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Genetic diversity for agronomic characters among quality protein maize inbred lines

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Abstract
Eighteen quality protein maize (QPM) inbred lines were evaluated in randomized block design with three replications during two seasons. An analysis of variance of the data pooled across the season revealed significant genetic differences among the inbred lines for days to 50% silking, days to 50% tasseling, days to 75% brown husk, plant height, ear height, cob length, cob girth, number of kernels in a row, number of kernels in a cob, test weight and grain yield per plant. The eighteen QPM inbred lines were divided into five groups using Mahalanobis D^2 statistics and the Tocher technique. Cluster I consisted of 10 QPM inbred lines, Cluster II had 4 QPM inbred lines, Cluster III had 2 QPM inbred lines and the rest of the clusters (IV and V) were mono-genotypic. As a result, the D^2 analysis proved to be a very useful tool for identifying different groups from the inbred lines under investigation.

Keywords: Genetic divergence, D^2 statistics, Tocher method, cluster distance

Introduction
Globally, maize is one of the most extensively grown crops. As maize crop is having higher adaptability, the range of environment in which maize can be cultivated is greater as compared to rice and wheat (Koutsika-Sotiriou, 1999) [8]. The productivity of maize is high as compared to other cereals; Thus maize is popularly famous as "Queen of cereals". Maize is known as the poor man's nutricereal because maize grain is a having an extensive nutritional value as its kernel is rich in various vitamins, proteins and carbohydrates. Maize is considered to fulfill 15% of the total protein for human population worldwide (Shiferaw *et al*., 2011) [20]. Although maize is considered to be good source of protein, it is deficient in lysine and tryphtophan, the two essential amino acids (Vasal *et al*., 1980) [22]. Since human body cannot synthesize these two essential amino acids, their dietary supplement is essentially needed. Several investigations have found maize mutants with high lysine and tryptophan levels. Normal maize types contain less than half of the necessary lysine and tryptophan levels for human nutrition. This issue was addressed by research based discoveries in the late 1990s, which led to the development of quality protein maize (QPM) with twice the amount of lysine and tryptophan as compared to regular maize.

The maize production has benefited from a healthy increase in land planted to maize in recent years. Because plant progress requires diversity, breeders must first investigate genotype diversity before beginning any breeding effort. When parents are divergent, it contributes to genetic enhancement through hybridization. Superior inbred lines are required for the development of promising hybrid varieties in crop plants. Therefore, as a necessity, inbred lines must be generated and evaluated for their divergent gene pool in order to develop high yielding hybrids in maize. Because genetically divergent parents can have substantial heterotic effects, genetic diversity between genotypes is crucial. The ability to pick genetically diverse parents is made possible by the quantification of genetic diversity using biometrical procedures. Breeders have successfully employed D^2 statistics to evaluate genetic divergence among genotypes. Crosses between genetically dissimilar parents yield more heterosis than crosses between closely related parents (Moll and Stuber, 1971) [12]. As a result, crop development program require genetic diversity in the parents. Keeping all above into consideration, the present investigation was carried out to assess genetic diversity for important agronomic characters amongst some QPM inbred lines.

Materials and Methods
The field experiments were conducted at research farm of Tirhut College of Agriculture, Dholi, under Dr. Rajendra Prasad Central Agricultural University, Pusa, (Bihar) during May to

October for Kharif season 2020 and from November to June for Rabi season 2020-21. The nucleus seed of eighteen inbred lines of quality protein maize were obtained from AICRP, Dholi (Table-1).

