



Evaluation of variations for phenotypic traits by multivariate techniques in sweet corn (*Zea mays* L. *saccharata*)

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Received : 05.08.2022 ; Revised : 22.11.2022 ; Accepted on 02.12.2022

DOI: <https://doi.org/10.22271/09746315.2023.v19.i1.1675>

ABSTRACT

An experiment was carried out in sweet corn (*Zea mays* L. *saccharata*) to estimate the extent of genetic diversity of 25 inbred lines in randomized block design (RBD) with two replications. Based on the observations of twelve yield and its attributing traits, four principal components (PCs) were created having eigen value of >1 and explaining 84.64% of total variation. In two-dimensional biplot, three groups of traits were distinguished in regard to first and second principal components (PC1 & PC2) with angles between vectors exhibiting high positive correlation of cob weight with and without husk with traits such as number of kernels per row, ear diameter, ear length and plant height. The dendrogram developed by Hierarchical clustering using Ward's method has assigned the inbred lines into six clusters, explaining the diverse nature of genotypes to assist in selection of parental material for further sweet corn breeding programmes.

Keywords: Cluster analysis, genetic diversity, multivariate analysis, principal component analysis, sweet corn.

Sweet corn (*Zea mays* L. *saccharata*) is a variety of corn having four to eight times the total sugar found in non-mutant corn (Tracy, 2001). It can be consumed in fresh and raw forms, in roasted form as well as after cooking, as delicious sweet corn soup, baby corn, candy prepared with immature kernels, as alcoholic beverage chichi from mature kernels, as raw material for deriving industrial products like starch syrup, dextrose and dextrin etc. and whole plant can be used as feed for livestock. With increase in consumption and development of many organized processing industries (Chhabra *et al.*, 2019) in India and other developing and developed countries, the demand has been increased for sweet corn in urban and peri urban areas of these countries.

In a cross-pollinated crop like sweet corn, hybrid development is of great importance owing to its popularity for high yielding nature than the existing cultivars. Knowledge about genetic diversity and relatedness among breeding materials is the baseline for any breeding programme to assist in crop improvement strategies (Aleem *et al.*, 2021). Evaluation of the developed inbred lines for their genetic diversity helps the breeder to downsize the available gene pool by eliminating some parents and to concentrate their efforts in reduced number of hybrid combinations.

Among the various methods of characterization such as morphological, biochemical, cytological and

molecular methods for the assessment; description and classification of germplasm collections, method of phenotypic characterization has been proven as the efficient method (Al-Naggar *et al.*, 2020; Fuzatto *et al.*, 2002; Singh *et al.*, 2020). Univariate and multivariate analyses can be used to estimate the extent of genetic diversity. Multivariate analyses have become popular in recent times to measure the amount of genetic diversity involving various characters.

In order to interpret the pattern and magnitude of variability (Bhandari *et al.*, 2017), multivariate statistics methods such as the Mahalanobis D² statistics, Principal component analysis (PCA) and Hierarchical cluster analysis based on the Ward's minimum variance have been reported to assess genetic divergence, to classify germplasm in to different groups and to select diverse parents for development of high yielding hybrids as well as inbreds.

PCA forms the basis of multivariate data analysis and has been widely applied in plant sciences for reduction of dimensionality of large datasets, increase of interpretability and minimization of information loss simultaneously as it does not discard any samples or characteristics (variables). To assess the genetic variability among genotypes of maize, PCA has been used by many researchers owing to its ability to retrieve small number of principal components (PCs) accounting for most of the variations in the data (Stephen *et al.*, 2016).

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How to cite : Chavan, S., Bhadraru, D., Swarnalatha, V. and Mallaiiah, B. 2023. Evaluation of variations for phenotypic traits by multivariate techniques in sweet corn (*Zea mays* L. *saccharata*). *J. Crop and Weed*, 19 (1): 164-172.

To facilitate the classification of data, cluster analysis is an important technique to divide the genetic material on the basis of morphogenetic traits into various homogenous groups. Hierarchical cluster analysis can be applied readily to assess genetic dissimilarity and similarity in germplasm collections by determining the distance and relatedness of any kind of samples characterized by any kind of descriptors and by displaying it in the form of a dendrogram (Peeters and Martinelli, 1989). In consideration of the aforementioned, the present study was envisaged to measure the genetic diversity of 25 sweet corn inbred lines using PCA and hierarchical cluster analysis using Ward's method.

