Introduction
Multivariate analysis is the analysis of observations on several correlated random variables, for a number of individuals. Such analysis becomes necessary when one deals with several variables simultaneously. Multivariate analysis by means of Mahalanobis D^2 Statistics and Euclidean distances is widely used for quantifying the degree of genetic divergence among the population. The genetic divergence analysis estimates the extent of diversity existed among selected genotypes (Mondal, 2003) [5]. The cluster analysis is to allocate a set of individuals to a set of mutually exclusive, exhaustive groups such that the individuals within a particular group are similar to one another while the individuals in the different groups are dissimilar. Precise information on the nature and degree of genetic diversity helps the plant breeder in choosing the diverse parents for purposeful hybridization (Samsuddin, 1985) [7]. Clustering is a distribution free non parametric technique that is no assumptions are made concerning the number of groups and grouping is done on the basis of similarities or distance i.e. dissimilarities (Chatfield and Collins 1990; Jhonson and Wichern 1996) [1, 3].

Materials and Methods
The material for this study is composed of 55 genotypes of maize. These lines were maintained at SKUAST-K Shalimar and KD station by Department of Genetics and Plant Breeding, SKUAST K Shalimar. Data collected for the study constitutes the data base for the present investigation. The data was subjected to different types of cluster analysis. The experiment was laid out in a RCBD consisting of two replications each containing 55 genotypes considered as treatment. The data generated from the experiment on maize conducted by DARS, Budgam, SKUAST-K, has been used for this study. Data comprised of 55 genotypes of maize, out of which 10 genotypes were individual plants and 45 were crosses and 12 characters were recorded for each of the genotypes. The characters recorded were: Plant height, Ear height, Days to 50% tasseling, Days to 50% silking, 75% HB, Cob length, Cob per plant, Rows per cob, Grains per row, Cob diameter, 100 seed weight, Yield per plant. Genetic diversity was studied using Mahalanobis (1936) [4] generalized distance (D^2) extended by Rao (1952) [6].

Result and Discussion
Agglomerative hierarchical cluster analysis that produces partitions by a series of successive fusions of the *n* individuals into groups have been carried out.

We start our analysis by computing the dissimilarity matrix containing the Euclidean distance of the plant characters on all 55 genotypes. The resulting 55×55 matrix can be inspected by the image plot (Figure 5) obtained by the function (*levelplot*). Correlation between the characters was observed. The function for obtaining the correlation is: (*cor*). Several characters were found to be highly correlated such as grain/row and yield (0.925), 50% Tasseling and 50% silking (0.939), cob length and grain per row (0.942), and 100 seed weight and Yield (0.895) as shown in Table 1 and Figure 1.

Table 1: Correlation between the traits

Correlation Matrix												
	PlHt	**ErHt**	**Tsl**	**Sil**	**HB**	**CobLn**	**Cobpt**	**Rowcob**	**GrnRow**	**Cobdia**	**Sdwt**	**YPlnt**
PlHt	1.0000											
ErHt	0.8919	1.0000										
Tsl	-0.0819	-0.0679	1.0000									
Sil	-0.1444	-0.1106	0.9387	1.0000								
HB	0.1233	0.2235	0.4243	0.4692	1.0000							
CobLn	0.6497	0.7524	0.0114	0.0044	0.1494	1.0000						
Cobpt	0.4810	0.3674	-0.0644	-0.1300	-0.1174	0.3977	1.0000					
Rowcob	0.6287	0.7313	-0.0063	-0.0188	0.1895	0.7328	0.2976	1.0000				
GrnRow	0.7255	0.8123	0.0444	0.0350	0.2373	0.9422	0.4662	0.8021	1.0000			
Cobdia	0.6908	0.7631	-0.0770	-0.1022	0.1334	0.8452	0.4867	0.8594	0.8603	1.0000		
Sdwt	0.7819	0.8609	0.0401	0.0226	0.1846	0.8130	0.3490	0.7542	0.8587	0.7973	1.0000	
YPlnt	0.7473	0.7961	0.0079	-0.0249	0.0993	0.8827	0.5951	0.8141	0.9253	0.8816	0.8947	1.0000

