

Assessment of Genetic Diversity of Maize (*Zea mays* L.) Inbreds Based on Morphological Traits

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Authors' contributions

This work was carried out in collaboration among all authors. Author MS designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors JPS and RS supervised the study and provided necessary resources. Author AS managed the analyses of the study. Authors PD and PS managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The screening of forty eight maize inbreds from International Maize and Wheat Improvement Center was carried out to estimate the genetic diversity and the results indicated presence of considerable diversity. The experiment was conducted in a randomized complete block design with three replication and phenotypic data were analyzed using twenty one agro-morphological traits. The forty eight maize inbreds grouped into eleven clusters based on Mahalanobis D^2 statistic. Among them cluster I accommodated maximum number of inbred lines (15) followed by cluster III and V (9). Existence of six solitary clusters showed larger genetic diversity. The character chlorophyll content showed highest contribution towards genetic divergence followed by anthesis to silking interval, cob girth, cob weight, hundred grain weight, number of kernels per row, days 75 per cent brown husk, plant height at 90 DAS, cob length and number of kernel rows per cob. Based

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upon the divergence studies suggesting crossing may be made between genotypes of cluster VIII (CAL1428) and cluster X (ZL154357, CAL1467, ZL153785, CAL1422, and ZL154346) and the hybrid in these cluster could be used as donor parents for new single cross maize hybrid development for improving yield.

Keywords: Genetic diversity; Mahalanobis D²; statistic; clusters.

1. INTRODUCTION

Maize or corn is known as “Queen of Cereals” because of its productivity and wider adaptability among all cereals. It is one of the world’s most widely cultivated crops and is the main staple food in many developing countries [1]. In India, corn is the third most important food crops following rice and wheat. Madhya Pradesh has the highest area (1.35 Million Hectares) and production (3.54 Million Tonnes) whereas Tamil Nadu has the highest productivity (59.84 Quintals/Hectare) (www.agricoop.nic.in).

Accurate information about the nature and degree of genetic divergence would assist the plant breeder in choosing the ideal parents for different breeding procedures. For developing high yielding single cross hybrids in maize, inbred lines need to be evaluated for their genetic diversity. Several studies on maize have shown that inbreds from genetically diverse parents tend to be more productive than inbred lines from same source [2]. The appearance of heterosis in the offspring is usually depends on the genetic divergence of two parents [3]. Therefore, characterization of genetic diversity of maize germplasm lines is of great importance in hybrid maize breeding [4]. It has become possible to quantify magnitude of genetic diversity among inbreds with the help of advanced biometrical methods such as multivariate analysis [5] based on Mahalanobis D² statistics [6]. With this view, genetic diversity among 48 maize inbreds was studied using multivariate approaches of analysis of variation to select out diverse to develop high yielding single cross hybrid.

2. MATERIALS AND METHODS

The experimental material comprised of 48 maize inbred lines which were obtained from International Maize and Wheat Improvement Center, Hyderabad, India. All maize inbreds were sown in randomized complete block design with three replications at the Agriculture Research Farm of Institute of Agricultural Sciences, Banaras Hindu University during Rabi 2019. Each inbred was planted in two rows with three

meters in length and a spacing of 60×25 cm with ten plants per row. Soil type of experimental site is sandy loam. The results obtained from the statistical analysis of the data from 48 maize inbreds for 21 characters viz., days to 50 per cent anthesis, days to 50 per cent silking, anthesis to silking interval, days 75 per cent brown husk, plant height 30DAS (days after sowing), plant height 60DAS, plant height 90DAS, number of cobs per plant, cob height, cob length, cob girth, number of kernel rows per cob, number of kernels per row, number of grains per cob, cob weight, hundred grain weight, shelling percentage, grain yield, chlorophyll content, relative water content and leaf area index.