Table1: List of the experimental materials along with their source

Sl. No.	Pedigree	Designation	Source
1.	[CL-G 2501×CML-170]-B-2-2-2-B-1-1-1-BBB#	QPML-01	AICRP, Dholi Centre
2.	CML-161×165-18-2-1-2-BBB-#	QPML-02	AICRP, Dholi Centre
3.	[CML-176×CLG 2501]-B55-1-5-2-BBB-#	QPML-03	AICRP, Dholi Centre
4.	[CLQ-6601×CL-0243]B-26-1-1-BB-1-B*6-#	QPML-04	AICRP, Dholi Centre
5.	CLQ-RCYQ 035-B*11-#	QPML-05	AICRP, Dholi Centre
6.	CML-161×165-3-2-3-B*4-#-B1	QPML-06	AICRP, Dholi Centre
7.	[CLQ-RCYQ 31×CLQ-RCYQ 35]-B-36-2-B*5-5	QPML07	AICRP, Dholi Centre
8.	G 33 QMH 103-3-1-5-1-B*14	QPML-08	AICRP, Dholi Centre
9.	P70CO-BBB-6-B*6#	QPML-09	AICRP, Dholi Centre
10.	CML-193-B*6#	QPML-10	AICRP, Dholi Centre
11.	[CML-161/CML-165]B-B-B-11-B-B-B/CML-193	QPML-11	AICRP, Dholi Centre
12.	POP 61 C1 QPM TEYF-51-2-1-2-2-B-1-B/CML-193	QPML-12	AICRP, Dholi Centre
13.	[CML-161×CLQ-RCYQ 31]-B-10-3-B-B	QPML-13	AICRP, Dholi Centre
14.	CML-161	QPML-14	AICRP, Dholi Centre
15.	CML-163	QPML-15	AICRP, Dholi Centre
16.	CML-165	QPML-16	AICRP, Dholi Centre
17.	Pool 34 C24 (Subtinty D QPM)-B-20-BB	QPML-17	AICRP, Dholi Centre
18.	Pool- 17 QPM	QPML-18	AICRP, Dholi Centre

Eighteen QPM inbred lines were evaluated randomized block design with three replications. Each plot consisted of two rows of four meters each, spaced 75 cm row to row and 20 cm plant to plant. Days to 50% silking, days to 50% tasseling and days to 75% brown husk were the characters studied on plot basis. Five plants were selected randomly for recording observations on plant height (cm), ear height (cm), cob length (cm), cob girth (cm), number of kernel in a row, number of kernels in a cob, test weight (g) and grain yield per plant. Statistical software WINDOSTAT version 9.2, created by Indostat Services Ltd., Hyderabad, India, was used to statistically analyze the data collected on eleven traits.

Analysis of genetic divergence

Analysis of genetic divergence was based on Mahalanobis' generalized distance (1936) [9] and D^2 statistics was used to analyze the data recorded on eleven agronomic characters.

Identification of group constellations and clusters

Tocher method, as described by Rao (1952) [16], was used to group the populations into several clusters. In this method, the clustering criterion is that any two variables belonging to the same cluster should have a lower D^2 value on average than those belonging to separate clusters. D^2 values of each genotype combination were presented in ascending order of magnitude in a tabular form for this purpose, as suggested by Singh and Chaudhary (1985) [19]. To begin, two populations with the shortest distance between them were selected, followed by a third population with the lowest D^2 value of the first two populations. The nearest fourth population was then considered and the process was repeated.

Average intra-cluster distance

The formula used to calculate intra-cluster distances was iDi^2/n, where iDi^2 was the sum of distances between all feasible combinations (n) of populations in a cluster.

Average inter-cluster distance

Clusters were examined one by one, and distances between them and other clusters were calculated. The distance between two clusters was calculated by multiplying the sum of D^2 values between members of the other cluster by the product of the number of genotypes in both clusters.

Contribution of individual character towards divergence

Each cluster was scored based on how close it was to divergence between two entries in all of the combinations. The largest mean difference received rank one, and the lowest received rank'd' where'd' signifies the total number of traits taken. The following formula was used to calculate the percentage contribution of each trait (P) to genetic divergence.

$$P = \frac{A \times 100}{B}$$

Where,

A= Number of genotype combinations where the character was ranked first.