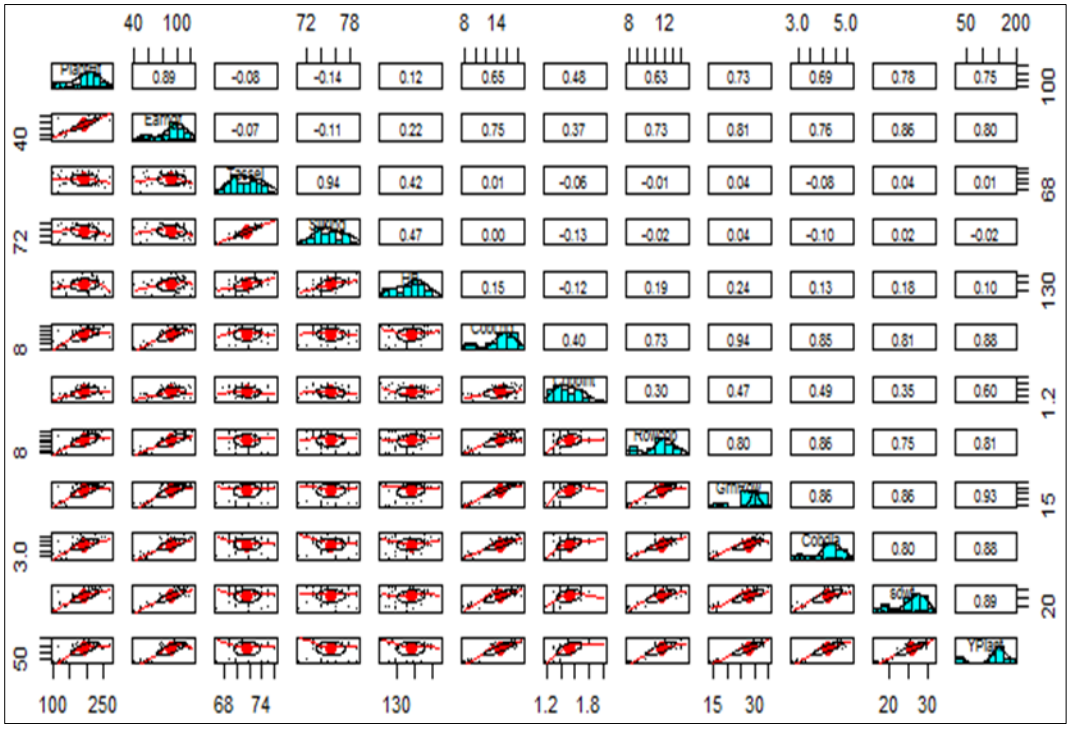


Fig 1: Correlation matrix plot

To begin with the euclidean distances are calculated using the (*dist*) function.

- *cluster=read.table("clipboard",header=T)*
- *dist(cluster[,-1])*
- *dist=dist(cluster[,-1])*

Here we have omitted the 1st column of the data matrix which specifies the genotypes of maize.

We now construct three series of partitions using single, complete, and average linkage hierarchical clustering. The function (*hclust*) performs all the three procedures based on the distance matrix of the data. The corresponding plot method draws a dendrogram.

- *hier=hclust(dist,method="single")*
- *hier=hclust(dist,method="complete")*
- *hier=hclust(dist,method="average")*

Here the output is named as *hier* and to plot the dendrogram the *plot* function is used

- *plot(hier)*. The output plots are shown in the figures 2, 3 and 4 below:

