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Research article

Genetic divergence studies of fodder yield and quality attributing characteristics in promising maize (*Zea mays* L.) composites

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Abstract

The objective of the current study was to assess the extent of genetic variation among twenty-seven maize varieties using morpho-agronomic data based on Principal Component Analysis (PCA) and to measure the genetic distance among these genotypes using hierarchical cluster analysis. Twenty-seven composites were grown in a randomised complete block design with three replications for two years. The experimental material was assessed for 15 morpho-agronomic traits. Green fodder yield depends on various other traits such as plant height, number of leaves, leaf length and fodder quality traits such as crude protein, acid detergent fibre and neutral detergent fibre. This study showed a positive correlation of green fodder yield with various such traits. A very high positive correlation was noticed between number of leaves and ear height (0.723) and between crude protein and *in vitro* dry matter digestibility (0.823). However, crude protein showed a significant negative correlation with acid detergent fibre (-0.739) and neutral detergent fibre (-0.678). Five principal components had more than one eigen value, contributing 75% variability among genotypes. PC_1 contributes 25.1% followed by PC_2 with 19.9%, PC_3 with 12.5%, PC_4 with 9.7% and PC_5 with 7.8%. The scree plot revealed that the experimental material could be divided into five clusters. The genotypes under cluster five could be used to improve green fodder yield. The minimum intra-cluster distance observed for cluster 1 was 48.008, and the maximum inter-cluster distance observed between clusters 2 and 5 was 259.45. The different groups obtained could be useful for deriving the inbred lines with diverse features, which could be used in various maize breeding programmes.

Keywords: Cluster analysis, Correlation, Principal Component Analysis, *Zea mays*

Introduction

Maize is a fast-growing, succulent, high-yielding, nutritional and toxin-free fodder crop (Arya *et al.,* 2020). Its superior fodder ensiling properties make it preferred over other cereal crops for the lean season, which safeguards the interests of small dairy farmers (Takawale *et al*., 2009). Maize fodder, a rich source of energy and protein for dairy animals, can safely be fed at all stages of growth (Chaudhary *et al.,* 2016). Still, its nutritional quality is significantly superior for cattle, particularly from the tasseling stage onwards (Prasad *et al.,* 2021).

In the present scenario, improving nutritional quality with high biomass production is much desired in fodder crops (Mahanta *et al*., 2020; Kumar *et al*., 2023). So adequate information regarding the extent of genetic divergence among the available germplasm is necessary to develop new high-yielding fodder maize composites or hybrids. The knowledge of genetic variability is precious in a planned breeding programme since it helps to choose the best yield attributes for selection or hybridization, which leads to greater chances for developing superior cultivars (Rathod *et al*., 2021). Keeping this in view, an attempt was made to evaluate the degree and extent of genetic divergence for several green fodder yield contributing traits among the available fodder maize genotypes. The magnitude of genetic diversity can be assessed using statistical tools such as principal component analysis (PCA) and cluster analysis. PCA is a simple, non-parametric method that can evaluate and extract meaningful information from complex data sets (Jain *et al*., 2022). PCA can calculate the significant contributor to the total genetic variation at each axis of differentiation (Singh *et al.,* 2021). The central idea of principal component analysis is to reduce the dimensionality of a data set with many interrelated variables, while retaining the variation in the data set as much as possible. This reduction is achieved by transforming to a new set of variables, the principal components, which are uncorrelated and ordered so that the first few retain most of the variation present in all of the original variables. The computation of the principal components reduces to the solution of an eigen value- eigen vector problem for a positive-semi-definite symmetric matrix (Rao, 1952). The purpose of the present study was to evaluate the genetic diversity among maize genotypes that can be exploited in future breeding programmes to improve fodder yield and its quality by using PCA and cluster analysis.

Materials and Methods

Experimental site and design: The experiment was conducted in the Forage, Millet and Nutrition Section, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. The Experiment was conducted for two consecutive years (*kharif* 2020 and *kharif* 2021). The experimental material comprised of twenty-seven composites made from inbreds belonging to two heterotic pools (*Makki safed* and *Tuxpeno planta baja*), and a few inbreds were derived from indigenous and exotic germplasm (Table 1). The maize composites were sown in a randomized complete-block design with three replications, each in a 5 m^2 plot with row-to-row spacing of 30 cm and plant-to-plant spacing of 22.5 cm. Data for fifteen morpho-agronomic traits *viz.*, green fodder yield (GFY), dry matter (DM), days to 50% pollen (DOP), days to 50% silk (DOS), plant height (PH), ear height (EH), number of leaves (NOL), stem girth (SG), leaf length (LL), leaf width (LW), leaf-stem ratio (LSR), crude protein (CP), acid detergent fibre (ADF), neural detergent fibre (NDF), *in-vitro* dry matter digestibility (IVDMD) was recorded/calculated.