B= All possible combinations of number of genotypes

Results and Discussion

The level of genetic divergence present in the material determines the success of a breeding program; the larger the diversity in the material, the better the possibilities of producing promising and desired types. The genotypic and phenotypic components of phenotypic variability displayed by a genotype or a collection of genotypes can be separated. Because genotypic components are the heritable fraction of total variability, their magnitude for yield and related traits influences the selection tactics of the breeders. Morphological markers based analysis is the simplest and quickest method for identifying or detecting exploitable variation in morphological characteristics for their further improvement. However, these qualities need to be researched in detail across regions and diverse environmental conditions, such as temperature and climate, as they are heavily influenced by environmental variables.

Statistical analysis for the design of the experiment revealed significant genetic differences among the eighteen QPM

inbred lines for all of the traits studied (Table-2). The occurrence of a great level of variability is owing to a variety of material sources as well as environmental influences, which dictated the phenotypic factor primarily. Number of kernels in a cob, test weight and plant height showed highly significant variability. This trend was followed by ear height, grain yield per plant, days to 50% silking and days to 50% tasseling. The presence of these significant disparities among the inbred lines revealed that significant phenotypic and genotypic differences existed. As a result, the presence of this variability in the current study revealed that there was plenty of opportunity for selection of these traits in improvement breeding program.

Table 2: Analysis of variance for eleven characters of QPM inbred lines

Character	Mean sum of squares		
	Replications (df=2)	Genotypes (df=17)	Error (df=85)
Cob girth	0.03	0.38**	0.08
Cob length (cm)	0.02	0.23**	0.07
Days to 50% silking	0.23	19.63**	6.10
Days to 50% tasseling	0.17	19.49**	6.34
Days to 75% brown husk	1.81	15.95**	5.37
Ear height (cm)	7.11	68.92**	26.65
Grain yield per plant	6.77	31.01**	22.62
Number of kernels in a cob	117.48	1243.99**	633.08
Number of kernels in a row	0.06	2.95**	0.97
Plant height	8.06	214.76**	92.54
Test weight (g)	65.58	267.14**	120.01

Table 3: Mean performance of eighteen QPM inbred lines for eleven characters

Inbred line	Cob girth	Cob length (cm)	Days to 50% silking	Days to 50% tasseling	Days to 75% brown husk	Ear height (cm)	Grain yield per plant	Number of kernels in a cob	Number of kernels in a row	Plant height	Test weight (g)
QPML-01	4.98	6.40	83.33	80.17	111.17	57.67	74.33	354.83	12.00	135.33	283.17
QPML-02	5.00	6.35	81.00	77.83	114.17	55.50	74.50	357.33	11.67	133.67	266.83
QPML-03	4.98	6.70	80.00	77.00	117.50	61.50	78.00	335.17	11.33	140.67	276.00
QPML-04	5.12	6.83	82.00	79.00	115.17	62.33	75.17	351.67	12.33	144.83	286.17
QPML-05	4.83	6.62	83.50	80.50	116.67	65.00	80.17	331.33	11.33	148.50	275.17
QPML-06	4.70	6.45	84.50	81.50	115.50	56.83	75.83	332.67	11.33	136.67	272.50
QPML-07	4.77	6.57	85.17	82.00	118.17	63.50	78.67	323.67	11.50	137.33	285.67
QPML-08	4.45	6.68	83.17	80.17	116.00	60.00	74.17	352.83	13.33	133.17	283.33
QPML-09	4.68	6.53	85.33	82.17	115.67	62.83	75.67	337.83	13.33	140.83	278.83
QPML-10	4.65	6.52	85.67	82.67	117.83	60.67	69.83	353.50	12.67	138.33	273.00
QPML-11	4.72	6.63	79.17	76.17	115.67	66.00	75.67	319.33	11.67	147.50	284.33
QPML-12	5.20	6.12	84.17	81.17	115.33	67.50	76.83	351.67	13.00	143.67	284.67
QPML-13	5.23	6.60	82.00	78.83	116.33	62.67	77.50	354.83	11.83	134.33	272.17
QPML-14	4.65	6.65	81.67	78.67	117.17	63.67	75.00	353.67	12.50	135.00	275.67
QPML-15	4.55	6.78	82.83	79.83	116.17	60.33	75.67	339.50	12.67	145.83	288.67
QPML-16	4.55	6.78	83.17	80.00	116.67	60.33	73.50	365.00	12.83	134.67	272.50
QPML-17	4.83	6.33	81.17	78.00	114.00	66.33	74.00	374.33	12.00	152.83	286.00
QPML-18	4.33	6.88	82.50	79.50	116.67	57.83	74.50	351.50	11.33	134.67	287.83
MEAN	4.79	6.58	82.80	79.73	115.88	61.69	75.50	346.70	12.15	139.88	279.58
CV	6.10	4.17	2.98	3.16	2.00	8.37	6.30	7.26	8.11	6.88	3.92