Chlorophyll content of leaves was measured by using SPAD chlorophyll meter (KONICA MINOLTA, SPAD 502 PLUS, Version: 1.20.0000) which measures the light attenuation at 430 nm (The peak wave length absorption by chlorophyll a and chlorophyll b) and at 750 nm (near infrared) with no transmittance. The unit-less value measured by the chlorophyll meter is termed as SCMR (SPAD chlorophyll meter reading) which indicates relative amount of leaf chlorophyll. The SPAD meter is a simple hand held instrument, which operates with DC power of three volts. The fully expanded second leaf from the top of the main stem was used to record SCMR. Observations were recorded from 9.00 AM to 11.00 AM in the morning. Selected leaf was clamped avoiding the mid rib region and inserted into the sensor head of SPAD meter. A gentle stroke was given to record the SPAD reading.

The relative water content was estimated as per the method of Barrs and Weatherly [7]. The discs from the third leaf from top were collected randomly in each genotype and weighed accurately up to three decimals on an electronic balance. This was considered as fresh weight. The weighed leaf discs were allowed to float on distilled water in a petridish and allowed to absorb water for four hours. After four hours, the leaf discs were blotted gently and weighed. This was referred to as the turgid weight. After taking turgid weight, the leaf discs were dried in an oven at 80°C for 48 hours and the dry weight

was recorded. The RWC was calculated by using the following formula.

$$\text{RWC (\%)} = \frac{\text{Fresh weight (g)} - \text{Dry weight (g)}}{\text{Turgid weight (g)} - \text{Dry weight (g)}} \times 100$$

The leaf area index was estimated by dividing leaf area per plant to the ground area covered by the plants [8].

$$\text{LAI} = \frac{\text{Leaf area per plant in cm}^2}{\text{Land area per plant in cm}^2}$$

The crop was raised as per the recommended agronomic package of practices. The statistical analysis of data based on the mean value of recorded observations on five random plant basis were done for determining the genetic diversity of the experimental inbred. The analysis was carried out by using INDOSTAT software version 9.2. The data collected on different characters were analyzed using Mahalanobis' D^2 statistics to determine the genetic divergence among the genotypes [9]. The grouping of genotypes into different clusters was done using Tocher's method as described by Rao [5].

3. RESULTS AND DISCUSSION

The percent contributions of individual characters towards total genetic divergence by the major contributing traits out of twenty one studied traits are presented in Table 1 and the analysis of variance for all the traits present in Table 6. The character SPAD value showed highest contribution (61.08) towards genetic divergence followed by anthesis to silking interval (17.46), cob girth (15.51), cob weight (2.66), hundred grain weight (1.86), number of kernels per row (0.09), days 75 per cent brown husk (0.18), plant height 90 DAS (0.09), cob length (0.09) and number of kernel rows per cob (0.09). The findings were supported by Singh et al. [10] and Maruthi et al. [11] who also identified above said characters as the principal components contributing maximum to the total variation in maize. These characters should be given importance for selecting diverse parents for yield breeding programmes in maize [12]. Because the traits chlorophyll has direct roles on photosynthesis and the trait anthesis to silking interval determines reproductive success. Hence above mentioned traits closely relates to development and yield of crops. The results are supported by many studies [13,14].

From the point of selecting the parents for hybridization, which are divergent enough for the

character of interest, estimation of the genetic distance is most important. The present estimation of genetic divergence gave clear idea about the diverse nature of the population. Wide range of variation was observed in cluster mean performance for most of the characters studied. All the genotypes were grouped into eleven clusters, using the Tocher's method with the criterion that the intra-cluster average D^2 values should be less than the inter-cluster D^2 values, indicating the presence of diversity for different traits. The distribution of 48 inbreds into eleven clusters is presented in Table 2 with the maximum number of 15 inbreds in cluster I. Whereas Cluster III and V is the second largest with nine inbreds and cluster II (CIL12137), IV (ZL154349), VI (CAL1475), VII (CAL1428), IX (ZL153773) and XI (CZL03003) consists of single inbred in each. The formation of distinct solitary clusters may be due to geographic barriers preventing gene flow otherwise intensive natural and artificial selection for diverse and adaptable gene complexes must be responsible for this genetic diversity. The clustering pattern of inbred lines showed that the inbred lines had significant genetic divergence among themselves for the studied traits. These results are in agreement with that of Mani and Deshpande [15] and Alam [16].

