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Diversity analysis of maize (*Zea mays* L.) genotypes under excess soil moisture condition

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Abstract
The screening of seventy CIMMYT maize hybrids was carried out to estimate the genetic diversity which was evaluated undermanaged excess moisture condition, indicated presence of considerable diversity. The experiment was conducted in an alpha lattice design with two replication and phenotypic data were analyzed using sixteen agro-morphological traits. The seventy single cross maize hybrids including five checks grouped into sixteen clusters based on Mahalanobis D^2 statistic. Cluster III (25) was the biggest one which accommodated maximum number of genotypes followed by cluster I (22), cluster XI (6) and cluster II (5). Presence of twelve solitary clusters indicated larger genetic diversity. Based upon the divergence studies suggesting crossing may be made between genotypes of cluster II (ZH17365, VH11128, HT5102, NK6240 and GM6) and cluster XV (ZH17380), and the hybrid in these cluster could be used as donor parents for new double cross maize hybrid development for improving yield under water logging condition.

Keywords: Genetic diversity, Mahalanobis D^2 statistic, water logging and clusters

Introduction
Maize (*Zea mays* L., 2n= 20) is the third most important cereal crop next to wheat and rice (Kumawat *et al*., 2020; Kumawat, 2020) [3, 4, 5]. Maize has the highest production potential among cereals and wider adaptability so it is also referred to as the "Queen of cereals" (Morris *et al*., 1999, Kumawat *et al*., 2020) [10, 3,4, 5]. As per the first Advance Estimate published by Ministry of Agriculture and Farmer Welfare on 26 September 2018, in India production of maize is 21.47 Mt. Among the various abiotic stresses, water-logging, caused by unforeseen flooding, continuous rainfall and inadequate soil drainage or a high water table, is one of the most imperative constraints for maize production in South Asia and many other countries of the world (Rathore *et al*., 1997) [12]. In India, water-logging is the second most serious constraint, after drought, where about 8.5 Mha of arable soil is affected by water logging problem. Out of the total 6.55 Mha area of maize, about 2.5 Mha are affected by excess soil moisture problems that cause, on average, 25-30% loss of national maize production almost every year (Zaidi *et al.,* 2016) [18]. So, by exploiting diverse lines in hybridization programme of maize which are excess moisture resistance/tolerance can reduce this stress losses. Precise information on the nature and degree of genetic divergence would help the researcher in selecting the best parents for different plant breeding procedures. For developing high yielding double cross hybrids in maize, single cross hybrid lines need to be evaluated for their genetic diversity. Several studies on maize have shown that single crosses from genetically diverse parents tend to be more productive than crosses of single crosses lines from same source (Singh *et al*., 2015) [14, 15]. The manifestation of heterosis in the progeny is usually depends on the genetic divergence of two parents (Saxena *et al*., 1998) [13]. Therefore, characterization of genetic diversity of maize genotypes is of great importance in hybrid maize breeding (Xia *et al*., 2005) [17]. It has become possible to quantify magnitude of genetic diversity among hybrids with the help of advanced biometrical methods such as multivariate analysis (Rao, 1952) [11] based on Mahalanobis (1936) [7] D^2 statistics. With this view, genetic diversity among 70 newly developed maize hybrids was studied using multivariate approaches of analysis of variation to select out diverse water logging tolerance single cross hybrids to develop high yielding double cross hybrid.

Material and Method
The experiment consisting of 70 maize hybrids, was conducted at Agricultural Research farm of Institute of Agricultural Sciences, Banaras Hindu University during *kharif* 2017-18.

