Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(5): 517-522
Received: 01-04-2020
Accepted: 02-05-2020

S Mallikarjuna
Research Scholar, Department of Genetics and Plant Breeding, Agricultural College, Bapatla, Guntur, Andhra Pradesh, India

V Roja
Assistant Professor, Department of Genetics and Plant Breeding, Agricultural College, Bapatla, Guntur District, Andhra Pradesh, India

I Sudhir Kumar
Scientist, Department of Plant Breeding, Agricultural Research Station, Peddapuram, East Godavari District, Andhra Pradesh, India

T Srinivas
Professor, Department of Genetics and Plant Breeding, Agricultural College, Bapatla, Guntur District, Andhra Pradesh, India

Corresponding Author:
S Mallikarjuna
Research Scholar, Department of Genetics and Plant Breeding, Agricultural College, Bapatla, Guntur, Andhra Pradesh, India

Genetic diversity studies in newly developed maize inbreds

S Mallikarjuna, V Roja, I Sudhir Kumar and T Srinivas

Abstract
Forty nine inbred lines of maize were evaluated for 14 parameters at the experimental field of Agricultural Research Station (ARS), Peddapuram, Andhra Pradesh, during 2018-19 to study the genetic divergence using multivariate analysis. The analysis indicated considerable genetic divergence among the 49 genotypes studied. D^2 analysis grouped the 49 maize genotypes into nine distinct clusters. Among those, cluster I was largest with 24 genotypes, while clusters IV, VI, VII, VIII, IX are smallest with single genotype in each. The intra-cluster distances in all the nine clusters were more or less low, indicating that the genotypes within the same cluster were closely related. The maximum inter cluster distance was observed between clusters II (KDML-115, PDML 15, VM 51, ADL 1619, PDML7470, CAL 1784, CAL 17834, VM 45, AL8127) and cluster VIII (CML 1505) indicating presence of maximum diversity among the genotypes falling within these clusters. Hence, the crosses VM51 × CML1505 is expected to result in heterotic hybrids and transgressive segregants.

Keywords: Newly developed, maize inbreds *Zea mays* L.

Introduction
Maize (*Zea mays* L.) is a monoecious and cross pollinated crop. It is a versatile cereal crop next to wheat and rice in the world. Moreover it is one of the most important agricultural food crop for humans and livestock. It also serves as the basic raw material in numerous industrial products that include starch, oil, protein, alcoholic beverages, food sweeteners, pharmaceutical, cosmetic, film, textile, gum, package, paper industries and so on (Nikkhoy and Shiri, 2017) [5].

Maize breeders are consistently emphasizing on diversity among parental genotypes because it is a significant factor contributing for development of heterotic hybrids. Hence, characterization of genetic diversity of maize germplasm is of great importance in hybrid maize breeding aimed at development of high yielding hybrids.

Magnitude of genetic diversity among germplasm can be estimated with the help of advanced biometrical techniques such as multivariate analysis based on Mahalanobis D^2 statistics which is a very useful technique in quantifying the degree of divergence between inbred lines or any biological population at genotypic level. It is also helpful in assessment of relative contribution of different components to the total divergence at both intra and inter-cluster level. Clustering method is used to separate the genotypes into sub groups in order to obtain homogeneity within the groups. Different clustering methods are used depending on the procedure that is most suitable for the data set. Data obtained from the clusters can then be used to select potential parents for production of heterotic hybrids in maize. In view of the above, the present investigation was taken up to assess the genetic diversity of 49 elite maize inbred lines towards identification of superior and divergent parents for the production of heterotic hybrids.

Materials and Methods
The present study was carried out during *kharif* 2018 at Agricultural Research Station (ARS), Peddapuram, Andhra Pradesh. The experimental material comprised of 49 inbred lines, including one check (BML-6) (Table 1). The genotypes were sown in Simple Lattice Design (SLD) with two replications. Each genotype was planted in two rows of 4m length each in each replication, with a spacing of 70 cm between rows and 20 cm within row. The crop was raised as per the recommended package of practices. Observations on various pre and post-harvest parameters were recorded on five plants selected at random from each entry in each replication for plant height (cm), ear placement height (cm), cob length (cm), cob girth (cm), kernel rows cob^{-1}, number of kernels row^{-1}, cob yield $plant^{-1}$, grain yield $plant^{-1}$, 100 grain weight and protein content (%).In contrast, observations for days to 50 per cent tasselling, days to 50 per cent silking, and anthesis silking interval were recorded on plot basis.