MATERIALS AND METHODS

The study pertaining to evaluation of sweet corn inbred lines using principal component analysis (PCA) and hierarchical cluster analysis was conducted at Maize Research Centre (MRC) situated at Rajendranagar, Hyderabad located at an altitude of 542.6 m at 79°23'E longitude and 17°19'N latitude during *rabi*, 2019-20. The twenty-five sweet corn inbred lines (Table 1) were grown in randomized block design with two replications at spacing of 60 x 20 cm in two rows of two meters length.

The data was collected for twelve yield and its attributing traits *viz.*, days to 50% pollenshed, days to 50% silking, ear length in cm, ear diameter in cm, number of kernel rows per ear, number of kernels per row, ear height in cm, cob weight with husk in kg ha⁻¹, cob weight without husk in kg ha⁻¹, plant height in cm and green fodder yield in kg ha⁻¹. Total soluble sugar (TSS) was measured in % by using portable digital brix refractometer. It works on the principle of passing light through a sample and measuring the refraction. TSS is measured by the amount that the light bends.

"R" software version 4.1.3 (2022) was used to perform the statistical analysis of data. Mean values were taken for analysis of variance as described by Panse and Sukhatme (1978). PCA was performed using the "FactoMineR" package (Husson *et al.*, 2020) and cluster analysis by using the Ward's method of hierarchical clustering technique (Ward, 1963).

RESULTS AND DISCUSSION

The mean and range for the twelve yield and its attributing traits are presented in Table 2. For the yield *i.e.*, cob weight with husk, the range was from 10429 kg ha⁻¹ to 25408 kg ha⁻¹ with a mean of 17329 kg ha⁻¹. For total soluble sugars, mean was observed to be 14.1% with a range from 11.7% to 17.2% and the green fodder yield, the mean was 30771 kg ha⁻¹ with a range from 24220 kg ha⁻¹ to 35072 kg ha⁻¹.

Principal component analysis (PCA)

PCA was performed to reduce the dimensionality of multivariate data by lowering the number of yield traits responsible to the maximum percentage of total variation into new variables (components) so as to maximize the information in first few components (Abdi and Williams, 2010; Stauffer *et al.*, 1985) among the sweet corn inbred lines.

Among the twelve traits used for performing principal components analysis, only four principal components (PCs) exhibiting eigen value of more than 1 attributed for majority of the variability registering about 84.64% variability among the traits studied in 25 inbred lines (Fig. 1). Therefore, these four PCs were given due importance for the further explanation in the present study. The amount of variation exhibited by PC1, PC2, PC3 and PC4 were 43.052, 18.004, 15.243 and 8.340 percent, respectively (Table 3). PC1, PC2 and PC3 accounted for majority of the variability with a cumulative of 76.3 percent.

The cut-off point was considered where absolute magnitude of the eigen vector coefficients exhibited value of equal to or more than 0.3 (Badu-Apraku *et al.*, 2006). A trait exhibiting coefficient of > 0.3 was considered to be an important trait as it had large enough effect. Traits having coefficient value of < 0.2 were regarded to be of no effect to the overall variation (Laude and Carena, 2015; Sharifi *et al.*, 2018). The PC1 explained 43.052% of total variation and had reported large loadings for six traits. Among the studied traits, highest positive contribution of PC1 were observed for cob weight with husk followed by cob weight without husk, ear length, ear diameter, no. of kernels per row and plant height. This component was the weighted average of the characters determining the yield level, thus reporting largest participation of these traits in divergence and there by variability. PC2 added 18.004% of total variation and loadings showed negative sign of the eigen vector coefficients for days to 50% silking followed by days to 50% pollenshed and total soluble sugars (%). Third PC registered 15.243% of the total variation with major contributions from number of kernels per row in positive sign. Whereas, the fourth PC explained 8.340% of total variation and the traits - total soluble sugars (%), plant height and ear height were found as the major contributors in this component. With regard to first and second principal components, characters under study were grouped into three group of characters. The first group which correlated positively with first two PCs comprised of cob weight with and without husk, ear diameter, ear length, green fodder yield, ear height and number of rows per cob. Second group composed of number of kernels per row and plant