The average intra and inter cluster D^2 values estimated as per the procedure was given by Singh and Chaudhary [17] and were presented in Table 3 and Fig. 2. Dendrogram represented the relationship among 48 maize genotypes in eleven clusters based on Mahalanobis's D^2 values are presented in Fig. 1. The intra and inter cluster D^2 values indicated that inter cluster D^2 values were higher than intra cluster D^2 values. The maximum intra cluster D^2 value was 1497.84 for cluster VII followed by 1481.09 for cluster V while it was zero for cluster II (CIL12137), IV (ZL154349), VI (CAL1475), VIII (CAL1428), IX (ZL153773) and XI (CZL03003). The high intra cluster distance in cluster VII indicated the presence of wide genetic diversity among the genotypes viz., ZL154351, ZL154377, CAL1427 and CZL03003.

Maximum inter cluster distance was recorded between the clusters VIII and X (18275.22) followed by cluster XI and X (14322.01) suggesting more variability in genetic makeup of the inbreds included in these clusters. Based on inter cluster D value crosses may be made between genotypes of clusters VIII (CAL1428) and cluster X (ZL154357, CAL1467, ZL153785,

CAL1422, ZL154346) followed by genotypes of clusters XI (CZL03003) and X (ZL154357, CAL1467, ZL153785, CAL1422, ZL154346) to obtain new desirable recombinants in maize. Minimum inter cluster distance was recorded between cluster I and VIII (6121.19). The genotypes from the clusters separated by high statistical distance could be utilized in hybridization programme for obtaining a broad spectrum of variation among the segregates. These findings are in agreement with the findings of Singh [2] and Marker and Krupakar [18].

Wide range of mean values among the cluster was recorded for different characters. Analysis of cluster means revealed the relative contribution of different characters to the total divergence by the different clusters. Based on range of means, it is possible to know the traits influencing divergence. It helps to identify clusters having different levels of variability for different traits,

based on final ranks it is possible to identify clusters having higher and lesser diversity for more number of traits. Utilization of low ranked clusters in crop improvement programme is anticipated to yield desirable lines in advanced generation of selection. The mean values of twenty one characters studied in 48 maize genotypes for eleven clusters are presented in Table 5.

Cluster VI had the highest mean value for plant height 30 DAS, plant height 60 DAS, plant height 90 DAS, cob height, number of kernels per row, number of grains per cob and relative water content. Cluster IV had the highest mean value for cob length, cob girth, hundred grain weight and grain yield. Cluster VIII had maximum mean value for days to 50 per cent anthesis, days to 50 per cent silking and days to 75 per cent brown husk. Cluster XI had the highest mean value for anthesis to silking interval, number of cobs per plant and cob weight.

Table 1. Contribution of different characters towards genetic divergence among 48 maize inbreds

| Characters | Contribution toward divergence |
|-------------------------------|--------------------------------|
| Anthesis to silking Interval | 17.46 |
| Days 75 per cent brown husk | 0.18 |
| Plant height 90DAS(cm) | 0.09 |
| Cob length (cm) | 0.09 |
| Cob girth (cm) | 15.51 |
| Number of kernel rows per cob | 0.09 |
| Number of kernels per row | 0.98 |
| Cob weight (g) | 2.66 |
| Hundred grain weight (g) | 1.86 |
| Chlorophyll content | 61.08 |