According to the results obtained from the mean performance, the best QPM inbred line for grain yield per plant is QPML-05; this inbred line was also superior for number kernels in a row having high test weight and moderately high cob length and cob girth (Table-3). The QPM inbred line which was observed to have lowest grain yield per plant was QPML-10. The QPM inbred line which was observed to have highest plant height was QPML-17 and lowest plant height was observed in QPML-08. The observation for cob girth was highest for inbred line QPML-13 and was lowest for QPML-18. The inbred line QPML-18 showed highest cob length and QPML-12 was observed to have the lowest value. Test weight was observed to be highest in inbred QPML-15 and lowest in inbred QPML-02. Highest value of kernels in a cob was observed for inbred QPML-17 and lowest value was observed for inbred QPML-11. The inbred QPML-08 and QPML-09 was observed to have maximum number of kernels in a row whereas QPML-03 and QPML-06 showed the minimum value. Highest value for ear height was observed in inbred QPML-12 and lowest value was observed for QPML-02. Early silking and tasseling was observed in QPML-11 and late silking and tasseling was observed in QPML-10.

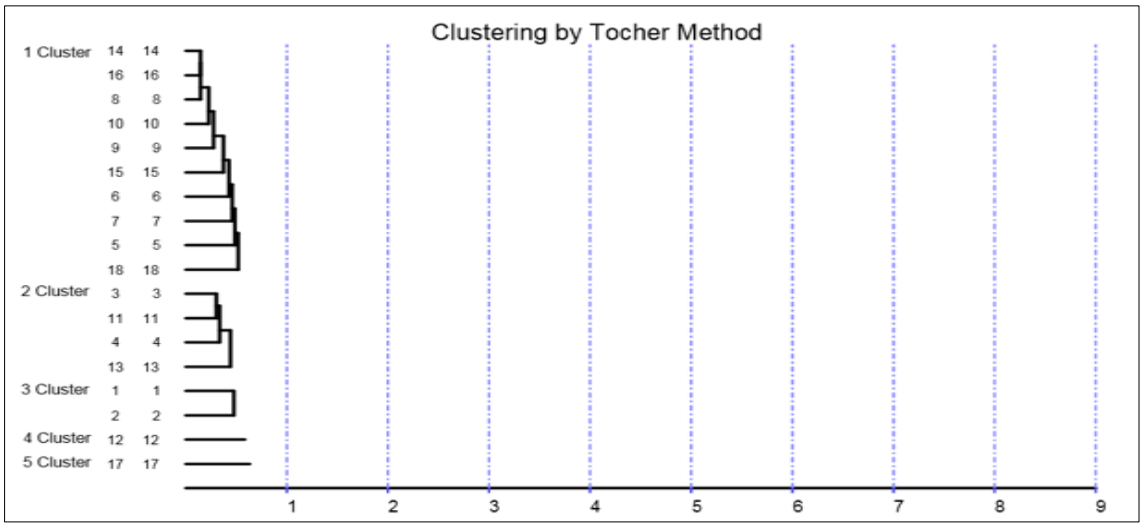

Fig 1: Tocher method-based clustering for distribution of 18 QPM inbred lines in five clusters