Sampling and laboratory analysis: The crude protein was estimated as per AOAC (2005). The dried ground samples were used for nitrogen content using Kjeldahl digestion procedure. The per cent crude protein content was estimated using the formula; crude protein (%) = N (%) × 6.25, while cell wall components were analysed by the method of Van Soest (1991). *In vitro* dry matter digestibility content was estimated as per the protocol of Tilley and Terry (1963).

Statistical analysis: The basic statistics, correlation matrix and principal component analysis (PCA) of morpho-agronomic traits were analysed using

Table 1. List of genotypes used in experiment

S. No.	Entry	Pedigree/source	S. No.	Entry	Pedigree/source	S. No.	Entry	Pedigree/source
1	**JC1**	Chain cross among the public hybrids	**10**	**JC9**	PMH 1 X J 1006	**19**	**JC17**	PMH 1 X Vijay
2	**JC2**	Seed bulk among indigenous collection	**11**	**JC10**	PMH 1 X African Tall	**20**	**JC18**	J 1006 X Partap Makka Chari-6
3	**JC3**	31 Y 45 X J 1006	**12**	**JC11**	PMH 3 X J 1006	**21**	**JC19**	African Tall X Partap Makka Chari-6
4	**JC4**	31 Y X African Tall	**13**	**JC12**	PMH 3 X African Tall	**22**	**JC20**	MS pool C_8
5	**JC5**	DKC 9108 X J 1006	**14**	**JC13**	Partap Makka Chari-6	**23**	**JC1464**	141243#
6	**JC6**	DKC 9108 X African Tall	**15**	**JC14**	Chain cross among the private hybrids	**24**	**JC1469**	Tux pool C_7 X MS pool C_7
7	**JC7**	P 3396 X J 1006	**16**	**JC15**	Vijay X J 1006	**25**	**JC21**	Chain cross among the private hybrids
8	**JC8**	P 3396 X African Tall	**17**	**JC16**	J 1006 X Vijay	**26**	**African Tall**	Selection from 7 varieties
9	**Parbhat**	(J54 X Suwan) X J54	**18**	**Kesri**	(Ludhiana Local X J 2014) X Parbhat	**27**	**J 1006**	MS_1 X Tuxpeno PBL

Minitab 17 statistical software (2010). The hierarchical cluster analysis was performed with the agglomeration method and the Ward linkage. The dendrogram based on Euclidean distances was developed by Ward's method (Ward, 1963) using Minitab 17 software.

Results and Discussion

Analysis of variance: The basic statistics of morpho-agronomic traits revealed considerable variability among twenty-seven genotypes. The pooled analysis of variance for 15 traits showed significant differences for all traits except stem girth and leaf width (Table 2). The presence of a large amount of variability might be due to the diverse sources of materials taken for the study. This indicated that there is ample scope for the selection of promising lines from the present gene pool for green fodder yield and its attributing traits. The germplasm characterization showed a high extent of phenotypic variability (Table 3). Several studies on maize landraces recorded similar results showing high phenotypic variability (Al-Naggar *et al.,* 2020; Yadav *et al.,* 2022). Trait GFY varied from 176 q/ha to 489.2 q/ha with a mean value of 344.7 q/ha. DM ranged from 14.7 to 25.0 % with a mean value of 21.7%. Traits such as DOP and DOS varied from 49.5 to 58 days and 59.7 to 69.4 days after sowing with mean values of 53.9 and 64.5 days after sowing. PH, EH, NOL, SG, LL, LW and LSR ranged from 120 to 241.05 cm, 56.6 to 157.2 cm, 12.1 to 18.05, 1.5 to 2.2 cm, 61.7 to 99.4 cm, 6.2 to 9.1 cm and 0.19 to 0.59, respectively with mean values of 196.5 cm, 111.9 cm, 14.8, 1.7 cm, 85.5 cm, 7.6 cm and 0.37, respectively. The quality traits CP, ADF, NDF and IVDMD ranged from 6.3 to 8.3%, 34.1 to 38.7 %, 58.9 to 66% and 50.7 to 55.7%, respectively with mean values of 7.4%, 36.4%, 63.16% and 53.6% , respectively.