Table 1: List of 25 sweet corn inbred lines used in the present study

S. No.	Genotype no.	Name of genotype (Inbred line)
1	G1	SCGP-2
2	G2	SCGP-83-1
3	G3	SCGP-82
4	G4	SCGP-36-2-1
5	G5	SCGP-54
6	G6	SCGP-207-1
7	G7	SCGP-42
8	G8	SCGP-66-1
9	G9	SCGP-61-1-4
10	G10	SCGP-88
11	G11	VNR-SC-24
12	G12	SCGP-36-3-1
13	G13	SCGP-91
14	G14	SCGP-111
15	G15	SCGP-114-1
16	G16	SCGP-118
17	G17	SCGP-207
18	G18	BSL-4
19	G19	VNR-SC-7
20	G20	VNR-SC-12
21	G21	SCGP-80
22	G22	SCGP-44-2
23	G23	SCGP-210-2
24	G24	VNR-SC-23
25	G25	VNR-SC-29

Table 2: Mean and range for yield and its attributing traits in sweet corn

S.No.	Trait	Mean	Range	
			Minimum	Maximum
1	Days to 50% pollenshed	74	70	78
2	Days to 50% silking	77	72	81
3	Plant height (cm)	143	119	175
4	Ear height (cm)	46	32	67
5	Ear length (cm)	15	9	19
6	Ear diameter (cm)	12	9	14
7	Number of kernel rows per ear	15	12	20
8	Number of kernels per row	27	18	37
9	Cob weight with husk (kg ha ⁻¹)	17329	10429	25408
10	Cob weight without husk (kg ha ⁻¹)	13267	6581	20211
11	Total soluble sugars (%)	14.1	11.7	17.2
12	Green fodder yield (kg ha ⁻¹)	30771	24220	35072

height correlated positively with PC1 and negatively with PC2. Third group that correlated negatively with PC1 and positively with PC2 consisted of days to 50% pollenshed, days to 50% silking and total soluble sugars (%) (Table 4).

The genotypes (inbred lines) were plotted on biplot regarding the first two PCs to look over the diversity among them. The inbred lines had dispersed in all

directions in the biplot (Fig. 2). Lines that were closely located on the biplot, perceived as alike when rated on given attributes. The lines which were of more distance from the point of origin, more diverse are the lines from others. SCGP-61-1-4 clogged far away from the origin and was considered to be the diverse one among others.

The angles between the vectors gives an idea about the correlation between the traits. If angle between

Table 3: Eigen value, percentage of variance and cumulative percentage of variance for all the 12 principal components

Principal components (PCs)	Eigen value	Percentage of variance	Cumulative percentage of variance
1	5.166	43.052	43.052
2	2.161	18.004	61.057
3	1.829	15.243	76.300
4	1.001	8.340	84.640
5	0.719	5.991	90.631
6	0.477	3.971	94.602
7	0.338	2.814	97.416
8	0.152	1.271	98.686
9	0.083	0.689	99.375
10	0.060	0.497	99.872
11	0.012	0.103	99.975
12	0.003	0.025	100.000

Table 4 : Principal component analysis for 12 yield and its attributing traits

Character	PC1	PC2	PC3	PC4
Days to 50% pollenshed	-0.183	-0.581	0.166	-0.085
Days to 50% silking	-0.156	-0.589	0.203	-0.082
Plant height	0.324	-0.041	-0.293	0.329
Ear height	0.263	-0.191	-0.439	0.301
Ear length	0.358	0.035	0.245	0.184
Ear diameter	0.354	-0.080	-0.292	-0.267
Number of kernel rows per cob	0.088	-0.170	-0.488	-0.614
Number of kernels per row	0.340	0.105	0.332	-0.024
Cob weight with husk	0.395	-0.113	0.170	-0.094
Cob weight without husk	0.392	-0.115	0.191	-0.083
Total soluble sugars	-0.071	-0.359	-0.206	0.534
Green fodder yield	0.275	-0.274	0.226	-0.057

vectors of two characters is less than 90° , then those characters are considered to be positively correlated. Whereas, if the angle is more than 90° or equal to 90° , they are considered to be negatively correlated and independent respectively (Yan and Rajcan, 2002). Positive correlations were evident for cob weight with and without husk, ear height, green fodder yield, ear diameter, plant height, no. of rows per ear, ear length, and no. of kernels per row; and for traits like days to 50% pollenshed, total soluble sugars (%), days to 50% silking and no. of rows per ear, with the angles between them measuring less than 90° . However, cob weight with and without husk were highly correlated with ear diameter, plant height, number of kernels per row and ear length, with an angle measuring less than 45° between them. No traits were observed to have a large angle of close to 180° and thus no negative correlation was reported between any of the traits under study (Fig. 3).