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The 65 maize single cross hybrids developed from CIMMYT(International Maize and Wheat Improvement centers) projects, "Climate Resilient Maize for Asia (CRMA)" along with (five checks- 900MG (Monsanto), P3502 (Pioneer), HT5106 (Hytech), NK6240 (Syngenta) and GM6 (A.A.U.)). The experimental material was laid out in alpha lattice design with two replications. The sowing was taken up on 25th June 2017 and the ears were harvested on 12th October 2017. Each genotype was sown in two rows of three meters each in length with a spacing of 60x25cm with ten plants per row. Water-logging stress was enforced for one week at the 'Knee height stage' (V_6-V_7 growth stage). The depth of water level maintained at the level of 2.0-3.0 inches above the ground surface in the field (water stagnation). Proper bunding was done so that water remains within, and after one week, the plots were drained completely through surface drainage (Zaidi *et al,* 2016) [18]. The observations were recorded for sixteen characters like (pre-harvest) number of surface roots, number of nodes bearing brace roots, days to 50% anthesis, days to 50% silking, anthesis-silking interval, number of plants per plot, number of ears per plot, plant height, ear height and (post-harvest) ear length, ear diameter, number of kernel rows per ear, number of kernels per row, 100 seed weight and yield per plant.

Mahalanobis (1936) [7] D^2 statistics was used for estimating the pair wise genetic divergence among all the genotypes. The genetic divergence between any two populations was calculated using the formula.

$$D^2p = d^1 S^{-1}d$$

Where,

D^2p= Square of distance considering p traits
d = Vector of observed differences of the average values of p traits
d^1= Transpose of vector of observed differences of the average value of p traits
S^{-1}= Inverse of variance and covariance matrix

The genotypes were arranged into different clusters following Tocher's method as described by Rao (1952) [11]. The Intra-cluster distances were calculated by the formula suggested by Singh and Chaudhary (1977) [16].

$$\text{Intra-cluster distance} = \sqrt{\frac{\sum D^2_i}{n}}$$

Where,

$\sum D^2_i$ = sum of squared distance between all possible combinations of genotypes include in cluster
n = number of all possible combinations
The inter-cluster distances were calculated according to Singh and Chaudhary (1977) [16].

$$\text{Inter cluster distance} = \sqrt{\frac{\sum D^2_i}{n_i n_j}}$$

Where,

$\sum D^2_i$ = sum of squared distance between all possible combinations of genotypes include in clusters i and j.
n_i = number of genotypes in clusteri
n_j = number of genotypes in cluster j

Result and Discussion
Grouping of genotypes into various clusters
Genetic diversity is essential to develop genotype with increased yield, wider adaptation, and desirable qualities. The genetic divergence conducted to identify the genetic distance between the experimental genotypes. Inclusion of more diverse parents (within a limit) in hybridization supposed to increase the chance of obtaining greater heterosis and also provide a broad spectrum of variability in segregating generations. With this aim, an attempt was made in the present study to analyse genetic diversity using Mahalanobis D^2 statistic as suggested by Rao (1952) [11] among 70 genotypes of maize based on yield and yield attributing traits. A method suggested by Mahalanobis was used to groups the genotypes into different clusters based on the D^2 values and a dendrogram has been made. It grouped seventy genotypes including five checks into sixteen clusters (Table 1 and Fig 1) using the Tocher's method, indicating the presence of diversity for different traits. Similar result was found by Mani and Deshpande (2016) [8] and Kumar *et al.* (2017) [2]. Out of sixteen clusters, Cluster III was the biggest, consisting of twenty-five genotypes followed by cluster I (twenty two genotypes), cluster XI (six genotypes) and cluster II (five genotypes). Genotype ZH17386, ZH17375, ZH12112, ZH17373, ZH17223, ZH17354, ZH17351, ZH17192, ZH17367, ZH17371, ZH17380 and ZH17384 formed a distinct cluster IV, V, VI, VII, VIII, IX, X, XII. XIII, XIV, XV and XVI respectively and these were named as a mono-genotypic cluster. The pattern of distribution of genotypes into various clusters was at random indicating that geographical and genetic diversity were not related. The pattern of group constellations indicated significant variability among the genotypes.