Genetic variability and heritability: The phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) of 15 morpho-agronomic traits revealed that the estimated values of PCV were higher than the GCV values for all traits indicating greater influence of environment on the expression of traits. Higher PCV and GCV values were recorded for green fodder yield and ear height. However, low phenotypic and genotypic coefficients were observed for days to 50% silk, ADF, NDF and IVDMD (Table 3). The results obtained through the estimation of GCV along with heritability revealed that selection was the most suitable method for the improvement of green fodder yield and its contributing traits. The values for broad sense heritability ranged from 36.5% (GFY) to 99.8% (EH). These results were in agreement with previous studies documenting the phenotypic and genotypic coefficients along with heritability (Goyanka *et al.,* 2022). Thus, the study of genetic parameters like the GCV, PCV and heritability provided a transparent picture of the magnitude of variability present in a set of germplasm.

Correlation coefficients: Correlation coefficients revealed positive associations among traits (Table 4). Correlation studies explained that selection of one trait could improve the correlated traits, while the negative correlation showed the presence of linkage drag. These negative correlations must be broken for the simultaneous improvement of negatively correlated traits. Green fodder yield had a positive correlation with dry matter (0.341), ear height (0.308), stem girth (0.217), leaf length (0.314), crude protein (0.312) and *in vitro* dry matter digestibility (0.277) and negative and significant correlation with leaf stem ratio (-0.305) and ADF (-0.241). Days to 50% pollen had a significant and positive correlation with days to 50% silk (0.526). Plant height showed a significant and positive correlation with ear height (0.654), number of leaves (0.713) and leaf length (0.556). The results of correlated traits like DOP and DOS, and PH and EH were in agreement with Belalia *et al.* (2019). Ear height exhibited a positive association with the number of leaves (0.723), SG (0.312), LL (0.430), CP (0.349) and IVDMD (0.412). Number of leaves was associated with stem girth (0.304) and leaf length (0.448). Stem girth revealed an association with leaf length (0.306) and leaf width (0.498). Leaf length was positively correlated with NDF (0.315). The CP exhibited a highly positive and significant correlation with IVDMD (0.873) and negatively significant with ADF (-0.739) and NDF (-0.678). ADF had a positive correlation with NDF (0.618) and significantly negative correlation for IVDMD (-0.687). NDF had a negative and significant association with IVDMD (-0.498). These results were in agreement with that of More (2003) and Kapoor (2017).

Principal components analysis: The principal component analysis (PCA) was performed to reduce the data set for 15 morpho-agronomic traits under investigation. The Eigen values for all traits were determined along with the proportion of each principal component contributing to the variation (Table 5). Five principal components (PCs) had an Eigen value of more than 1 (Fig 1). These five PCs contributed 75% of the total variability amongst the

Table 2. Pooled analysis of variance for forage and quality traits in maize

Source	DF	GFY	DM	DOP	DOS	PH	EH	NOL	SG	LL	LW	LSR	CP	ADF	NDF	IVDMD
Year	1	514.6	1.2	5.6	5.4	103.4	1802.9	8.6	0.001	36.1	0.064	0.1	4.4	91.3	2.4	54.5
Rep within year	4	417.3	1.4	8.2	4.2	1090.9	65.2	2.7	0.017	4.2	0.3	0.01	0.6	4.0	30.7	16.2
Genotypes	26	43135.8	33.1	30.0	41.0	6386.2	3603.4	10.5	0.1	554.6	3.4	0.04	1.6	8.6	27.8	12.0
Genotypes x Year	26	594.2	5.1	0.7	2.6	229.5	18.5	1.5	0.04	176.3	0.05	0.05	0.3	2.8	11.2	4.1
Pooled error	104	133.7	0.7	0.9	0.1	276.4	0.9	0.6	0.009	0.253	0.007	0.002	0.07	0.1	0.3	0.4

GFY: Green fodder yield (q/ha); DM: Dry matter (%); DOP: Days to 50% pollen; DOS: Days to 50% silk; PH: Plant height (cm); EH: Ear height (cm); NOL: Number of leaves; SG: Stem girth, LL: Leaf length (cm); LW: Leaf width (cm); L:S: Leaf-stem ratio; CP: Crude protein (%):, ADF: Acid detergent fibre (%); NDF: Neural detergent fibre (%); IVDMD: *In-vitro* dry matter digestibility (%)