The observation of Principal components has revealed more and instant contribution of the characters viz., no. of kernels per row, ear length, plant height, cob weight with and without husk and ear diameter towards variability. Thus, these traits will be considered first in selection followed by other traits in future hybridization programmes. The other traits did not make considerable contribution to the variation within the inbred lines under study and can be dropped under similar analysis.

Similar findings were also reported by identifying principal components (PCs) whose eigen values are more than one in maize by Bhusal *et al.* (2016) indicating 78.12% of total variation by first five PCs, Singh *et al.* (2020) reporting 75.89% of cumulative variation by first three PCs, Al Nagggar *et al.* (2020) and Shashibhushan *et al.* (2021) indicated 88.54% and 88.50% of total variation by first five and four PCs respectively. Saleh *et al.* (2022) showed first three PCs contributed to 77.3% and 81.2% of cumulative variance in two different locations under study.

Table 5 : d values based on phenotypic yield and its attributing traits analysis among 25 sweet corn inbred lines

	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	G15	G16	G17	G18	G19	G20	G21	G22	G23	G24	
G2	4.11																								
G3	3.59	4.25																							
G4	3.99	5.19	3.49																						
G5	3.64	5.69	6.34	7.10																					
G6	3.00	4.63	5.37	4.74	4.04																				
G7	2.04	4.81	3.56	3.27	4.34	2.73																			
G8	4.32	5.70	6.00	7.06	3.26	4.03	4.19																		
G9	3.26	4.30	3.40	5.15	4.80	4.66	4.04	5.17																	
G10	3.57	4.69	5.91	6.58	2.22	3.53	3.96	2.42	4.86																
G11	3.93	5.66	5.75	4.51	5.78	2.14	3.23	5.28	5.88	5.10															
G12	4.86	6.32	6.96	6.03	4.56	2.67	3.87	4.03	6.41	3.87	3.59														
G13	2.52	3.82	2.83	4.58	4.35	3.74	2.64	3.40	2.92	3.77	4.69	5.16													
G14	4.66	6.38	5.53	4.74	6.50	4.13	3.57	5.04	5.87	5.71	4.03	4.51	4.10												
G15	6.50	7.92	8.81	8.65	4.68	5.10	6.04	4.05	8.23	4.32	5.91	3.51	6.66	6.82											
G16	5.62	6.08	7.11	7.85	4.50	5.00	5.32	2.45	6.85	3.40	6.04	4.38	4.70	5.60	3.54										
G17	3.29	4.16	4.23	5.11	3.71	2.84	3.01	3.09	3.25	3.25	4.28	3.81	2.48	4.74	5.47	4.41									
G18	3.01	4.87	4.38	3.84	5.03	2.18	1.84	4.05	4.90	4.15	2.09	3.40	3.07	3.03	5.70	4.97	3.23								
G19	3.71	4.86	5.25	4.19	5.27	3.16	2.87	4.78	6.22	4.29	3.12	3.55	4.24	4.31	5.20	4.74	4.29	2.44							
G20	4.75	5.86	3.34	3.02	7.41	5.14	3.63	6.44	5.06	6.77	4.70	6.15	4.10	4.12	8.41	7.23	4.57	3.84	4.94						
G21	3.27	5.42	4.68	3.18	5.13	3.89	2.88	6.20	4.93	5.21	4.54	4.80	4.79	5.72	7.12	7.20	4.46	4.03	3.94	5.10					
G22	2.53	4.89	5.09	4.65	3.57	3.82	3.06	4.85	5.03	3.72	4.87	4.58	4.11	5.47	5.74	5.40	4.25	4.14	3.35	5.89	2.97				
G23	4.71	5.50	5.00	2.99	7.00	5.35	4.38	7.99	5.71	6.77	5.75	6.44	5.91	6.50	9.13	8.68	5.92	5.33	5.18	5.42	2.77	4.72			
G24	2.87	4.79	3.92	2.64	5.21	3.86	2.68	6.10	3.97	5.08	4.61	5.08	4.19	5.18	7.62	7.16	4.12	3.93	4.29	4.41	1.65	3.26	2.30		
G25	4.89	3.85	4.87	4.18	6.42	4.18	4.52	6.53	4.95	5.59	4.96	5.27	5.03	6.33	7.92	7.27	4.10	4.49	4.84	5.26	4.02	5.47	4.02	3.72	