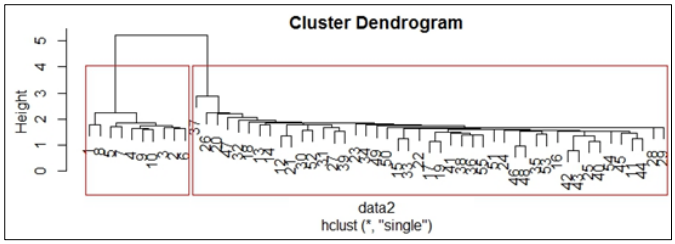


Fig 2: Single Linkage Clustering

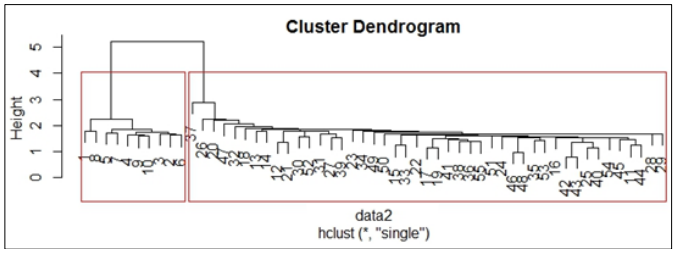


Fig 3: Complete Linkage Clustering

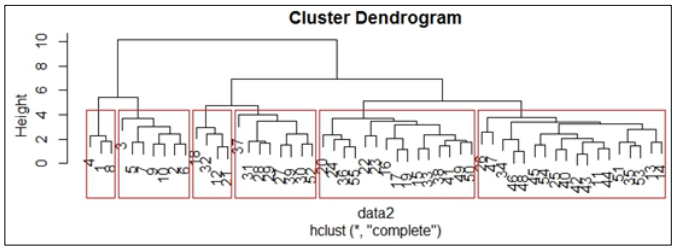
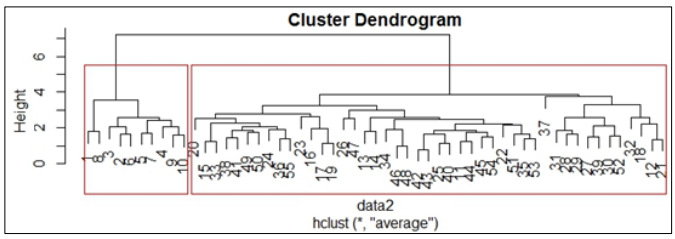


Fig 4: Average Linkage Clustering



Here cluster analysis using single linkage classified our data set into two types or groups viz. Type 1 and Type 2. Type 1 containing the individual plants and Type 2 containing the crosses. The dendrogram suggests that the cluster 1 contains 10 genotypes which are the individual plants and Cluster 2 contains all the rest of the genotypes which are crosses. The level plot obtained for the Euclidean distances is shown in Fig 5. The diagonal elements of the dissimilarity matrix are dark coloured indicating zero distance while as the pale values indicate greater distance. The level plot indicates that there are at least two distinct groups with large inter cluster distance whereas much larger distance can be observed for the other cells.

Many algorithms have been proposed for cluster analysis. Here the hierarchical techniques have been used which produce a dendrogram as shown in Figure 2, Figure 3 and Figure 4. The method starts with the calculation of distances of each individual to all other individuals. Groups are then formed by the process of agglomeration or division. Agglomerative hierarchical clustering techniques differ primarily in how they measure the distances between or similarity of two clusters (where a cluster may, at times, consist of only a single individual). Two simple intergroup measures are:

$$d_{AB} = min(d_{ij}),$$
$$d_{AB} = max(d_{ij}),$$

Where, d_{AB} is the distance between two clusters A and B, and d_{ij} is the distance between individuals i and j. This is the Euclidean distance. The first intergroup dissimilarity measure above is the basis of single linkage clustering, the second that of complete linkage clustering. A further possibility for measuring intercluster distance or dissimilarity is:

$$d_{AB} = \frac{1}{nAnB} \sum\sum d_{ij}$$

Where, n_A and n_B are the number of individuals in clusters A and B. This measure is the basis of a commonly used procedure known as group average clustering. All three intergroup measures described here are illustrated by the dendrograms:

Single Linkage: Single linkage measures the shortest distance between the two clusters. A dendrogram for the single linkage clusters results in the Fig. 2 and Table 2

Complete Linkage: Complete linkage measures the longest distance between the two clusters. A dendrogram for the complete linkage clusters results in the Fig. 3 and Table 3

Average Linkage: Single linkage measures the average distance between the two clusters. A dendrogram for the average linkage clusters results in the Fig. 4 and Table 4

A level plot for the Euclidean distances is shown in Fig. 5.