Table 3. Pooled descriptive statistics for forage and quality traits in maize

Variable	Minimum	Maximum	Mean	SD	Variance	PCV	GCV	Heritability
GFY (q/ha)*	176.0	489.2	344.7	86.1	7423.6	25.1	24.7	0.36
DM(%)	14.7	25.0	21.7	2.5	6.5	12.5	11.2	0.80
Days to 50% pollen	49.5	58.0	53.9	2.4	6.7	4.0	3.9	0.94
Days to 50% silk	59.7	69.4	64.5	2.5	6.7	3.9	3.8	0.95
Plant height	120.0	241.0	196.5	35.6	1270.5	16.3	16.2	0.99
Ear height	56.6	157.2	111.9	24.4	596.6	22.3	22.3	0.99
No. of leaves	12.1	18.0	14.8	1.5	2.2	9.9	9.4	0.89
Stem girth	1.5	2.280	1.7	0.2	0.0	12.0	11.7	0.94
Leaf length	61.7	99.4	85.5	11.0	120.9	12.9	12.9	0.99
Leaf width	6.2	9.1	7.6	0.7	0.5	10.2	10.2	0.99
Leaf stem ratio	0.1	0.5	0.3	0.0	0.0	25.6	17.3	0.45
CP (%)	6.3	8.3	7.4	0.5	0.2	8.7	8.3	0.90
ADF (%)	34.1	38.7	36.4	1.2	1.4	3.9	3.7	0.89
NDF (%)	58.9	66.0	63.1	2.117	4.4	3.9	3.9	0.95
IVDMD (%)	50.7	55.7	53.6	1.3	1.9	3.3	3.1	0.90

*10 quintals (q) = 1 ton

Table 4. Correlation matrix of various forage and quality traits in maize

Attributes	**GFY**	**DM**	**DOP**	**DOS**	**PH**	**EH**	**NOL**	**SG**	**LL**	**LW**	**L:S**	**CP(%)**	**ADF(%)**	**NDF(%)**	**IVDMD(%)**
GFY	**1**														
DM	0.341	**1**													
DOP	-0.107	-0.222	**1**												
DOS	0.181	-0.294	0.526*	**1**											
PH	0.198	0.151	-0.063	0.057	**1**										
EH	0.308	0.227	-0.234	0.208	0.654*	**1**									
NOL	0.097	0.198	-0.021	0.089	0.713*	0.723	**1**								
SG	0.217	0.015	-0.257	-0.071	0.156	0.312	0.304	**1**							
LL	0.314	0.074	0.004	0.217	0.556*	0.430	0.448	0.306	**1**						
LW	0.061	-0.011	-0.113	-0.254	0.136	0.067	0.167	0.498	0.034	**1**					
L:S	-0.305	-0.207	-0.002	-0.059	-0.003	-0.260	0.035	0.141	-0.051	0.134	**1**				
CP (%)	0.312	0.149	0.098	0.244	0.163	0.349	0.092	0.025	0.036	0.076	-0.154	**1**			
AD F(%)	-0.241	-0.057	-0.040	-0.241	0.216	-0.194	0.166	0.164	0.197	0.065	0.294	-0.739*	**1**		
NDF (%)	0.021	-0.087	0.049	0.023	0.077	-0.238	0.019	0.076	0.315	0.024	0.266	-0.678*	0.618	**1**	
IVDMD (%)	0.277	0.237	-0.058	0.214	0.266	0.412	0.162	0.059	0.032	0.039	0.014	0.873**	-0.687	-0.498*	**1**

genotypes selected under investigation. The remaining components contributed only 25% towards the genetic diversity. Pavithra *et al.* (2022) also recorded 70.30% variability in 4 principal components in 93 inbreds of fodder maize. Priyanka *et al.* (2022) recorded similar results in oat germplasm with 72.18% variability due to 4 principal components. The PC_1 contributed maximum variability towards the total variability (25.1%) followed by PC_2 (19.9%), PC_3 (12.5%), PC_4 (9.7%) and PC_5 (7.8%). The similar variability for PC_1 (23.23%) was estimated by Kujur *et al.* (2017).

For PC_1, traits like GFY, PH, EH, NOL, CP and IVDMD revealed significant and positive factor loadings, on the contrary ADF and NDF showed negative loadings. Traits like PH, EH, NOL, SG, LL, ADF and NDF exhibited positive loadings for PC_2 while CP showed negative loadings (Table 5). This revealed that the traits like GFY, PH, EH; NOL and quality traits (CP, ADF, NDF and IVDMD) were the major contributing traits towards diversity. Kumari *et al.* (2017) reported similar results, while studying 75 diverse maize accessions using 12 quantitative traits where maximum variability was observed in the first two PCs with plant height, ear height, ear width, number of kernels per row and kernel rows were major contributing traits towards phenotypic diversity. PC_3 was related to diversity among genotypes due to DOP and DOS with positive loadings and LW with negative loadings. PC_4 was elucidated by diversity among genotypes for LW and LSR with positive loadings and DM with negative loadings. PC_5 was elucidated by diversity among genotypes for negative loadings of GFY and with positive loadings for the number of leaves.