Intra- and inter-cluster divergence
The average intra and inter cluster D^2 values estimated as per the procedure was given by Singh and Chaudhary (1977) [16] and were presented in Table 2 and Fig 2. The intra and inter cluster D^2 values indicated that inter cluster D^2 values were higher than intra cluster D^2 values. The intra-cluster distances indicate the divergence among all the genotypes within the clusters and inter-cluster indicates diversity between clusters. The intra cluster D^2 value ranged from 0.00 to 569.30. The cluster XI had the maximum D^2 value (23.86) followed by cluster III (456.68) cluster II (165.12) and cluster I (153.76) while it was zero for cluster IV (ZH17386), cluster V (ZH17375), cluster VI (ZH12112), cluster VII (ZH17373), cluster VIII (ZH17223), cluster IX (ZH17354), cluster X (ZH17351), cluster XII (ZH17192), cluster XIII (ZH17367), cluster XIV (ZH17371), cluster XV (ZH17380) and cluster XVI (ZH17384). The high intra cluster distance in cluster V indicated the presence of wide genetic diversity among the genotypes *viz.,* ZH17347, ZH17352, ZH17379, ZH17383, ZH17391, P3502.

The inter cluster D values of the sixteen clusters observed that highest inter cluster distance was between cluster II and cluster XV (108.60) followed by cluster XI and XV (88.69), VI and XVI (79.82), XIII and XVI (78.21) (Table 3) suggesting more variability in genetic makeup of the hybrids included in these clusters. The information obtained from inter-cluster distances might be used to select genetically diverse and superior genotypes. Based on inter cluster D value crosses may be made between genotypes of clusters II (ZH17365, VH11128, HT5102, NK6240, GM6) and cluster XV (ZH17380) followed by genotypes of clusters XI

(ZH17347, ZH17352, ZH17379, ZH17383, ZH17391, P3502) and cluster XV (ZH17380) to obtain new desirable recombinants in maize. Minimum inter cluster distance was recorded between cluster IV and VII (11.50). The genotypes belonging to the clusters separated by high statistical distance could be used in hybridization programme for obtaining a wide spectrum of variation among the segregates. These findings are in conformity with the findings of Singh *et al*., (2015) [14, 15] Ganesan *et al*. (2010) [1] and Marker and Krupakar (2009) [9].

Mean performance of characters within clusters
Based on range of means, it is possible to know the characters influencing divergence. It helps to identify clusters having different levels of variability for different characters, based on final ranks it is possible to identify clusters having higher and lesser diversity for more number of characteristics. Utilization of low ranked clusters in breeding programme is expected to yield desirable lines in advanced generation of selection.

Cluster VI was showed maximum number of surface roots and cluster V showed maximum number of nodes bearing brace roots among the all cluster studied (Table 4). Cluster IV should be selected for ear length and cluster V for ear diameter, and number kernel rows per ear. With respect to yield, cluster II exhibited highest value whereas cluster XV, a monogenic cluster exhibited lowest value. Cluster VIII was showing highest100 seed weight and cluster VI showing maximum field weight among the entire cluster studied. Cluster means were always greater than the overall mean of the genotypes. Therefore, while developing hybrids for water-logging tolerance, combination of desirable characters should be kept in mind for selecting tolerant hybrids in subsequent generations of breeding programme. Similar results also found by Ganesan *et al*. (2010) [1]. Therefore, it is advised that most diverse clusters may be exploited as parents in hybridization programme to develop high yielding maize hybrids (Lahane *et al*., 2016) [6].

Table 1: The distribution of the 70 maize (*Zea mays* L.) genotypes into different clusters on the basis of D^2 statistics