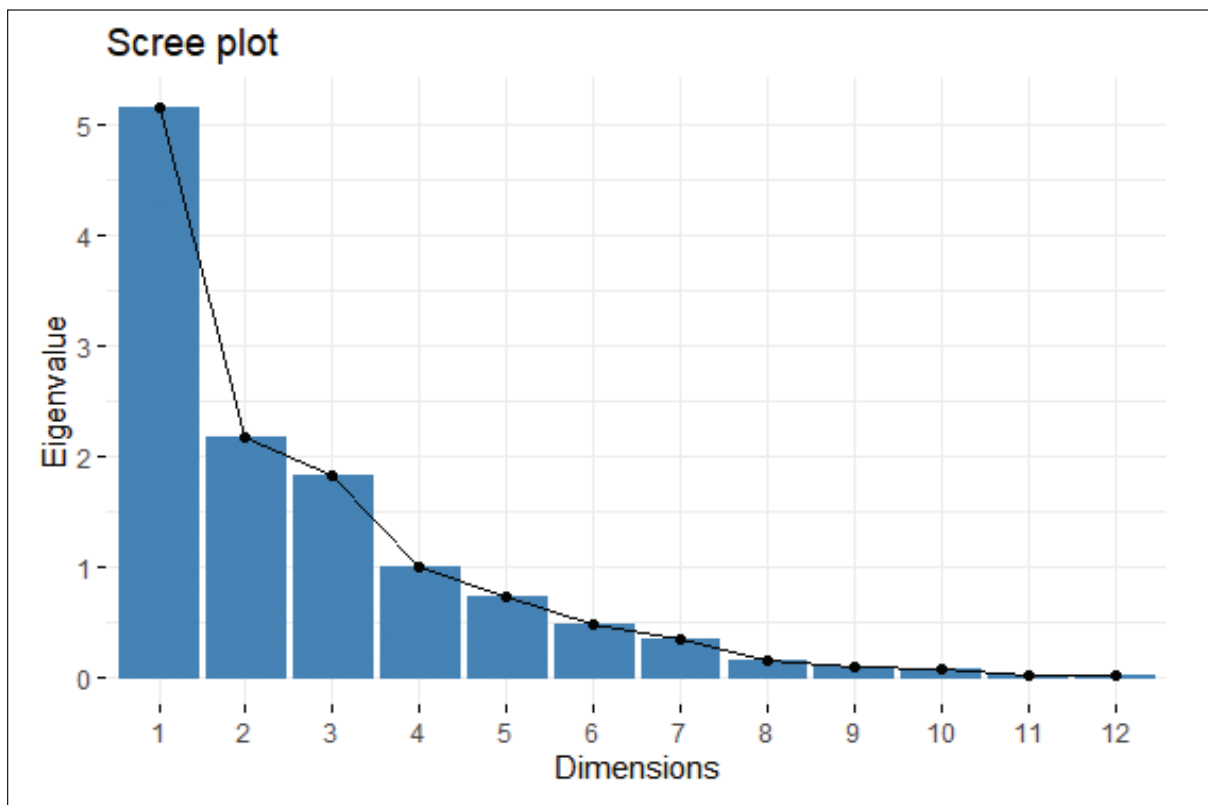


Fig. 1: Scree plot of 12 principal components (PCs) against its eigen values

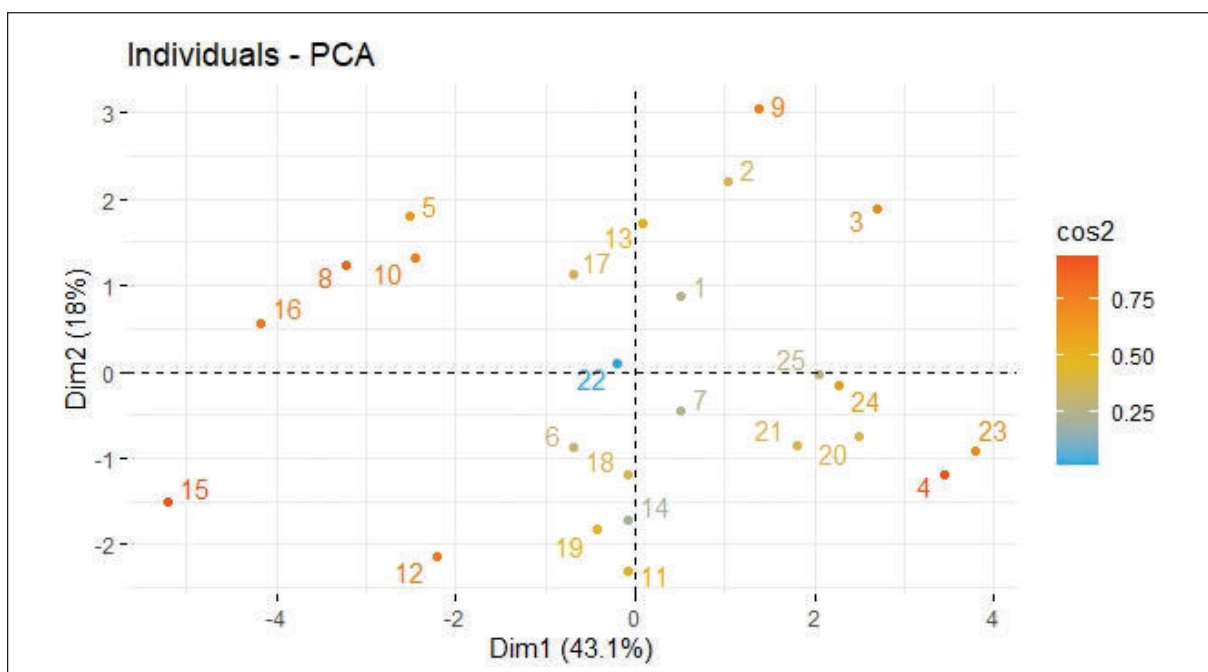


Fig. 2 : Biplot dispersion graph of 25 sweet corn inbred lines

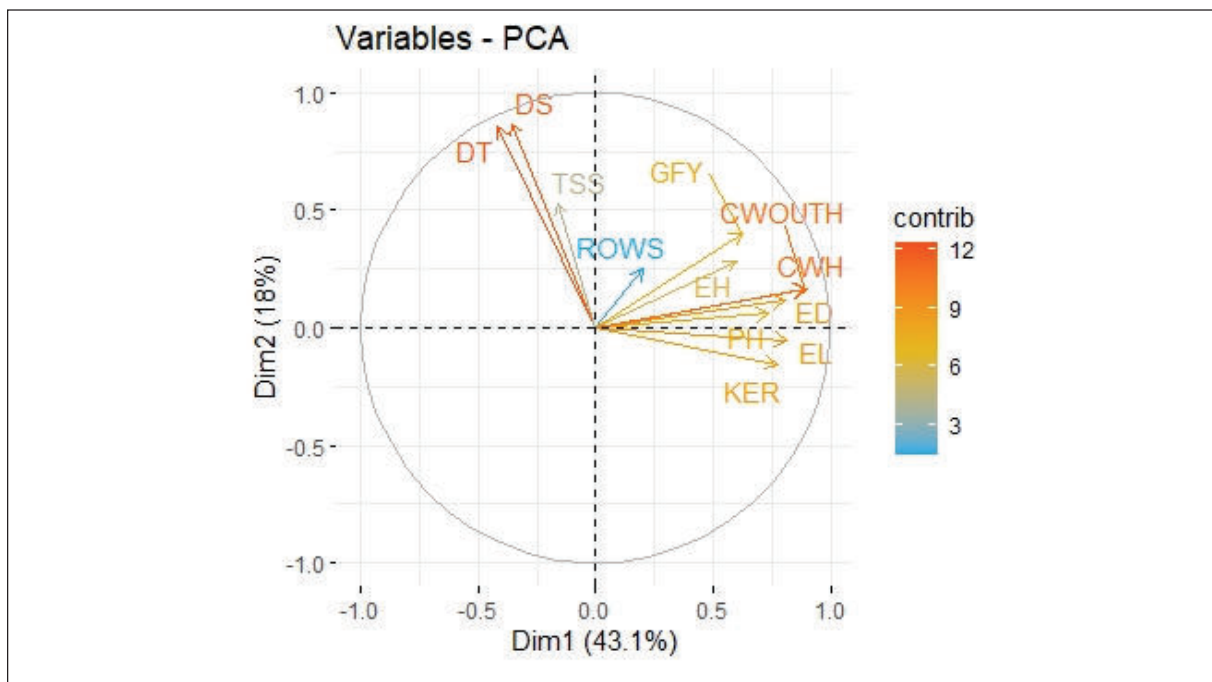


Fig. 3: Two dimensional biplot of 12 yield and its attributing traits contribution on principal component axes