A hierarchical cluster analysis was performed with the agglomeration method using Ward method (Ward, 1963). Twenty seven maize genotypes were grouped into 5 clusters using Elbow method based on 15 quantitative traits (Fig 2). Cluster analysis revealed that cluster 1 was comprised of 6 genotypes, cluster 2 of 5, and cluster 3 of 1, while cluster 4 contained 10 and cluster 5 contained 5 genotypes (Table 6). Cluster 2 comprised of genotypes with higher values for ADF. The third cluster was comprised of genotypes having higher values for DOP, DOS and LSR. Cluster 4 was comprised of genotypes with maximum values for PH, NOL and NDF. The members of cluster 5 (JC13, JC19, JC20, JC1464, African Tall) were characterized by higher values for GFY, DM, EH, SG, LL, LW, CP and IVDMD. Cluster 1 showed reasonable values for most of the traits under investigation (Table 7).

Table 5. Eigen value, contribution of variability and eigen vectors for the principal component axis of forage maize

Attributes	PC1	PC2	PC3	PC4	PC5
Eigen values	**3.770**	**2.984**	**1.878**	**1.454**	**1.169**
Proportion	0.251	0.199	0.125	0.097	0.078
Cumulative proportion	0.251	0.450	0.575	0.672	0.750
GFY	**0.266**	0.054	-0.010	**-0.295**	**-0.583**
DM	0.178	0.056	-0.296	**-0.437**	0.039
DOP	-0.050	-0.126	**0.534**	0.139	-0.065
DOS	0.134	-0.085	**0.599**	0.101	-0.206
PH	**0.279**	**0.358**	0.124	-0.007	**0.300**
EH	**0.408**	**0.214**	0.034	-0.068	0.181
NOL	**0.269**	**0.364**	0.095	0.063	**0.328**
SG	0.126	**0.289**	-0.224	**0.335**	**-0.398**
LL	0.186	**0.363**	0.244	-0.094	-0.180
LW	0.060	0.172	**-0.314**	**0.443**	**-0.292**
LSR	-0.151	0.117	-0.011	**0.532**	0.162
CP (%)	**0.408**	**-0.273**	-0.004	0.185	-0.021
ADF (%)	**-0.290**	**0.415**	0.023	-0.043	0.105
NDF (%)	**-0.256**	**0.345**	0.178	-0.095	-0.250
IVDMD (%)	**0.408**	-0.200	-0.044	0.194	0.066

Table 6. Number and name of genotypes in different clusters

Cluster	Number of gentoypes	Genotypes
I	6	JC1 ,JC4, JC5, Parbhat, JC16, Kesri
II	5	JC2,JC6, JC8, JC1469, JC1464
III	1	JC3
IV	10	JC7, JC9, JC11, JC12, JC14, JC15, JC17, JC18, JC21, J 1006
V	5	JC13, JC19, JC20, JC1464, African Tall