S.N.	Cluster number	Genotypes
1	Cluster I	ZH17346, ZH17349, ZH17350, ZH17356, ZH17359, ZH17360, ZH17361, ZH17362, H17366, ZH17368, ZH17376, ZH17378, ZH17381, ZH17387, ZH17390, ZH17393, ZH17394, ZH17395, ZH138256, ZH17398, 900MG
2	Cluster II	ZH17365, VH11128, HT5102, NK6240, GM6
3	Cluster III	ZH17344, ZH17345, ZH17348, ZH17353, ZH17355, ZH17357, ZH17358, ZH17363, ZH17364, ZH138025, ZH17369, ZH17370, ZH14595, VH112976, ZH17372, ZH17374, ZH17377, ZH17382, ZH17385, ZH17388, VH142037, ZH17392, ZH17396, ZH17397, ZH17399
4	Cluster IV	ZH17386
5	Cluster V	ZH17375
6	Cluster VI	ZH12112
7	Cluster VII	ZH17373
8	Cluster VIII	ZH17223
9	Cluster IX	ZH17354
10	Cluster X	ZH17351
11	Cluster XI	ZH17347, ZH17352, ZH17379, ZH17383, ZH17391, P3502
12	Cluster XII	ZH17192
13	Cluster XIII	ZH17367
14	Cluster XIV	ZH17371
15	Cluster XV	ZH17380
16	Cluster XVI	ZH17384

Table 2. Average intra (bold) and inter-cluster D^2 values for sixteen clusters in 70 genotypes of maize (*Zea mays* L.).

Cluster	ClusterI	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII	Cluster VIII	Cluster IX	Cluster X	Cluster XI	Cluster XII	Cluster XIII	Cluster XIV	Cluster XV	Cluster XVI
Cluster I	**153.76**	482.24	846.81	376.75	350.06	379.47	305.20	286.62	265.04	781.20	598.78	385.34	1168.96	1305.38	3955.15	1830.13
Cluster II		**165.12**	3307.40	739.30	500.42	1367.52	701.19	491.95	636.55	2513.02	650.76	1265.94	3453.91	4139.64	11793.96	3614.41
Cluster III			**456.68**	1593.61	1943.93	1293.12	919.30	1501.56	1069.94	777.85	2376.56	813.39	914.46	877.94	837.52	1840.41
Cluster IV				**0.00**	231.04	907.82	132.25	730.08	801.46	724.15	734.41	836.94	2785.73	1420.54	5126.56	1921.95
Cluster V					**0.00**	955.43	236.85	431.39	384.16	1893.12	1022.08	982.82	2194.92	1163.49	7130.11	1644.30
Cluster VI						**0.00**	1202.01	555.07	1145.82	1288.81	2059.34	663.58	560.74	2328.06	4884.61	6371.23
Cluster VII							**0.00**	358.72	794.68	811.11	827.71	606.14	1466.12	575.52	3504.64	523.95
Cluster VIII								**0.00**	883.28	2564.41	814.53	562.64	837.52	1918.44	5759.29	2268.62
Cluster IX									**0.00**	556.96	632.52	821.97	1740.56	1564.20	4856.70	2153.89
Cluster X										**0.00**	1802.00	2102.22	1534.29	1156.00	1854.16	3274.13
Cluster XI											**569.30**	1374.93	4303.36	3547.39	7865.92	2202.42
Cluster XII												**0.00**	1198.54	2114.16	3011.81	2303.04
Cluster XIII													**0.00**	1568.16	2659.46	6116.80
Cluster XIV														**0.00**	1340.29	466.13
Cluster XV															**0.00**	3139.36
Cluster XVI																**0.00**

Table 3: The nearest and farthest s from each based on D values among with the maize (*Zea mays* L.) studied

S.N.	Cluster No	Nearest with D value	Farthest with D value
1	Cluster I	XIV (16.28)	XVI (42.78)
2	Cluster II	VIII(22.18)	XV (108.60)
3	Cluster III	XII (28.52)	XI (48.75)
4	Cluster IV	VII(11.50)	XIII (52.78)
5	Cluster V	VII (15.39)	XV (84.44)
6	Cluster VI	VIII (23.56)	XVI (79.82)
7	Cluster VII	VIII (18.94)	XV (59.20)
8	Cluster VIII	XII (23.72)	XV (75.89)
9	Cluster IX	X (23.60)	XV (69.69)
10	Cluster X	XIV (34.00)	XVI (57.22)
11	Cluster XI	XII (37.08)	XV (88.69)
12	Cluster XII	XII (34.62)	XV (54.88)
13	Cluster XIII	XIV (39.60)	XVI (78.21)
14	Cluster XIV	XVI (21.59)	XV (36.61)
15	Cluster XV	III (28.94)	II (108.60)
16	Cluster XVI	XIV (21.59)	VI (79.82)