Genetic divergence among 18 QPM inbred lines was evaluated based on the eleven agro-morphological traits, namely, plant height (cm), ear height (cm), cob length (cm), cob girth (cm), days to 50% silking, days to 50% tasseling, days to 75% brown husk, number of kernels in a row, number of kernels in a cob, test weight (g) and grain yield per plant. With the help of the evaluated data obtained from the genetic divergence, all the eighteen inbred lines were classified into five separate clusters (Table-4). Dendrogram thus obtained with the help Tocher method indicated the distribution of ten out of eighteen inbred lines in the cluster I, followed by cluster II with four QPM inbred lines, cluster III consisting of two QPM inbred lines, and cluster IV and V, with only one QPM inbred lines (Mono-genotypic) in each cluster (Figure-1). Singh and Choudhary (2001) [18], More *et al*. (2006) [13], Bhoite and Dumbre (2007) [4], Farzana Jabeen *et al*. (2007) [5], Ganesan *et al*. (2010) [6], Astha Gupta and Singh (2011) [7], Alam and Alam (2013) [1] have suggested the use of this method for assessment of genetic divergence.

Table 4: Clustering pattern of eighteen QPM inbred lines on the basis of D^2 statistics

Cluster No.	Number of inbred lines	Inbred lines
Cluster-I	10	QPML- 05, QPML-06, QPML-07, QPML-08, QPML-09, QPML-10, QPML-11, QPML-14, QPML-16, QPML-18
Cluster-II	04	QPML-03, QPML-04, QPML-11, QPML-13
Cluster-III	02	QPML-01, QPML-02
Cluster-1V	01	QPML-12
Cluster-V	01	QPML-17

The average intra cluster distance ranged from 1.26 to 1.37 (Table-5). Cluster II (1.37) had the highest intra cluster distance, which was followed by Cluster III (1.27) and Cluster I (1.26). Cluster I had the least distance with Cluster II (2.02), whereas it had the maximum distance with Cluster III (2.89). Cluster II was having least distance with Cluster V (2.12) and showed highest divergence from cluster III (2.83). Cluster III exhibited close relatedness (Figure-2) to Cluster V (1.99), whereas it showed highest divergence from Cluster IV (2.20). For Cluster IV, the most divergence was observed with Cluster V (1.68). Farzana Jabeen *et al*. (2007) [5], Nehvi *et al*. (2008) [14], Astha Gupta and Singh (2011) [7] and Maruthi *et al*. (2015) [11] also derived similar type of inferences based on their findings in maize.

Table 5: Mean inter and intra cluster distances among five clusters of 18 QPM inbred lines

	Cluster-01	Cluster-02	Cluster-03	Cluster-04	Cluster-05
Cluster-I	1.26	2.02	2.89	2.36	2.71
Cluster-II	2.02	1.37	2.83	2.46	2.12
Cluster-III	2.89	2.83	1.27	2.20	1.99
Cluster-1V	2.36	2.46	2.20	0.00	1.68
Cluster-V	2.71	2.12	1.99	1.68	0.00

The data on cluster means for different agronomic characters revealed that (Table-6) the cluster mean ranged from a minimum value of 4.62 present in Cluster-I to the highest value of 5.20 in Cluster-IV for cob girth. For cob length, this value ranged from a minimum of 6.12 present in Cluster-IV to the highest value of 6.69 in Cluster-II. The value of cluster means for days to 50% silking ranged from 80.79 to 84.17 days. Cluster-II showed early silking as the cluster mean was minimum, it took 80.79 days for the emergence of silk. The value of cluster means for days to 50% tasseling ranged from 77.75 to 81.17 days. Cluster-II showed early tasseling as the cluster mean was minimum, and took 77.75 days for the emergence of tassel. The value of cluster means for days to 75% brown husk ranged from 112.17 to 116.65 days. Cluster-III consisted of inbred lines that attained the brown husk stage earlier whereas; Cluster-I included inbred lines that have