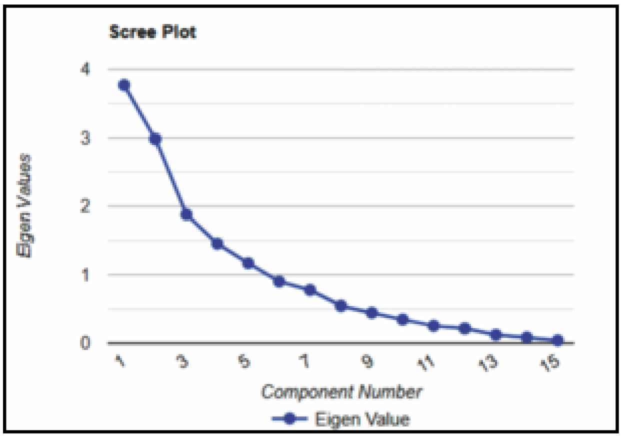

Fig 1. Scree plot on the basis of Eigen values and number of components

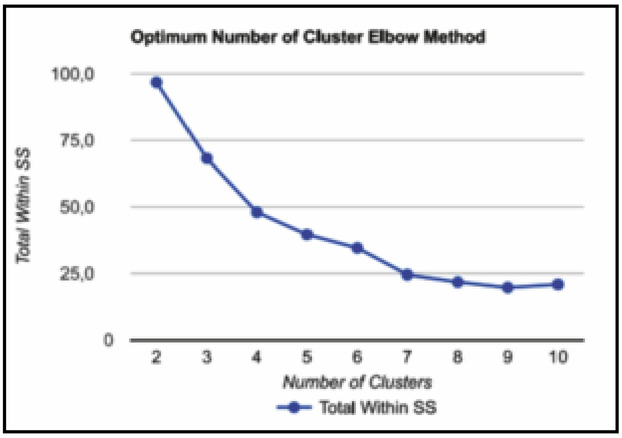

Fig 2. Optimum number of clusters using Elbow method

Table 7. Estimated mean values of five clusters based on different quantitative traits

Cluster	GFY	DM	DOP	DOS	PH	EH	NOL	SG	LL	LW	L:S	CP(%)	ADF(%)	NDF(%)	IVDMD(%)
I	287.325	22.267	53.267	63.283	170.833	103.775	14.317	1.763	75.917	7.332	0.378	7.183	36.558	63.192	53.342
II	195.538	19.563	54.950	64.513	199.375	102.425	15.150	1.700	89.538	7.851	0.444	7.413	36.775	62.888	53.225
III	337.900	17.650	57.950	68.150	120.000	64.050	12.100	1.535	61.750	7.210	0.455	7.500	34.950	61.100	53.500
IV	381.155	22.232	54.155	64.718	212.918	119.773	15.450	1.741	86.936	7.637	0.354	7.441	36.673	63.418	53.595
V	454.140	22.490	52.910	64.880	204.410	121.500	14.700	1.862	95.750	7.886	0.356	7.800	35.740	63.230	54.390

Table 8. Average intra and inter-cluster distance (Euclidean D^2 values)

Cluster	I	II	III	IV	V
I	**48.008**				
II	97.158	**61.489**			
III	83.664	169.866	**0**		
IV	104.675	186.958	119.629	**48.618**	
V	172.243	259.459	158.639	74.053	**88.140**

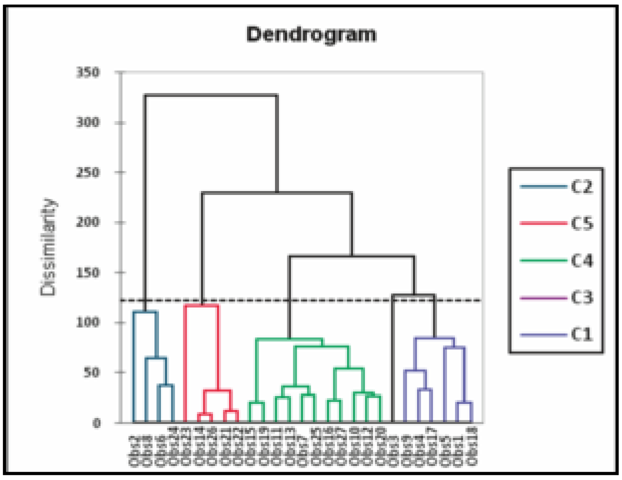

Fig 3. Dendrogram showing the relationship of 27 maize genotypes in five clusters based on Euclidean D^2 distance (Observation no. corresponds to entry number)

The dendrogram explained the clusters (Fig 3). Cluster 3 was solitary with an intra-cluster distance of zero because of its mono-genotypic nature. Cluster 1 had a minimum intra-cluster Euclidean distance value of 48.008 followed by cluster 4 (48.618), cluster 2 (61.489) and cluster 5 (88.140). The maximum inter-cluster distance was observed between clusters 2 and 5 (259.45) followed by clusters 2 and 4 (186.95), clusters 1 and 5 (172.24), clusters 2 and 3 (169.86) and clusters 3 and 5 (158.63) indicating the presence of maximum divergence between genotypes of these clusters (Table 8). The genotypes present in one cluster differed entirely from those present in other clusters.

Conclusion

Based on cluster mean and divergence observed in the present study, the genotypes *viz.* JC13, JC 19, J 20, JC 1464 and African Tall were found distinct and diverse. These genotypes could be classified as promising and might be used in crossing programme to achieve desired segregants in forage maize**.** The desirable correlations and high level of genetic variation present for most of the traits could be exploited for the derivation of genotypes having high fodder yield with good quality.

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