Table 2. The distribution of the 48 maize (*Zea mays* L.) inbreds into different clusters on the basis of D² statistics

| Clusters | Number of genotypes included | Name of genotypes |
|----------|------------------------------|--|
| I | 15 | CAL1411, CIL12105, CAL1447, VL108308, ZL11412, ZL153771, ZL14475, CIL1224, ZL136820, CIL12146, CZL0721, ZL12139, ZL153780, CAL1476, ZL154350 |
| II | 1 | CIL12137 |
| III | 9 | ZL154347, CIL1218, ZL154344, ZL11266, VL062625, CIL12161, L154341, ZL12903, CAL1444 |
| IV | 1 | ZL154349 |
| V | 9 | ZL154362, ZL153787, CZL0617, ZL1622, CAL1441, CAL1415, ZL154371, CAL1423, ZL153781 |
| VI | 1 | CAL1475 |
| VII | 4 | ZL154351, ZL154377, CAL1427, CZL03003 |
| VIII | 1 | CAL1428 |
| IX | 1 | ZL153773 |
| X | 5 | ZL154357, CAL1467, ZL153785, CAL1422, ZL154346 |
| XI | 1 | CZL03003 |

Table 3. Average intra (bold) and inter-cluster D² values for eleven clusters in 48 inbreds of maize (*Zea mays* L.)

| | Cluster I | Cluster II | Cluster III | Cluster IV | Cluster V | Cluster VI | Cluster VII | Cluster VIII | Cluster IX | Cluster X | Cluster XI |
|--------------|------------------|-------------------|--------------------|-------------------|------------------|-------------------|--------------------|---------------------|-------------------|------------------|-------------------|
| Cluster I | 984.84 | 1496.45 | 2349.01 | 1479.52 | 2288.16 | 1447.5 | 2347.16 | 6121.19 | 1806.82 | 4808.91 | 3879.51 |
| Cluster II | | 0 | 3573.58 | 2600.04 | 914.25 | 2499.9 | 2890.48 | 2303.15 | 4538.26 | 8704.03 | 1161.75 |
| Cluster III | | | 1204.41 | 1947.4 | 4104.92 | 4429.44 | 4910.32 | 9615.62 | 2189.18 | 3108.17 | 7879.73 |
| Cluster IV | | | | 0 | 2631.73 | 1662.05 | 1895.92 | 7066.89 | 1826.29 | 4431.08 | 5923.16 |
| Cluster V | | | | | 1481.09 | 3075.87 | 3283.88 | 2563.96 | 5287.26 | 9500.69 | 2219.9 |
| Cluster VI | | | | | | 0 | 1055.08 | 6788.98 | 1916.68 | 6218.9 | 4032.86 |
| Cluster VII | | | | | | | 1497.84 | 6786.92 | 3203.24 | 7595.82 | 4210.26 |
| Cluster VIII | | | | | | | | 0 | 11981.83 | 18275.22 | 1723.93 |
| Cluster IX | | | | | | | | | 0 | 1639.05 | 8424.35 |
| Cluster X | | | | | | | | | | 1397.31 | 14322.01 |
| Cluster XI | | | | | | | | | | | 0 |

Table 4. The nearest and farthest clusters from each cluster based on D values in maize (*Zea mays* L.) genotype

| Cluster No. | Nearest cluster with D values | Farthest cluster with D value |
|--------------------|--------------------------------------|--------------------------------------|
| Cluster I | VI (1447.5) | VIII(6121.19) |
| Cluster II | V (914.25) | X (8704.03) |
| Cluster III | IV (1947.4) | VIII (9615.62) |
| Cluster IV | I (1479.52) | VIII (7066.89) |
| Cluster V | II (914.25) | X (9500.69) |
| Cluster VI | VII (1055.08) | VIII (6788.98) |
| Cluster VII | VI (1055.08) | X (7595.82) |
| Cluster VIII | XI (1723.93) | X (18275.22) |
| Cluster IX | X (1639.05) | XI (8424.35) |
| Cluster X | IX (1639.05) | VIII (18275.22) |
| Cluster XI | II (1161.75) | X (14322.01) |

Table 5. Mean values of eleven clusters by Tocher's method for 48 genotypes of maize (*Zea mays* L.)