The data obtained was subjected to stardard statistical procedures. Genetic divergence analysis was done following the D^2 statistics proposed by Mahalanobis (1928) [3] and described by Rao (1952) [7]. The analysis was carried out using the Statistical Analysis Software (SAS) 9.2 version and WINDOSTAT 9.1.

Results and Discussion

Analysis of variance exhibited significant differences among the inbreds for all the traits studied, indicating presence of considerable variability among the 49 inbreds studied for yield and yield component characters (Table.2).

A perusal of the results on divergence using Mahanlobis D^2 values revealed grouping of the 49 genotypes studied into nine clusters using the Tocher's method (Table. 3 and Figure 1) with the criterion that the intra-cluster average D^2 values should be less than the inter cluster D^2 values. Among nine clusters, cluster I was observed to be the largest comprising of 24 genotypes representing collections from different centers, namely, Peddapuram and Hyderabad. Cluster II and cluster V comprised of nine and six genotypes, respectively, from Peddapuram and Hyderabad. Further, cluster III comprised of five genotypes included from Peddapuram and Hyderabad. The remaining clusters, IV, VI, VII, VIII and IX were solitary and monogenotypic clusters with zero intra cluster D^2 values. The mode of distribution of genotypes from different geographical regions into various clusters was observed to be at random indicating no relationship of geographic and genetic diversity. Genotypes chosen from the same eco-geographical region were observed to be present in different clusters as well as in same cluster, while genotypes from diverse geographical regions were included in the same cluster. The findings are in conformity with the reports of Rohman *et al*. (2015) [8].

The results on inter- and intra- cluster distances are presented in Table 4 and Figure 2. Intra-cluster D^2 values in the present study ranged from zero (cluster IV, VI, VII, VIII, IX) to 31.51 (cluster V). The high intra-cluster distance in cluster V indicates the presence of genetic diversity among the inbreds present within this cluster. However, genotypes grouped in the other clusters were relating similar while the intra cluster D^2 values for the monogenotypic clusters, IV, VI,VII and IX was noticed to be Zero.

Inter-cluster distances in the present study ranged from 16.25 (between clusters VI and VII) to 110.57 (between clusters II and VIII). Maximum inter-cluster distance was observed between the clusters II and VIII (110.57), followed by clusters III and VIII (97.01) suggesting wide genetic diversity between these clusters and hence, the desirability of crosses between the genotypes of these clusters in production of desirable and heterotic hybrids, also likely to yield transgressive segregants as suggested by Falconer, (1964) [1]. Lower inter cluster distance indicated closeness among accessions of the clusters. These results are in conformity with the experimental findings of Hassan *et al*. (2018) [2], Rafique *et al*. (2018) [6] and Najar *et al*. (2018) [4].

Based on the inter cluster distances, nearest and farthest clusters for each of the nine clusters were identified and presented in the Table 5. Cluster IV (26.70) was closest to cluster I with 24 genotypes followed by cluster V (34.74) and cluster VIII (63.63) was the farthest cluster followed by cluster IX (47.09). Cluster II comprised of nine genotypes and its nearest cluster was cluster VII (38.58) followed by cluster I (41.23), while the farthest was cluster VIII (110.57) followed by cluster V (83.40). Cluster III had five genotypes and cluster I (36.87) was the nearest cluster, followed by cluster VII (39.89) and cluster VIII (97.01) was farthest, followed by the cluster II (63.75). Cluster IV is monogenotypic (PDML124) and was nearest to cluster VI (23.28) followed by cluster VII (25.45) and was farthest from cluster II (60.18), followed by cluster IX (59.79). Cluster V comprised of six genotypes, and was nearest to Cluster I (34.74), followed by Cluster IV (45.82) and was farthest from Cluster II (83.40), followed by cluster VII (75.72). Cluster VI was monogenotypic (ADL8070) and was closest to cluster VII (16.25), followed by cluster IV (23.28) and was farthest from cluster VIII (84.20), followed by cluster V (73.01). Cluster VII was also monogenotypic (PDML19-46) and was closest to cluster VI (16.25) followed by cluster IV (25.45) and was farthest from cluster VIII (94.54), followed by cluster V (75.72). Cluster VIII was also monogenotypic (CML 1505) and was closest to cluster V (50.75), followed by cluster IV (59.13) and was farthest from cluster II (110.57) followed by cluster III (97.01). Cluster IX was also monogenotypic (TA5084) and was closest to cluster VII (38.77), followed by cluster I (47.09) and was farthest from cluster VIII (75.30), followed by cluster V (73.32).