taken maximum days to attain the brown husk stage. For ear height cluster mean value ranged from a minimum of 56.58 present in Cluster-III to the highest value of 67.50 in Cluster-IV. For grain yield cluster mean value ranged from a minimum of 74.00 present in Cluster-V to the highest value of 76.83 in Cluster-IV. For number of kernels in a cob cluster mean value ranged from a minimum of 340.15 present in Cluster-II to the highest value of 374.33 in Cluster-V. For number of kernels in a row cluster mean value ranged from a minimum of 11.79 present in Cluster-II to the highest value of 13.00 in Cluster-IV. For plant height cluster mean value ranged from a minimum of 134.50 present in Cluster-III to the highest value of 152.83 in Cluster-V. For test weight cluster mean value ranged 275.00 present in Cluster-III to the highest value of 286.00 in Cluster-V. Clusters having high mean value can be selected for the combination of the desired traits with the help of hybridization technique. Singh *et al*. (2005) [17], Marker and Krupakar (2009) [10] and Alam and Alam (2013) [1] also expressed that clusters having high mean value should be considered while selecting the parental genotypes for hybridization.

Table 6: Cluster mean for eleven morphological traits of QPM inbred lines

	Cob girth	Cob length (cm)	Days to 50% silking	Days to 50% tasseling	Days to 75% brown husk	Ear height (cm)	Grain yield per plant	Number of kernels in a cob	Number of kernels in a row	Plant height	Test weight (g)
Cluster-I	4.62	6.65	83.75	80.70	116.65	61.10	75.30	344.15	12.28	138.50	279.32
Cluster-II	5.01	6.69	80.79	77.75	116.17	63.13	76.58	340.25	11.79	141.83	279.67
Cluster-III	4.99	6.38	82.17	79.00	112.67	56.58	74.42	356.08	11.83	134.50	275.00
Cluster-1V	5.20	6.12	84.17	81.17	115.33	67.50	76.83	351.67	13.00	143.67	284.67
Cluster-V	4.83	6.33	81.17	78.00	114.00	66.33	74.00	374.33	12.00	152.83	286.00

The percentage of contribution of the eleven agronomic characters was calculated in order to determine their relative importance in relation to manifestation of total divergence amongst the inbred lines under evaluation (Table-7). It is evident from the pertinent data that the number of kernels in a row (20.26%) contributed the most to total divergence, followed by kernels in a cob (15.69%), cob girth (15.03%), test weight (11.76%), days to 50% silking and days to 75% brown husk (10.46%), ear height and plant height (5.23%), cob length and grain yield per plant (2.61%) and days to 50% tasseling (0.65%).

A total of 153 combinations was obtained in which the number of kernels in a row ranked 1st (31 times) and contributed the most to total divergence, followed by kernels in a cob ranked 1st (24 times), cob girth ranked 1st (23 times), test weight ranked 1st (18 times), days to 50% silking and days to 75% brown husk ranked 1st (16 times) each, ear height and plant height ranked 1st (8 times) each, cob length and grain yield per plant ranked 1st (4 times) each and days to 50% tasseling ranked 1st (1 time only). Anderson (1957) [2], Rao (1952) [16], Nehvi *et al*. (2008) [14], and Ganesan (2010) [6] all made similar observations.