| | Days to 50 per cent anthesis | Days to 50 per cent silking | Anthesis to silking Interval | Days 75 per cent brown husk | Plant height 30DAS (cm) | Plant height 60DAS (cm) | Plant height 90DAS (cm) | Number of cobs per plant | Cob height (cm) | Cob length (cm) |
|--------------|-------------------------------------|------------------------------------|-------------------------------------|------------------------------------|--------------------------------|--------------------------------|--------------------------------|---------------------------------|------------------------|------------------------|
| Cluster I | 63.85 | 65.89 | 2.04 | 111.2 | 92.31 | 163.23 | 173.23 | 1.78 | 94.79 | 16.09 |
| Cluster II | 59.93 | 62.17 | 2.23 | 104.33 | 94.4 | 164.4 | 174.4 | 2.07 | 78.5 | 16.6 |
| Cluster III | 63.82 | 65.78 | 1.96 | 111.07 | 92.4 | 164.91 | 174.91 | 1.7 | 89.31 | 17.14 |
| Cluster IV | 62.57 | 64.33 | 1.77 | 109.33 | 85.07 | 155.07 | 165.07 | 1.27 | 80.7 | 22.9 |
| Cluster V | 64.23 | 66.17 | 1.94 | 111.03 | 92.04 | 169.65 | 179.65 | 1.71 | 98.57 | 18.05 |
| Cluster VI | 55.27 | 57.33 | 2.07 | 109.67 | 100.07 | 170.07 | 180.07 | 2.03 | 123 | 17.27 |
| Cluster VII | 62.13 | 63.79 | 1.66 | 109.23 | 88.37 | 157.87 | 167.87 | 1.17 | 85.45 | 18.78 |
| Cluster VIII | 66 | 68.17 | 2.17 | 117.13 | 82.73 | 146.07 | 156.07 | 2.07 | 93.4 | 17.2 |
| Cluster IX | 63.3 | 65.33 | 2.03 | 110 | 93.8 | 163.8 | 173.8 | 1.33 | 106.33 | 15.07 |
| Cluster X | 63.64 | 65.7 | 2.06 | 109.07 | 78.19 | 135.79 | 145.79 | 1.51 | 89.9 | 13.79 |
| Cluster XI | 59.83 | 62.33 | 2.5 | 96.47 | 72.87 | 129.53 | 139.53 | 2.17 | 96.6 | 14.4 |

| | Cob girth (cm) | Number of kernel rows per cob | Number of kernels per row | Number of grains per cob | Cob weight (g) | Hundred grain weight (g) | Shelling percentage (%) | Grain yield (kg/ha) | Chlorophyll content | Relative water content | Leaf area index |
|--------------|-----------------------|--------------------------------------|----------------------------------|---------------------------------|-----------------------|---------------------------------|--------------------------------|----------------------------|----------------------------|-------------------------------|------------------------|
| Cluster I | 14.61 | 14.76 | 27.08 | 398.99 | 97.97 | 26.81 | 76.28 | 3839.95 | 48.75 | 74.22 | 2.47 |
| Cluster II | 14.9 | 15.5 | 24.4 | 378.47 | 104.4 | 21.7 | 74.3 | 3902.5 | 43.97 | 68 | 2.5 |
| Cluster III | 13.05 | 15.24 | 27.33 | 422.39 | 98.33 | 26.11 | 76.73 | 3852.5 | 55.23 | 75.86 | 2.49 |
| Cluster IV | 16.2 | 15.3 | 29.33 | 450.3 | 105.7 | 35.8 | 78.87 | 4410 | 52.73 | 75.8 | 2.43 |
| Cluster V | 14.21 | 14.25 | 28.81 | 408.93 | 111.02 | 24.84 | 73.49 | 3998.98 | 46.1 | 74.13 | 2.46 |
| Cluster VI | 15.2 | 15.2 | 31.23 | 474.77 | 107.4 | 29.2 | 79.33 | 4395 | 51 | 78.3 | 2.47 |
| Cluster VII | 13.95 | 14.58 | 25.55 | 371.39 | 120.55 | 26.63 | 65.38 | 3829.75 | 55.37 | 74.96 | 2.64 |
| Cluster VIII | 13.3 | 13 | 31 | 403.07 | 108.4 | 21 | 78 | 4310 | 32.97 | 77.6 | 2.33 |
| Cluster IX | 13.33 | 15.37 | 25.23 | 387.8 | 78.33 | 19.2 | 79.33 | 3200 | 49.6 | 77.6 | 2.4 |
| Cluster X | 11.82 | 14.46 | 25.97 | 371.01 | 65.18 | 20.11 | 73.85 | 2345 | 51.27 | 75.46 | 2.53 |
| Cluster XI | 14.3 | 13.3 | 27 | 359.77 | 131.4 | 20.7 | 52.2 | 3835.83 | 49.53 | 71.1 | 2.27 |