Cluster means indicated average performance of all genotypes present in a particular cluster. The cluster mean values for 14 characters studied in the present investigation are presented in Table 6. A perusal of these results revealed considerable differences between the clusters for all characters under study. Days to 50 per cent tasseling ranged from 47.33 days (cluster II) to 59.80 days (cluster III); days to 50 per cent silking from 49.33 days (cluster II) to 62.30 days (cluster III); anthesis silking interval ranged from 1 day (cluster VI) to 2.50 (cluster III); days to maturity from 99.78 days (cluster II) to 117 days (cluster III); plant height from 174.44 cm (cluster II) to 229.00 cm (cluster VIII); ear placement height from 69 cm (cluster VII) to 108 (cluster VIII); cob length from 12.05 cm (cluster IX) to 17.26 cm (cluster I); cob girth from 11.80 cm (cluster IX) to 15.65 cm (cluster IV); kernel rows cob^{-1} from 13.78 (cluster II) to 16.00 (clusters VII and VIII); number of kernels row^{-1} ranged from 31.00 (cluster VI) to 36.50 (cluster VIII); cob yield $plant^{-1}$ from 136.00 g (cluster IX) to 242.00 g (clusters VI and VII); grain yield $plant^{-1}$ from 90.50 g (cluster IX) to 193.50 g (clusters VI and VII); 100 grain weight from 36.44 g (cluster II) to 44.00 g (clusters IV and VI); and protein content from 7.7 per cent (cluster VIII) to 11.40 per cent (cluster IV). Selection of genotypes from clusters with high mean for the respective traits is suggested for their utilization in hybridization programmes aimed at improvement of the respective traits. Perusal of these results also revealed that there was no single cluster with all the desirable traits, which ruled out the possibility of direct selection of genotypes for immediate use. Therefore, hybridization between the selected genotypes from divergent clusters is suggested for judicious combination of all the targeted traits.

Information on the relative contribution of various plant characters towards genetic divergence was reported to aid the breeder in choice of parents for hybridization and effective selection. The per cent contribution of all the characters studied in the present investigation towards genetic divergence is presented in Table 7. Among the characters studied, days to 50 per cent tasseling (31.63%) was observed to contribute maximum contribution followed by ear placement height (12.84%), plant height (10.88%), grain yield $plant^{-1}$ (7.14%), cob girth (6.72%), 100 grain weight (6.29%), protein content (5.19%), cob yield $plant^{-1}$ (4.00%), number of

kernels row^{-1} (3.83%), cob length (3.57%), kernel rows cob^{-1} (3.32%), days to 50 per cent silking (2.38%), anthesis silking interval (1.53%) and days to maturity (0.68%). Similar findings of maximum contribution towards diversity by plant height and ear placement height reported were also reported earlier by Uday kumar *et al.* (2013) [9]. Hence, the characters days to 50 per cent flowering, ear placement height and plant height need to be given priority in the selection of diverse parents for breeding programmes.

Summary and Conclusion

D^2 analysis categorized the 49 maize inbreds into nine clusters with cluster I being largest comprising of 24 genotypes followed by cluster II and cluster V with nine and six genotypes, respectively. The clusters IV, VI, VII, VIII, IX comprised of single genotype only. Hybridization between VM51 of cluster II and CML1505 of cluster VIII is suggested for the production of heterotic hybrids and transgressive segregants, based on inter cluster distance and cluster means.