Table 4: Mean values of sixteen characters in 70 genotypes of maize (*Zea mays* L.)

| Cluster	Number of surface roots	Number of nodes bearing brace roots	Days to 50% anthesis	Days to 50% silking	Anthesis-silking Interval	Plant height (cm)	Ear height (cm)	Number of plants per plot	Number of ears per plot	Field weight (g)	Ear length (cm)	Ear diameter (cm)	Number of kernel rows per ear	Number of kernels per row	Yield per plant (g)	100 seed weight (g)
I	10.91	3.36	58.77	60.39	1.61	125.68	55.34	12.41	11.23	0.88	11.52	3.69	12.68	20.20	69.53	25.55
II	8.50	2.30	60.20	62.30	2.10	119.00	52.00	13.20	11.50	1.15	10.95	3.78	13.32	18.88	82.00	28.14
III	10.36	3.36	58.90	61.04	2.14	119.40	52.10	9.76	8.92	0.63	10.79	3.51	12.80	18.84	49.29	24.12
IV	11.50	5.00	62.00	63.00	1.00	125.00	62.50	12.50	10.50	0.90	13.25	3.23	13.00	22.60	73.75	24.79
V	8.00	6.00	58.00	60.00	2.00	145.00	55.00	16.50	12.50	1.26	12.83	4.06	14.00	20.60	74.10	27.38