Table 6. Analysis of variance for the twenty one studied traits in maize inbreds

| Source | D.f | Days to 50 per cent anthesis | Days to 50 per cent silking | Anthesis to silking Interval | Days 75 per cent brown husk | Plant height 30DAS (cm) | Plant height 60DAS (cm) | Plant height 90DAS (cm) | Number of cobs per plant | Cob height (cm) | Cob length (cm) | Cob girth (cm) |
|-------------|-----|------------------------------|-----------------------------|------------------------------|-----------------------------|-------------------------|-------------------------|-------------------------|--------------------------|-----------------|-----------------|----------------|
| Replication | 2 | 1244.11 | 1235.28 | 0.10 | 710.52 | 1076.28 | 4536.67 | 4536.67 | 1.13 | 1254.11 | 9.37 | 8.88 |
| Treatment | 47 | 33.09 | 32.95 | 0.26 | 45.27 | 258.10 | 1212.87 | 1212.87 | 0.52 | 737.72 | 20.12 | 7.56 |
| Error | 94 | 2.80 | 2.68 | 0.11 | 1.81 | 72.78 | 18.77 | 18.77 | 0.06 | 10.72 | 0.35 | 0.37 |
| SEm | | 0.97 | 0.95 | 0.19 | 0.78 | 4.93 | 2.50 | 2.50 | 0.14 | 1.89 | 0.34 | 0.35 |
| C.V. (%) | | 2.64 | 2.50 | 16.53 | 1.22 | 9.48 | 2.70 | 2.54 | 14.19 | 3.51 | 3.53 | 4.36 |
| C.D. 5% | | 2.71 | 2.65 | 0.53 | 2.18 | 13.83 | 7.02 | 7.02 | 0.39 | 5.31 | 0.96 | 0.98 |
| C.D. 1% | | 3.59 | 3.52 | 0.70 | 2.89 | 18.31 | 9.30 | 9.30 | 0.51 | 7.03 | 1.27 | 1.30 |

| Source | D.f | Number of kernel rows per cob | Number of kernels per row | Number of grains per cob | Cob weight (g) | Hundred grain weight (g) | Shelling percentage (%) | Grain yield (kg/ha) | Chlorophyll content | Relative water content | Leaf area index |
|-------------|-----|-------------------------------|---------------------------|--------------------------|----------------|--------------------------|-------------------------|---------------------|---------------------|------------------------|-----------------|
| Replication | 2 | 10.93 | 23.08 | 24472.15 | 1214.62 | 47.75 | 454.51 | 713063.67 | 229.73 | 116.26 | 0.13 |
| Treatment | 47 | 8.53 | 104.69 | 26916.01 | 1006.24 | 84.88 | 161.90 | 1545416.81 | 118.52 | 30.03 | 0.10 |
| Error | 94 | 0.35 | 0.93 | 664.40 | 14.61 | 1.47 | 7.17 | 38504.80 | 3.09 | 5.82 | 0.03 |
| SEm | | 0.34 | 0.56 | 14.88 | 2.21 | 0.70 | 1.55 | 113.29 | 1.02 | 1.39 | 0.09 |
| C.V. (%) | | 4.03 | 3.53 | 6.42 | 3.82 | 4.78 | 3.60 | 5.25 | 3.52 | 3.23 | 6.55 |
| C.D. 5% | | 0.96 | 1.57 | 41.79 | 6.20 | 1.96 | 4.34 | 318.12 | 2.85 | 3.91 | 0.26 |
| C.D. 1% | | 1.27 | 2.07 | 55.33 | 8.21 | 2.60 | 5.75 | 421.24 | 3.78 | 5.18 | 0.35 |