Table 1: List of maize genotypes employed in the present investigation

S. No.	Inbreds / Genotypes	Source
1	VML 15036	Winter Nursery Centre, Hyderabad
2	VML 16008	Winter Nursery Centre, Hyderabad
3	OML 17-47	Winter Nursery Centre, Hyderabad
4	CAL1511	CIMMYT, Regional Center, Hyderabad
5	ZL161032	Winter Nursery Centre, Hyderabad
6	ZL1381388081	Winter Nursery Centre, Hyderabad
7	CAL1711830	CIMMYT, Regional Center, Hyderabad
8	VML 15028	Winter Nursery Centre, Hyderabad
9	ADL1608	Winter Nursery Centre, Hyderabad
10	ADL8070	Winter Nursery Centre, Hyderabad
11	ADL1620	Winter Nursery Centre, Hyderabad
12	AL8178	Winter Nursery Centre, Hyderabad
13	CML08-292	CIMMYT, Regional Center, Hyderabad
14	PDML9644	Agricultural Research Station, Peddapuram
15	PDML124	Agricultural Research Station, Peddapuram
16	KDML-115	Winter Nursery Centre, Hyderabad
17	CAL1784	CIMMYT, Regional Center, Hyderabad
18	CAL17834	CIMMYT, Regional Center, Hyderabad
19	ADL1619	Winter Nursery Centre, Hyderabad
20	AL8127	Winter Nursery Centre, Hyderabad
21	ADL8106	Winter Nursery Centre, Hyderabad
22	VM 45	Winter Nursery Centre, Hyderabad
23	VM 51	Winter Nursery Centre, Hyderabad
24	PDML7470	Agricultural Research Station, Peddapuram
25	PDML506	Agricultural Research Station, Peddapuram
26	PDML15	Agricultural Research Station, Peddapuram
27	CAL1612	CIMMYT, Regional Center, Hyderabad
28	TA5084	Winter Nursery Centre, Hyderabad
29	CAL1810	CIMMYT, Regional Center, Hyderabad
30	CML1505	CIMMYT, Regional Center, Hyderabad
31	CML28708	CIMMYT, Regional Center, Hyderabad
32	CML28208	CIMMYT, Regional Center, Hyderabad
33	PDML8082	Agricultural Research Station, Peddapuram
34	PDML19-34	Agricultural Research Station, Peddapuram
35	PDML19-35	Agricultural Research Station, Peddapuram
36	PDML19-36	Agricultural Research Station, Peddapuram
37	PDML19-37	Agricultural Research Station, Peddapuram
38	PDML19-38	Agricultural Research Station, Peddapuram
39	PDML19-39	Agricultural Research Station, Peddapuram
40	PDML19-40	Agricultural Research Station, Peddapuram
41	PDML19-41	Agricultural Research Station, Peddapuram
42	PDML19-42	Agricultural Research Station, Peddapuram
43	PDML19-43	Agricultural Research Station, Peddapuram
44	PDML19-44	Agricultural Research Station, Peddapuram
45	PDML19-45	Agricultural Research Station, Peddapuram
46	PDML19-46	Agricultural Research Station, Peddapuram
47	PDML19-47	Agricultural Research Station, Peddapuram
48	PDML19-48	Agricultural Research Station, Peddapuram
49	BML6	PJTSAU, Hyderabad

Table 2. Analysis of variance for grain yield and yield attributing characters in maize (*Zea mays* L.)

Source of variation	Df	DT	DS	ASI	DM	PH	EPH	CL	CG	KR	KPR	CYP	GYP	100 GW	PC
		Mean Sum of Squares													
Replications	1	1.02	1.23	0.01	40.5	36.73	102.04	1.78	0.97	0.16	4.94	5527.51	4039.72	0.26	1.4
Treatments (Unadj.)	48	28.97**	30.16**	0.50*	59.44**	837.15**	314.89**	4.29*	1.06**	1.79**	21.02**	1488.52*	1100.80*	27.76**	2.19**
Blocks within replications (Adj.)	12	4.57	4.09	0.28	20.92	60.07	30.23	2.61	0.39	0.59	23.82	1028.96	760.27	9.59	0.90
Error	36	1.67	1.56	0.28	4.47	81.87	30.12	2.06	0.43	0.69	7.94	813.28	540.46	8.59	0.88
Total	97	36.23	37.04	1.07	125.33	1015.82	477.28	10.74	2.85	3.23	57.72	8858.27	6441.25	46.20	5.37

** Significant at 1% level
*Significant at 5% level

Df: Degrees of freedom; DT: Days to 50 per cent Tasseling; EPH: Ear Placement Height; CYP: Cob Yield Plant^{-1}; DS: Days to 50 per cent Silking; CL: Cob Length; GYP: Grain Yield Plant^{-1}; ASI: Anthesis Silking Interval; CG: Cob Girth; 100 GW:100 Grain Weight; DM: Days to Maturity; KR: Kernel Rows Cob^{-1}; PC: Protein Content; PH: Plant Height: KPR: Kernels.

