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MORPHOLOGICAL AND MOLECULAR DIVERSITY OF SWEET CORN INBRED LINES

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ABSTRACT

Genetic divergence among 46 inbred lines was assessed using 11 morphological traits and 18 microsatellite markers at PJTAU, Rajendranagar. Mahalanobis D² analysis grouped inbreds into 12 clusters, with total soluble sugars, days to 50% tasseling and number of kernels per row contributing most to overall diversity. Twelve polymorphic SSR markers detected 35 alleles (ranging from 2-6 per locus, with an average of 2.9 alleles per marker). Polymorphic information content values ranged from 0.201 to 0.766 with a mean of 0.406 and heterozygosity values ranged from 0.226 to 0.796 with a mean of 0.468. Clustering analysis resulted in two major groups comprising 8 and 38 genotypes, respectively. This study contributes novel parental lines for hybrid breeding, enhancing sweetness and yield in sweet corn programs.
Keywords : Clustering, D² analysis, NTSys, PIC, SSRs, Tocher method, UPGMA.

Introduction

Sweet corn, known for its distinct flavour, texture and aroma, is a popular choice for consumption as both a fresh and processed vegetable. Consumer satisfaction and market demand for sweet corn rely heavily on its sweetness, which is determined by sucrose levels and starch content in kernels. Extensive research has been conducted on various mutants of sweet corn, with fourteen mutants thoroughly studied and eight currently meeting the market demands. These mutants can be categorised into two classes: Class-I mutants, such as *shrunken-1* (*sh1*), *shrunken-2* (*sh2*), *shrunken-4* (*sh4*), *brittle-1* (*bt1*) and *brittle-2* (*bt2*), have increased sugar content and reduced carbohydrate concentration, resulting in significantly sweeter kernels compared to field corn. On the other hand, Class II mutants, including *amylose extender* (*ae*), *dull* (*du*), *sugary1* (*su1*), *sugary2* (*su2*) and *waxy* (*ex*), have altered type and quantity of accumulated starch. Class I mutants (*sh2, bt1*, and *bt2)* are more popular commercially due to their high sugar content up to 35% (Boyer and Shanon, 1984). Maize breeders have bestowed their special attention on two genes, *su1* and *sh2*, which have been widely used for developing "super sweet" corn hybrids due to their elevated sugar levels (Creech, 1965; James *et al*., 1995). These *sh2*-based mutants, while having higher sweetness and lower starch content, exhibit a collapsed and shrunken appearance of kernels when mature (Garwood and Creech, 1972).

Sweet corn is cultivated for various markets, including fresh consumption, the canning and frozen products. Despite the growing demand and potential financial benefits associated with sweet corn cultivation, farmers face some serious challenges like high perishability as it is harvested before physiological maturity (Rowe and Garwood, 1978 and Andrew, 1982), loss of sweetness, dehydration and post-cooking browning (Becerra-Sanchez and Taylor, 2021). Therefore, there is a pressing need to develop new genotypes with improved quality and high yield that sustain the challenges and ensure profitable cultivation and a stable market.

To ensure successful hybridisation programs, it is crucial to understand the genetic divergence within germplasm (Anusha *et al.,* 2022). The Mahalanobis D^2

statistic is an essential tool for quantifying the level of genetic diversity present in the sweet corn material, revealing the association between geographical distribution and genetic variation (Mahalanobis, 1936). Maize breeders consistently emphasise the importance of diverse parental genotypes as a significant factor contributing to the development of heterotic hybrids (Ahloowalia and Dhawan, 1963; Ihsan *et al*., 2005 and Abdul Hussein Al-Badeiry *et al*., 2014).

In addition, molecular markers, particularly Simple Sequence Repeats (SSRs), play a vital role in identifying specific genes or genomic regions, as well as conducting diversity studies. SSRs are highly effective due to their co-dominant inheritance, high polymorphism, and widespread presence in genomes. However, despite the importance of molecular markers, the availability of improved sweet corn hybrids remains limited compared to regular corn, underscoring the need for new genetically diverse sweet corn hybrids that offer enhanced sweetness and higher yield (Mehta *et al*., 2017).

In traditional breeding approaches, genotypes are selected based on morphological traits of the kernels. However, relying solely on morphological traits can lead to misleading estimates due to their susceptibility to environmental influences (Mahato *et al*. 2018). Hence, integrating both morphological and molecular markers provides a more informative assessment of sweet corn diversity. In this context, the present study was undertaken to evaluate the morphological and molecular diversity in sweet corn inbreds that contribute to the development of improved hybrids with superior characteristics.