*DT = days to 50% pollenshed, DS = days to 50% silking, TSS = total soluble sugars, ROWS = no. of kernel rows per ear, GFY = green fodder yield, CWOUTH = cob weight without husk, CWH = cob weight with husk, EH = ear height, ED = ear diameter, PH = plant height, EL = ear length, KER = no. of kernels per row

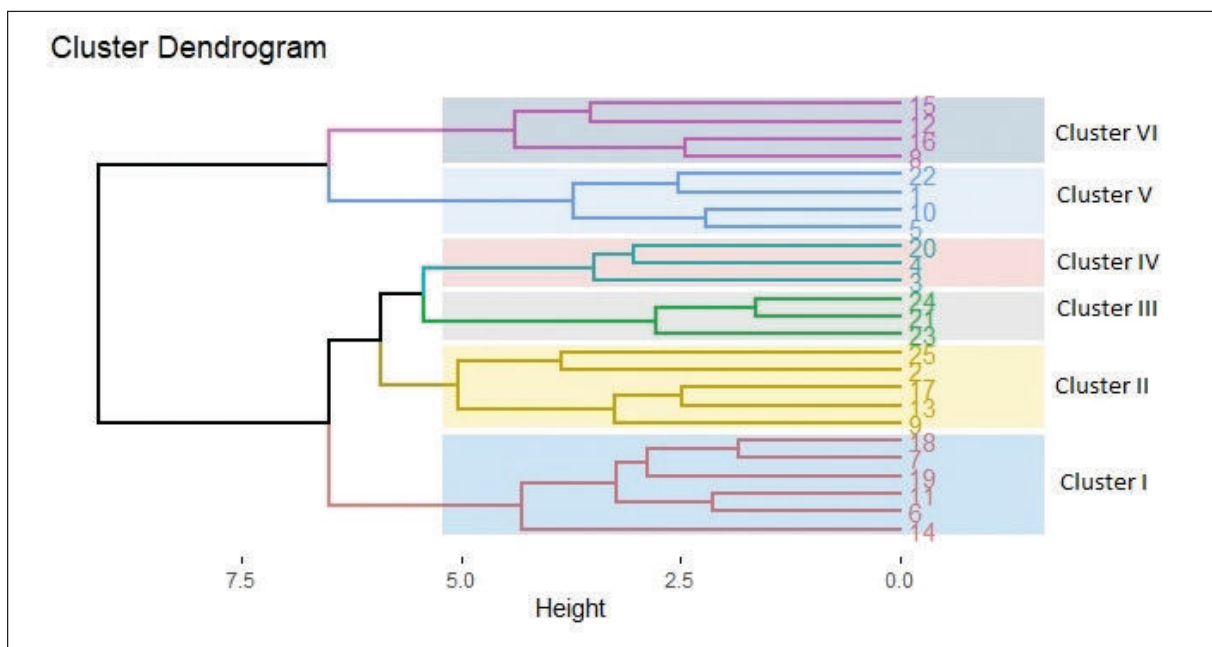


Fig. 4: Dendrogram based on Hierarchical clustering method for 25 sweet corn inbred lines evaluated for 12 yield and its attributing trait

Whereas in sweet corn, Yuvaraja *et al.* (2017), Stansluos *et al.* (2019) and Hemavathy (2020) indicated 73.80%, 86.76% and 83.63% of cumulative variance by first three, four and six PCs respectively.