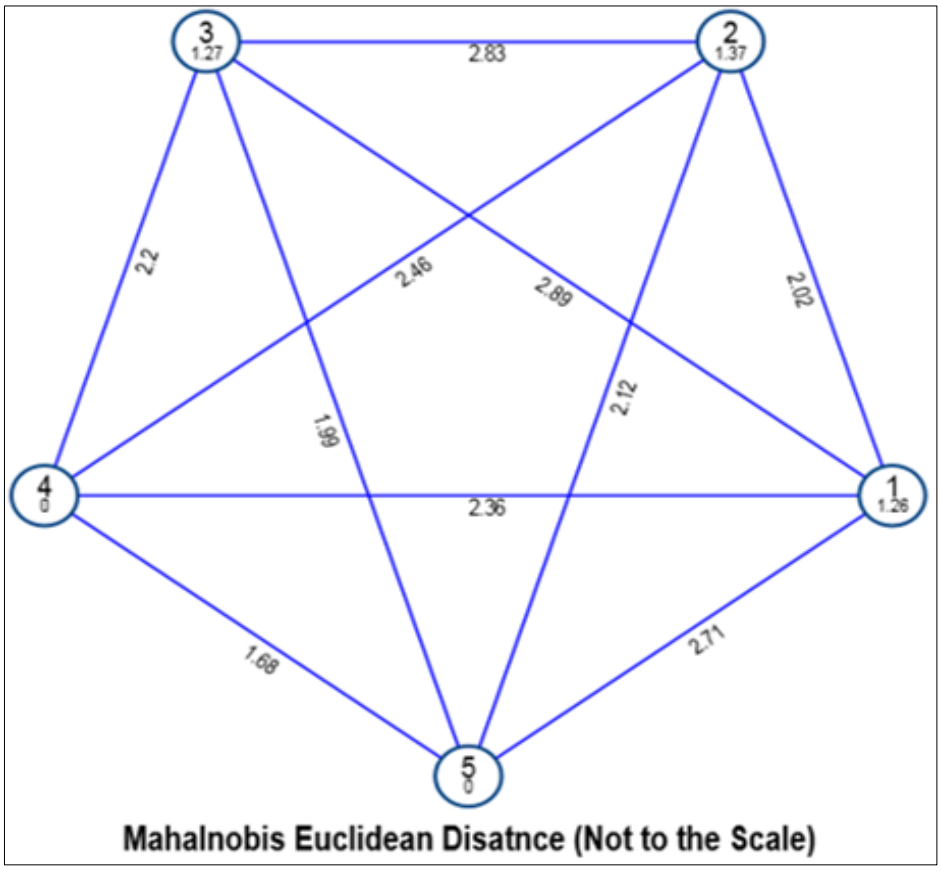

Fig 2: Spatial distribution pattern of clusters

Table 7: Percentage contribution of eleven characters towards genetic divergence

Source	Times ranked first	Contribution %
Cob girth	23	15.03%
Cob length (cm)	04	2.61%
Days to 50% silking	16	10.46%
Days to 50% tasseling	01	0.65%
Days to 75% brown husk	16	10.46%
Ear height (cm)	08	5.23%
Grain yield per plant	04	2.61%
Number of kernels in a cob	24	15.69%
Number of kernels in a row	31	20.26%
Plant height (cm)	08	5.23%
Test weight (g)	18	11.76%

Conclusion

According to the analysis of variance, statistically significant heterogeneity existed among 18 QPM inbred lines evaluated in the present study. These QPM inbred lines were grouped into five clusters based on agronomic characters dependent genetic divergence analysis. Mahalanobis statistic-based clustering pattern revealed that Cluster IV had the highest mean value for the important agronomic traits like cob girth, ear height, grain yield per plant, number of kernels in a cob plant height and test weight, thus cluster-IV can be selected for these agro-morphological traits for further hybridization process. For earliness in emergence of silk and tassel cluster-II was considered best. Cluster-II which consisted of QPML-03, QPML-04, QPML-11 and QPML-13 inbred lines, was observed to have highest intra-cluster distance and as a result the inbred lines in this cluster can be further used for recombination breeding techniques. As cluster IV and V was mono-genotypic and cluster-I was having the highest number of QPM inbred lines i.e. 10. So if any of the QPM inbred lines present in cluster-I is crossed with the mono-genotypic cluster, it may result in combinations with enhanced heterotic hybrids. Since, D^2 analysis has proven to be a more precise and reliable technique for assessing genetic diversity quantitatively, parent selection that is based on genetic divergence can result in greater success.

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