Table 3: Clustering pattern of 49 maize (*Zea mays* L.) newly developed inbreds by Tocher'smethod

Cluster No	No. of genotypes	Name of genotypes/ Inbreds
I	24	ZL1381388081, ADL 1608, VML 16008, CAL 1511, OML17-47, AL 1620, CML28208, CAL1810, VML 15028, PDML19-38, ZL 161032, PDML19-47, PDML8082, PDML9644, PDML 506, PDML19-45, CAL 1612, ADL 8106, VML 15036, PDML 19-42, PDML 19-43, PDML 19-41, PDML 19-36, PDML 19-37
II	9	KDML-115, PDML 15, VM 51, ADL 1619, PDML 7470, CAL 1784, CAL 17834, VM 45, AL8127
III	5	PDML19-35,PDML19-44,PDML19-34,BML6, CAL1711830
IV	1	PDML124
V	6	CML08-292, CML28708, ADL1608, PDML19-40, PDML19-48, PDML19-39
VI	1	ADL8070
VII	1	PDML19-46
VIII	1	CML 1505
IX	1	TA5084

Table 4: Average intra and inter cluster distances (D^2 values)

Cluster No.	I	II	III	IV	V	VI	VII	VIII	IX
I	18.61	41.23	36.87	26.70	34.74	40.34	35.57	63.63	47.09
II		20.74	63.75	60.18	83.40	60.56	38.58	110.57	51.66
III			26.37	43.56	51.11	63.02	39.89	97.01	49.27
IV				0.00	45.82	23.28	25.45	59.13	59.79
V					31.51	73.01	75.72	50.75	73.32
VI						0.00	16.25	84.20	60.69
VII							0.00	94.54	38.77
VIII								0.00	75.30
IX									0.00

Note: Diagonal values are intra cluster distances. Off-diagonal values are inter cluster distances.

Table 5: The nearest and farthest cluster from each cluster based on D^2 using Tocher's method.

Cluster No.	Nearest cluster with D^2 values	Farthest cluster with D^2 values
I	IV (26.70)	VIII (63.63)
II	VII (38.58)	VIII (110.57)
III	I (36.87)	VIII (97.01)
IV	VI (23.28)	II (60.18)
V	I (34.74)	II (83.4)
VI	VII (16.25)	VIII (84.20)
VII	VI (16.25)	VIII (94.54)
VIII	V (50.75)	II (110.57)
IX	VII (38.77)	VIII (75.3)

Table 6: Cluster mean values for yield and yield components in maize

Cluster No.	Days to Tasseling	Days to Silking	Anthesis Silking Interval	Days to Maturity	Plant Height (cm)	Ear Placement Height (cm)	Cob Length (cm)	Cob Girth (cm)	Kernel Rows per Cob	Number of Kernels per Row	Cob Yield per Plant (g)	Grain Yield per Plant (g)	100 Grain Weight (g)	Protein Content (%)
I	54.94	56.83	1.90	112.19	202.81	87.46	17.26	14.54	14.46	33.94	228.44	182.58	38.83	9.99
II	47.33	49.33	1.94	99.78	174.44	73.50	16.40	14.34	13.78	33.17	205.94	163.80	36.44	10.48
III	59.80	62.30	2.50	117.00	188.80	69.60	16.13	14.37	13.80	34.30	200.00	165.70	37.50	9.49
IV	56.50	58.50	1.50	115.00	225.50	79.00	16.80	15.65	15.00	32.00	215.00	172.00	44.00	11.40
V	58.83	60.92	1.67	116.08	220.00	100.92	17.24	14.14	13.83	35.50	218.17	167.08	38.67	9.77
VI	53.50	55.50	1.00	113.00	201.00	89.50	12.50	15.20	16.00	31.00	242.00	193.50	44.00	9.65
VII	53.50	55.50	2.00	109.00	186.50	69.00	13.75	15.15	16.00	34.00	242.00	193.50	40.00	9.05
VIII	55.50	57.50	1.50	114.00	229.00	108.00	15.75	14.50	14.00	36.50	215.00	121.00	43.00	7.70
IX	53.50	55.50	2.00	113.50	188.50	69.50	12.05	11.80	14.00	35.00	136.00	90.50	43.00	8.40