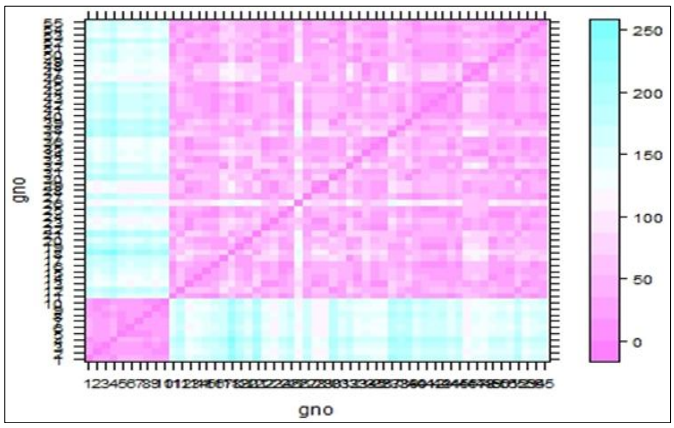


Fig 5: Level Plot

It displays the all distances of the Euclidean matrix of order 55× 55. The x axis represents the total number of observations while as the y axis represents the distances. When the cluster is cut a distance of 4 it specifies two distinct clusters as shown in the dendrogram. The cluster 1 contains individual genotypes (P1, P2, P3, P4, P5, P6, P7, P8, P9 and P10) while as the cluster 2 contains crosses as shown in Fig. 2 and Table 2. The selection of parents should also consider the special advantage of each cluster and each genotype within a cluster depending on specific objective of hybridization (Chahal and Gosal, 2002) [2].

Table 2: Clusters specifying the genotypes (single linkage basis)

Cluster	Genotypes
1	P1,P2,P3,P4,P5,P6,P7,P8,P9,P10
2	P11,P12,P13,P14, P15,P16,P17,P18, P19,P20,P21,P22, P23,P24,P25,P26 P27,P28,P29,P30, P31,P32,P33,P34 P35,P36,P37,P38, P39,P40,P41,P42, P43,P44,P45,P46, P47,P48,P49,P50, P51,P52,P53,P54,P55

Table 2 indicates that the genotypes in their respective clusters are homogenous amongst themselves and heterogeneous in between.

For Complete linkage using R the *hclust* function specified 6 distinct clusters when the dendrogram was cut a distance of 4 as shown in Fig. 2.

Table 3: Clusters specifying the genotypes (complete linkage basis)

Cluster	Genotypes
1	P1,P8
2	P4,P10,P5,P7,P3,P2,P6,P9
3	P37,P31,P28,P29
4	P18,P12,P21,P27,P39,P30,P52
5	P13,P14,P47,P51,P53,P11,P14,P34,P46,P48, P45,P54,P42,P43,P25,P40
6	P20,P24,P41,P36,P38,P55,P22,P15,P33,P49, P50,P17,P19,P16,P26,P35,P22,P23

Similarly, for Average linkage using R the *hclust* function specified distinct clusters when the dendrogram was cut a distance of 4 as shown in Fig. 3.

Table 4: Clusters specifying the genotypes (Average linkage basis)

Cluster	Genotypes
1	P1,P8,P3,P2,P6,P5,P7,P9,P4,P10
2	P20,P31,P28,P29,P36,P38,P55,P41,P24,P15,P33,P49, P50,P34,P47,P22,P23,P17,P19,P16,P26,P35,P13, P14,P45,P54,P42,P43,P25,P40,P51,P11,P44,P53.P46,P48
3	P37,P27,P39,P30,P52,P32,P18,P12,P21

Conclusion

The present investigation provided considerable information useful in genetic improvement of Maize. There is significant genetic variability among tested genotypes that indicates the presence of excellent opportunity to bring about improvement through wide hybridization by crossing genotypes in different clusters. Thus multivariate clustering can be employed to increase the efficiency of hybridisation programmes.

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