Thus, the aforesaid characters which load high positively or negatively in PCA became important in representing the clusters as they have contributed more to genetic diversity.

Cluster analysis

Hierarchical clustering technique based on twelve quantitative traits data using Ward's method grouped 25 sweet corn inbred lines into six clusters (Figure 4). Dissimilarity coefficients based on the phenotypic traits among these genotypes ranged from 1.65 to 9.13. The dissimilarity distances showed that the genotype G15 (SCGP-114-1) was the most dissimilar with G23 (SCGP-210-2), followed by with G3 (SCGP-82), G4 (SCGP-36-2-1), G20 (VNR-SC-12) and G9 (SCGP-61-1-4), suggesting that these pairs of genotypes were the most unrelated ones and would show good heterosis when crossed. Similarly, the combination of G16 (SCGP-118) and G23 (SCGP-210-2) also exhibited high dissimilarity. Whereas, the pair of genotypes such as G21 (SCGP-80) and G24 (VNR-SC-23) followed by G7 (SCGP-42) and G18 (BSL-4); G1 (SCGP-2) and G7 (SCGP-42) and the combination G11 (VNR-SC-24) and G18 (BSL-4) showed lowest dissimilarity distances, suggesting them as the most related genotypes in this study owing to the common parents involved in its development (Table 5).

Among the six clusters formed in the analysis, cluster I comprised of six genotypes namely G6 (SCGP-207-1), G7 (SCGP-42), G11 (VNR-SC-24), G14 (SCGP-111), G18 (BSL-4) and G19 (VNR-SC-7) characterized by high green fodder yield and ear length. Cluster II contained of five genotypes, including G2 (SCGP-83-1), G9 (SCGP-61-1-4), G13 (SCGP-91), G17 (SCGP-207) and G25 (VNR-SC-29) having high ear diameter, green fodder yield and no. of kernel rows per ear. Cluster III included three genotypes which were characterized by early types with less days to 50% pollenshed and silking, large ear diameter and no. of kernel rows per cob such as G21 (SCGP-80), G23 (SCGP-210-2) and G24 (VNR-SC-23). Cluster IV comprised of three genotypes namely G3 (SCGP-82), G4 (SCGP-36-2-1) and G20 (VNR-SC-12) having high yield traits such as cob weight with and without husk, no. of kernels per row and the green fodder yield. Cluster V consisted of four genotypes namely G1 (SCGP-2), G5 (SCGP-54), G10 (SCGP-88) and G22 (SCGP-44-2) having high total soluble sugars (%) and the cluster VI included four genotypes characterized by low plant height and ear height such as G8 (SCGP-66-1), G12

(SCGP-36-3-1), G15 (SCGP-114-1) and G16 (SCGP-118). The result was reinforced by Yuvaraja *et al.* (2017), Stansluos *et al.* (2019) and Hemavathy (2020) by constructing dendrograms and reported the formation of three, four and eight clusters respectively in their studies on sweet corn. Whereas, Patel *et al.* (2020) and Inyang *et al.* (2021) reported formation of seven and three clusters in their studies on maize.

To develop a hybrid with early maturity and low plant and ear height, inbreds belonging to cluster III and VI can be selected for crossing. Green fodder yield can be improved by selecting inbreds from the clusters I, II and IV. While, if the emphasis is on the sweetness, inbreds from cluster V can be selected as one of the parents.

CONCLUSION

The present investigation in sweet corn inbred lines using multivariate techniques displayed considerable amount of genetic diversity for yield and its attributing traits. PCA has identified number of kernels per row, ear length, plant height, cob weight with and without husk and ear diameter as the main characters describing the variation within the inbred lines. The classification of inbred lines will help in identification of desired diverse lines for utilizing in further hybrid development programs. The cluster analysis classified the 25 sweet corn inbred lines into six clusters. Crossing of inbred lines from different clusters with desired traits would ensure the greater chances of obtaining hybrids with high heterosis pertaining to different yield and its attributing traits.

ACKNOWLEDGEMENT

The first author acknowledges the receipt of stipend from Professor Jayashankar Telangana State Agricultural University, Telangana during her course of Post Graduate Degree programme.

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