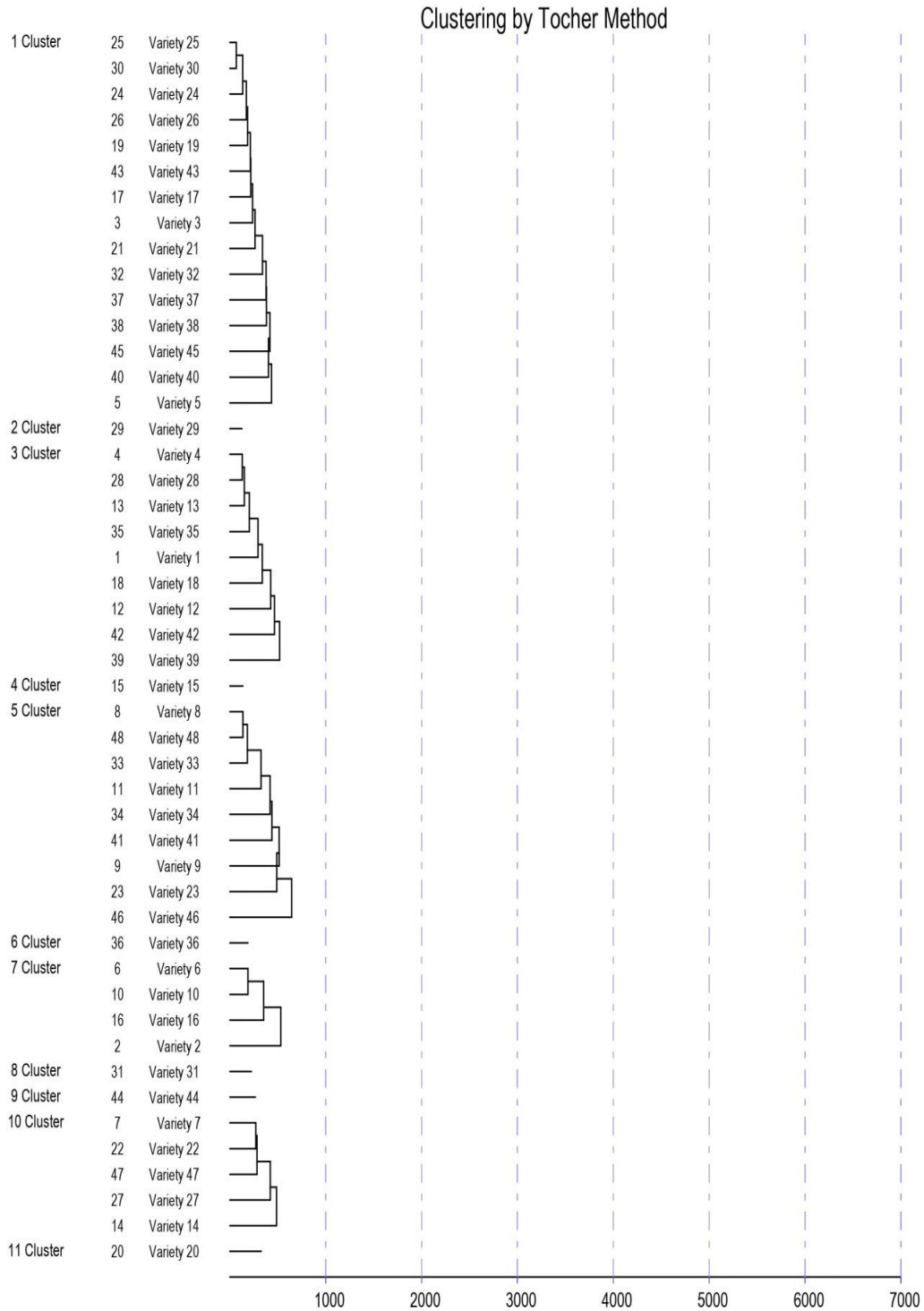


Fig. 1. Dendrogram showing relationship among 48 maize (*Zea mays* L.) inbreds in eleven clusters based on Mahalanobis's D^2 values

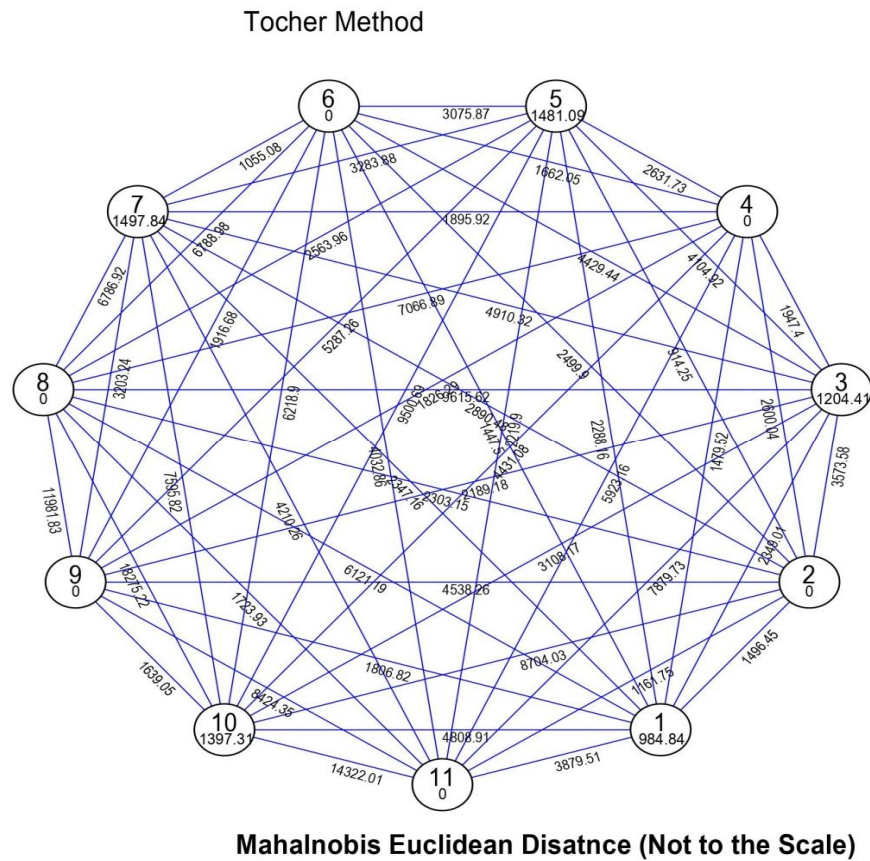


Fig. 2. Intra and inter-cluster distance of 48 maize (*Zea mays* L.) inbreds in eleven clusters based on euclidean D^2 distances

Cluster IX had the highest mean value for shelling percentage. Similar outcome also reported by ganesan et al. [19]. Therefore, it is suggested that most diverse clusters may be used as parents in hybridization programme to develop high yielding hybrids [20].

4. CONCLUSION

Genetic divergence of inbreds should be assessed in evaluating potential crosses particularly for single cross hybrid. Considering the estimation of genetic diversity the present investigation, crossing among the maize inbreds between cluster VIII (CAL1428) and cluster X (ZL154357, CAL1467, ZL153785, CAL1422, and ZL154346) may be recommended which can throw out superior segregants in hybridization. So the genotypes belonging to these clusters can be exploited in future breeding programmes for improving yield.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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