Note: Bold figures indicate minimum and maximum values in each character

Table 7: Contribution of different characters towards genetic divergence

S. No.	Source	Times ranked first	Contribution per cent
1	Days to 50 per cent tasseling	372	31.63
2	Days to 50 per cent silking	28	2.38
3	Anthesis silking interval	18	1.53
4	Days to maturity	8	0.68
5	Plant height (cm)	128	10.88
6	Ear placement height (cm)	151	12.84

7	Cob length (cm)	42	3.57
8	Cob girth (cm)	79	6.72
9	Kernels rows cob^{-1}	39	3.32
10	Number of kernels row^{-1}	45	3.83
11	Cob yield plant^{-1} (g)	47	4.00
12	Grain yield plant^{-1} (g)	84	7.14
13	100 grain weight (g)	74	6.29
14	Protein content (%)	61	5.19

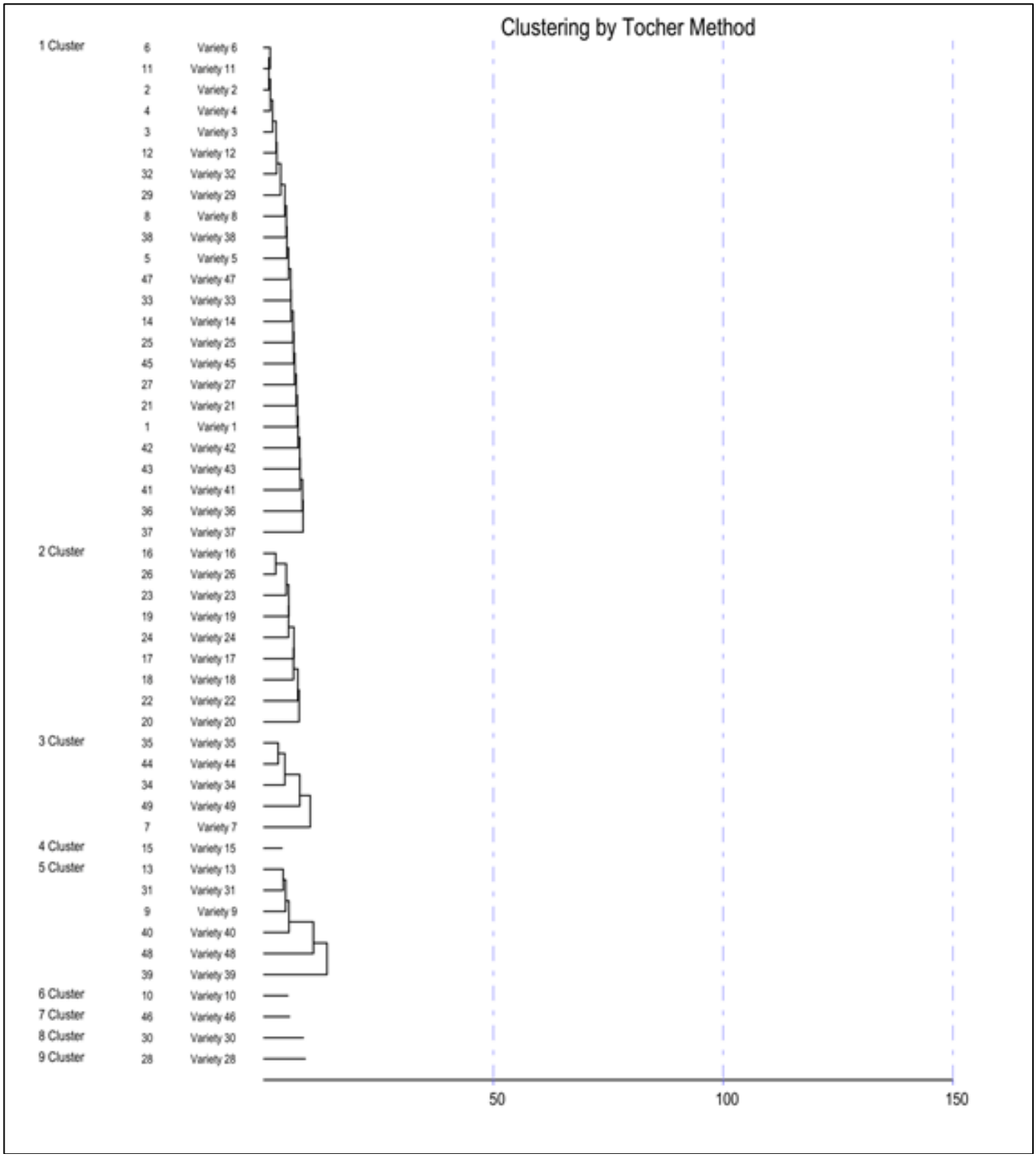

Fig 1: Dendrogram showing relationship among 49 maize inbreds in nine clusters based on Mahalanobis' D^2 values