VI	15.00	2.50	53.50	55.50	2.00	142.50	65.00	14.00	18.00	1.60	12.01	3.79	13.50	20.80	78.90	23.86
VII	11.50	2.50	62.00	64.50	2.50	142.50	67.50	13.00	9.00	0.58	12.35	3.55	13.60	19.20	63.45	25.97
VIII	13.00	2.50	55.00	58.00	3.00	140.00	70.00	13.50	12.00	1.17	13.03	3.84	12.80	21.70	76.00	33.06
IX	6.00	5.00	58.50	60.50	2.00	95.00	37.50	15.50	11.50	0.60	11.57	3.95	13.20	21.00	67.50	24.99
X	13.50	2.50	60.50	62.50	2.00	82.50	42.50	15.50	8.00	0.32	12.06	3.35	13.00	22.60	52.60	17.73
XI	9.83	2.92	59.50	61.58	2.08	110.83	42.92	7.92	5.83	0.45	12.11	3.47	12.67	20.63	73.79	26.24
XII	4.00	3.50	58.50	60.00	1.50	150.00	77.50	8.50	13.00	1.02	11.53	3.65	12.60	22.20	74.51	25.48
XIII	13.00	3.00	49.50	56.50	7.00	132.50	80.00	17.00	14.00	1.07	11.05	3.76	13.40	18.80	61.00	26.44
XIV	12.00	3.50	63.50	69.00	5.50	127.50	50.00	12.00	10.50	0.69	12.17	3.74	11.80	20.30	42.25	22.92
XV	10.00	2.50	62.00	62.50	0.50	100.00	45.00	5.00	5.00	0.75	11.61	3.52	12.80	21.20	27.90	23.49