Material and Methods

Plant material

The experimental material consisted of 46 sweet corn genotypes obtained from the Maize Research Unit, Rajendranagar, Hyderabad. These genotypes were grown in a randomised block design with two replications during *kharif* 2022 at College Farm, College of Agriculture, Rajendranagar, located at 78.40^0 longitude and 17.30^0 latitude.

Morphological characterisation

The morphological traits assessed were days to 50% tasseling, days to 50% silking, plant height (cm), ear height (cm), cob girth (cm), cob length (cm), number of kernels per row, number of kernel rows per ear, cob weight with husk (g), cob weight without husk (g) and total soluble sugars (TSS) content (%), measured using a TSS meter. The days to 50% tasseling and silking were recorded on a plot basis, while the remaining traits were recorded on five randomly selected plants and the average values were considered for analysis. The Mahalanobis D^2 analysis was conducted using Tocher's method (Rao, 1952) with the INDOSTAT software.

Molecular characterisation

Genomic DNA was isolated from young leaves following a modified CTAB protocol as described by Doyle and Doyle (1987). For diversity analysis, a set of 18 simple sequence repeats (SSRs) was used. PCR amplifications were carried out in a 10 µl reaction volume containing approximately 40-50 ng of genomic DNA. The PCR products were resolved on a 3% Seakem LE agarose gel under a steady voltage of 120 V for approximately 1 hour and 30 minutes. The DNA fragments were visualised using a UV-transilluminator and documented using a documentation system (GELSTAN). The sizes of amplified fragments were estimated during electrophoresis using a 100 bp ladder (Takara) as a reference. From the initial set of 18 SSR markers, 12 markers that exhibited polymorphism were chosen and their allelic data represented in binary format (1 for presence of the allele and 0 for absence), were utilized to assess the molecular diversity using the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) and SAHN (Sequential, Agglomerative, Hierarchical, and Nested) clustering algorithm with NTSYS-pc version 2.02 software (Rohlf, 1998).

Results and Discussion

Morphological diversity

The Mahalanobis D^2 statistic grouped the 46 sweet corn inbreds into twelve clusters (Figure1) by Tocher's method, with varying numbers of entries in each cluster. Cluster I had 11 genotypes, while cluster V, IV, and IX contained 8 and 7 genotypes, respectively. Cluster X and cluster VII consisted of 4 and 3 genotypes, respectively. Clusters II, III, VIII, XI and XII were solitary clusters, indicating higher heterogeneity among those genotypes. This finding aligns with a study by Freeman *et al.* (2019) that also reported the formation of twelve clusters using Mahalanobis D^2. Cluster IX displayed the highest intra-cluster distance value (100.10) and the clusters II, III, VI, VIII, XI and XII exhibited zero intra-cluster distances, signifying the presence of a single genotype that is more divergent (Table 1 & Figure 2). The highest inter-cluster distance was recorded between cluster XI and cluster XII (378.57) and lowest was seen between cluster II and cluster III (24.04).

From Table 2, it is evident that cluster VII recorded the lowest mean for days to 50% tasseling

(49.17) and silking (52.33), suggesting that the genotypes within this cluster tend to mature earlier. Cluster XII registered the highest mean value for cob weight with husk (267.70) and without husk (204.00), while cluster XI exhibited the highest mean value (18.30) for the trait TSS. Crossing genotypes from clusters exhibiting high genetic divergence will result in better heterotic expression as well as a greater range of diversity in succeeding segregating populations. The genotype SCGP-34 from cluster XII, exhibiting high cob weight but low TSS content, can be crossed with SCGP-15, a genotype from cluster XI with high TSS but low cob weight, in order to produce a hybrid with both traits improved (Table 1, 2).

Contribution of individual characters towards divergence

The relative percentage contributions of individual traits to the genetic diversity among genotypes were assessed (Table 3). The trait TSS (22.61%) showed highest contribution to genetic divergence, followed by days to 50% tasseling (21.16%), number of kernels per row (17.10%), cob girth (17.00%) and cob length (15.65%). Da Silva Coutas *et al.* (2021) reported a significant contribution from the number of kernels per row (17.63%), while Antony *et al.* (2021) found that ear height contributed 3%. The contributions of TSS (22.61%), cob weight without husk (1.55) and cob weight with husk (0.19) were consistent with the results of Shikha *et al.* (2018). The contributions of plant height (0.77) and number of kernel rows per ear (0.77) were comparable to the findings of Mani and Deshpande (2016), while cob girth (17.00) matched with Suman *et al.* (2020).