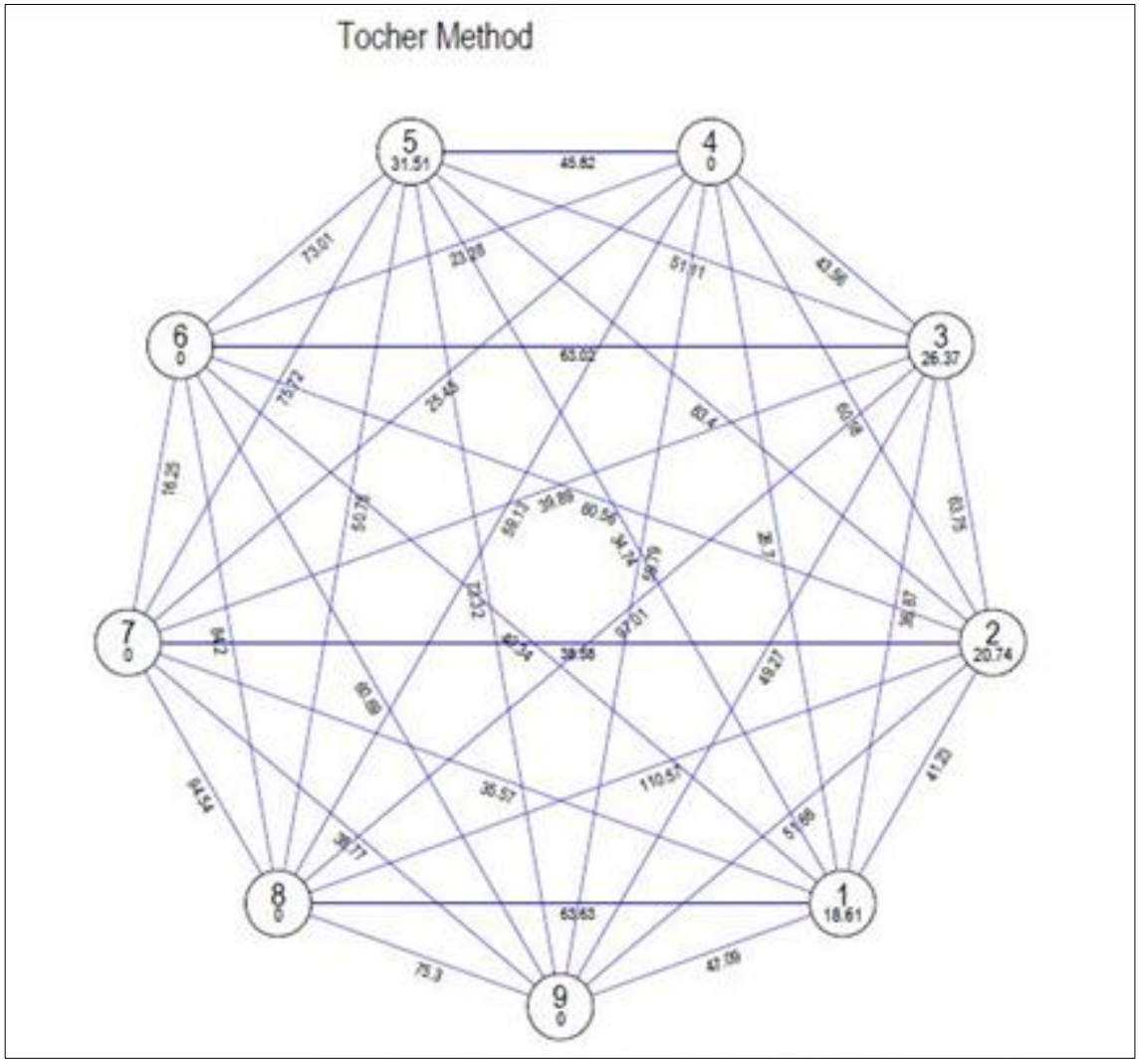

Fig 2: Intra and inter cluster distances of 49 maize inbreds in nine clusters based on Tocher's method

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