XVI	8.00	3.00	68.00	71.00	3.00	140.00	37.50	6.00	4.00	0.12	11.28	3.53	11.80	18.00	41.30	26.30
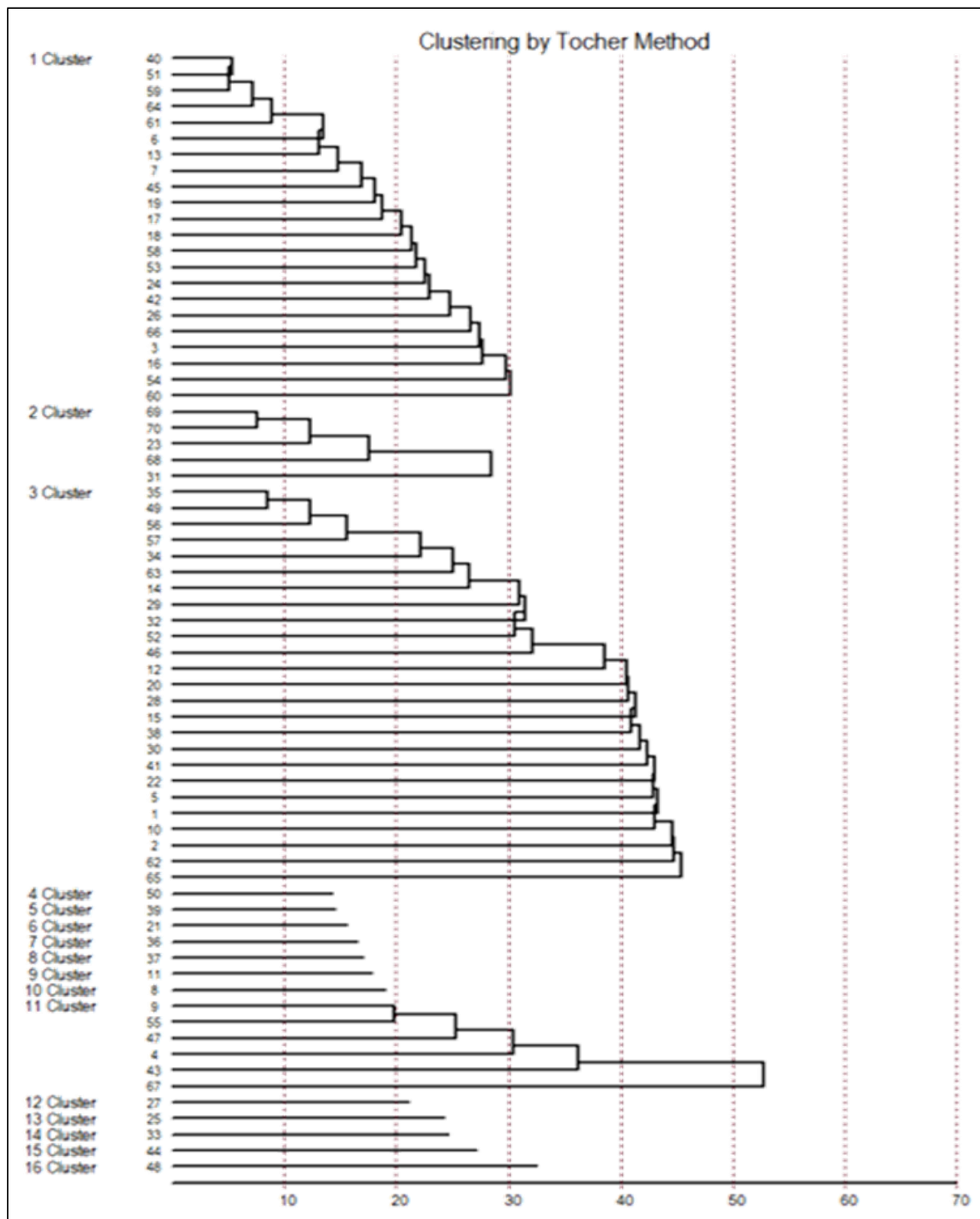
Fig 1: Dendrogram showing relationship among 70 maize genotypes in sixteen clusters based on Mahalanobis's D^2 values.
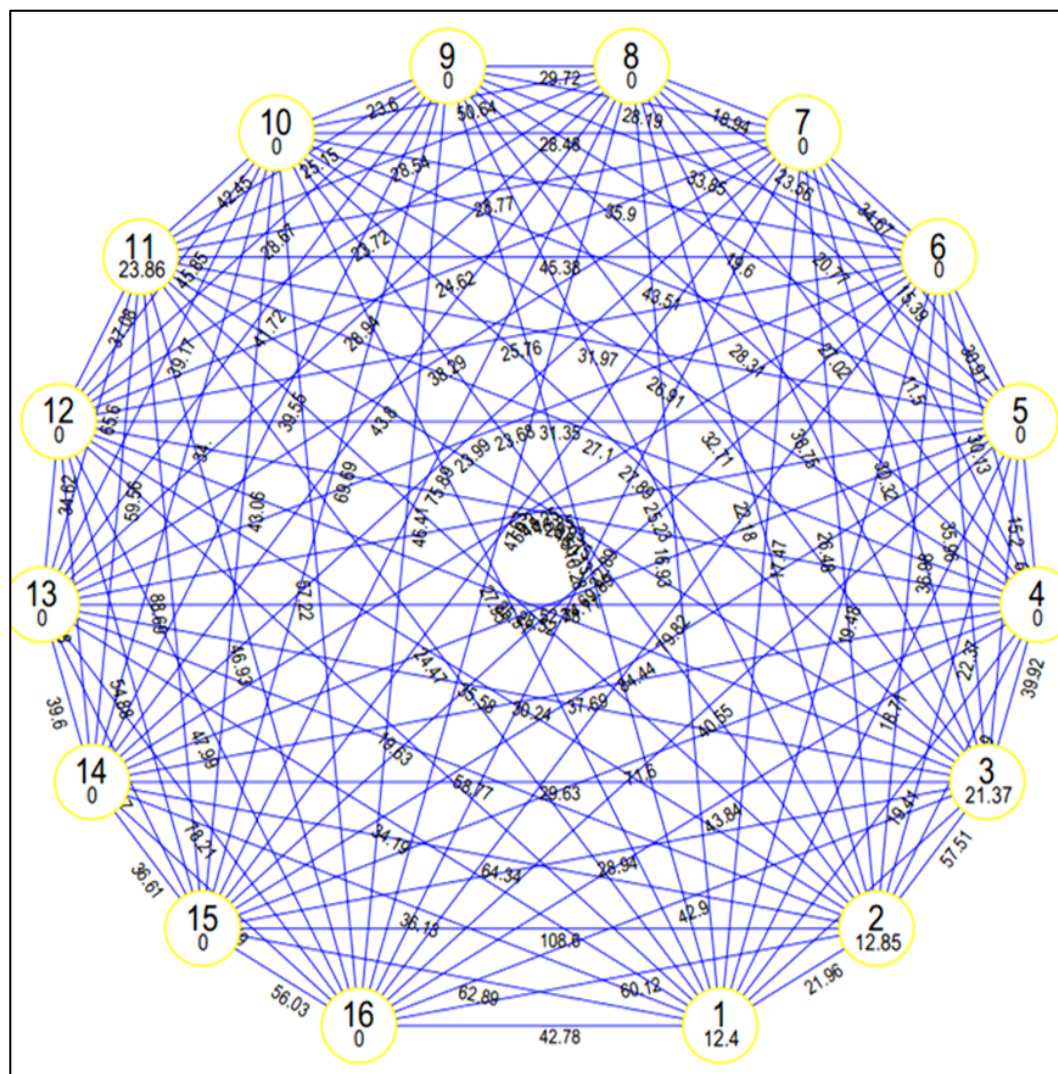
Fig. 2: Intra-and Inter-cluster distances of 70 maize in sixteen clusters based on Euclidean D^2 distances

Conclusion
Genetic divergence study is essential to develop cultivars with higher yields, wider adaptation, desirable qualities, abiotic and biotic resistance. The present investigation indicated maximum difference between cluster II (ZH17365, VH11128, HT5102, NK6240 and GM6) and cluster XV (ZH17380) were recommended maximum diversity. So the genotypes belonging to these clusters can be exploited in future breeding programmes for improving yield under water logging condition.

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