Molecular diversity

The molecular analysis of 12 polymorphic SSR markers resulted in the detection of 35 alleles, ranging from 2 to 6 alleles per locus (Table 4). The average number of alleles per marker was 2.9, which is in line with the findings of Mahato *et al.* (2021), Joshi *et al.* (2020) and Iboyi *et al.* (2020). Among the markers, bnlg1185 exhibited the highest number of alleles (6), while phi015, umc1582, umc2309, bnlg1305 and umc1066 markers had the lowest number of alleles (2). The polymorphic information content (PIC) values ranged from 0.2011 to 0.766. The mean of PIC value recorded was 0.406, similar to the findings of Lopes *et al.* (2015), Iboyi *et al.* (2020) and Mahato *et al.* (2021). Notably, bnlg1185 marker displayed the highest PIC value of 0.766. The markers bnlg1185 and umc2232 are highly valuable since it is a known fact that the markers with PIC values greater than 0.5 are considered the most informative for grouping lines and hence considered very efficient.

Heterozygosity values ranged from 0.2268 to 0.796 in the study, with a mean of 0.468 (Table 4). The marker bnlg1185 showed the highest heterozygosity value of 0.796, while umc1066 exhibited the lowest. These findings are in agreement with the studies conducted by Comertpay *et al.* (2012) and Mahato *et al.* (2021). The elevated heterozygosity of bnlg1185 could be attributed to residual heterozygosity retained among the inbred lines at that particular locus during the process of their development and maintenance.

The polymorphism among the inbred lines was depicted using Figure 3. This analysis classified the genotypes into two primary clusters at a similarity coefficient of 0.62, as shown in Figure 4. The formation of these two clusters aligns with the previous findings of Diwan *et al.* (2015) and Suyadi *et al.* (2024), confirming the consistency of the experimental results. Cluster 2 comprised the highest number of genotypes, with 38 inbred lines, while cluster 1 was the smallest, consisting of 8 inbred lines. Furthermore, both clusters were divided into subclusters 1a, 1b, 2a and 2b.

Conclusion

The present study revealed the presence of a considerable magnitude of morphological and molecular diversity in the evaluated sweet corn inbreds. The markers bnlg1185 and umc2232 were identified as the most informative markers. The lines SCGP-34, SCGP-15, SCGP-34-1, SCGP-10-2, SCGP-70-2, and SCGP-9-1 were highly divergent, exhibiting superior performance for various yield-attributing traits. These genotypes will be utilized as parents to develop superior hybrids combining high yield and sweetness.

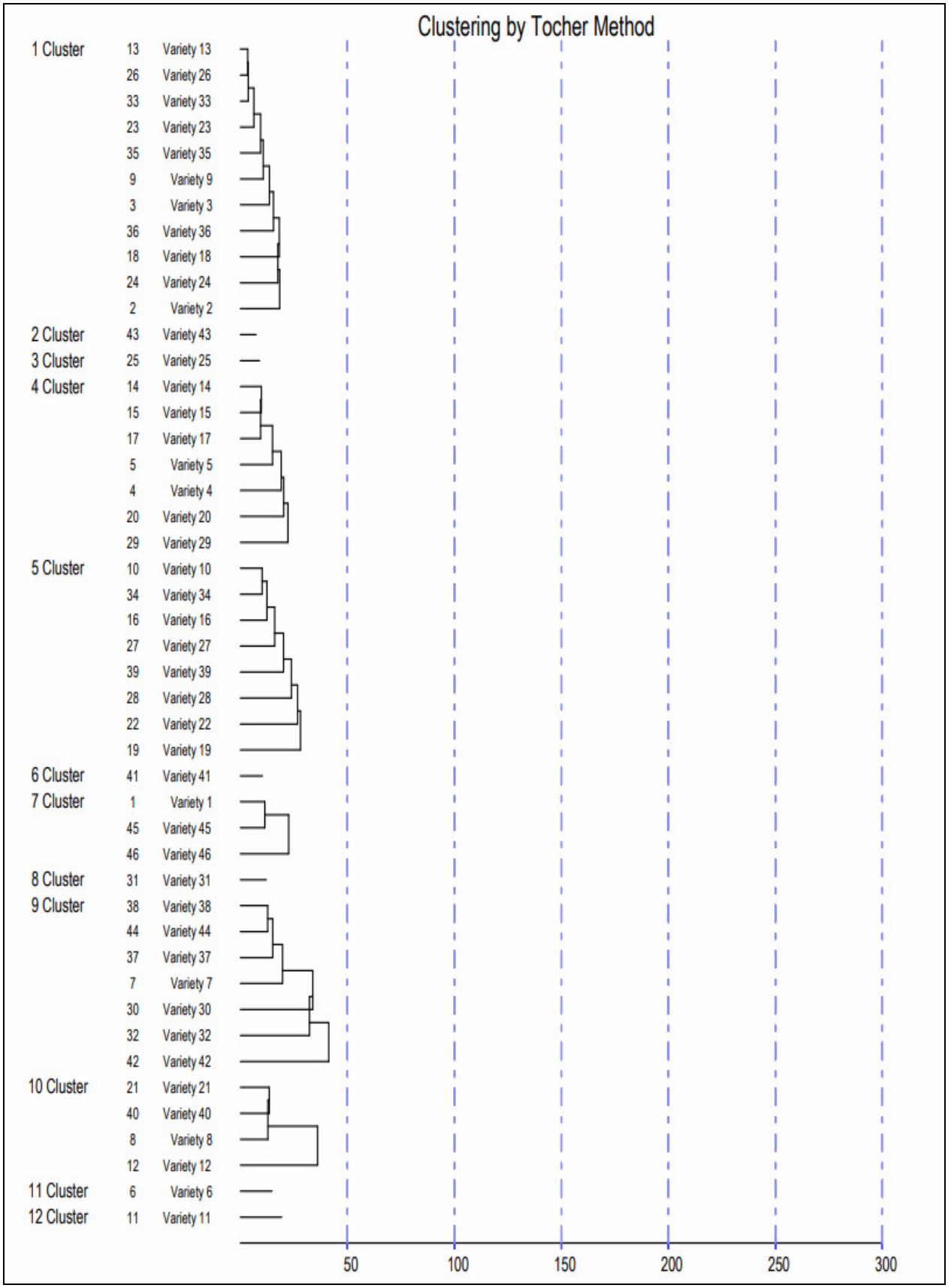

Fig. 1 : Clustering Pattern of genotypes by Tocher's Method

Table 1 : Average intra (Bold values) and inter-cluster distances among the sweet corn genotypes

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII	Cluster VIII	Cluster IX	Cluster X	Cluster XI	Cluster XII
Cluster I	**44.05**	75.28	93.35	109.47	93.03	118.67	174.74	110.27	152.26	131.70	88.21	210.99
Cluster II		**0.00**	24.04	112.84	113.09	132.74	161.25	148.28	165.28	203.01	88.13	238.92
Cluster III			**0.00**	116.12	105.41	178.30	152.55	185.90	189.19	222.96	95.18	212.70
Cluster IV				**59.67**	102.87	95.78	113.10	76.08	171.02	246.22	135.36	139.87
Cluster V					**72.96**	174.71	174.74	143.64	145.60	135.25	189.50	99.27
Cluster VI						**0.00**	249.80	48.17	119.46	330.19	102.93	243.76
Cluster VII							**70.22**	174.94	363.81	283.32	189.24	260.67
Cluster VIII								**0.00**	151.32	262.99	94.60	231.74
Cluster IX									**100.10**	255.01	221.50	169.60
Cluster X										**87.13**	293.95	227.60
Cluster XI											**0.00**	378.57
Cluster XII												**0.00**

Table 2 : Cluster means of the sweet corn genotypes for yield traits

	DFT	DFS	PH	EH	CL	CG	NKRPE	NKPR	TSS	CWWOH	CWWH
Cluster I	58.64	61.68	110.58	42.92	15.88	14.36	14.73	28.09	17.55	143.39	190.50
Cluster II	59.00	62.00	103.20	44.60	13.85	11.85	10.00	24.50	16.30	104.00	128.00
Cluster III	59.50	62.50	109.50	47.15	14.25	12.25	14.00	29.00	15.80	117.80	149.50
Cluster IV	53.00	56.21	121.79	45.63	15.79	14.69	14.29	27.29	16.91	146.41	187.55
Cluster V	55.69	58.69	110.78	43.39	15.89	15.18	15.00	28.44	16.02	142.94	187.98
Cluster VI	57.00	60.00	**126.40**	44.70	13.85	14.35	14.00	22.50	18.05	150.35	188.50
Cluster VII	49.17	52.33	110.53	**48.27**	16.58	13.93	14.00	**30.00**	17.43	129.78	164.23
Cluster VIII	53.00	55.50	112.00	33.00	12.85	14.05	14.00	23.50	17.80	89.50	111.35
Cluster IX	**59.57**	**62.86**	113.13	38.43	13.61	14.24	14.29	22.14	15.84	129.20	169.51
Cluster X	56.38	59.75	83.68	34.08	15.79	14.58	13.50	26.88	16.39	140.95	194.41
Cluster XI	59.50	62.80	113.60	40.10	13.35	12.25	**16.00**	28.50	**18.30**	93.35	118.85
Cluster XII	53.00	56.50	120.10	45.70	**17.50**	**16.35**	**16.00**	27.00	14.55	**204.00**	**267.70**

DFT- Days to 50% tasseling, DFS- Days to 50% silking, PH- Plant height, EH- Ear height, CL- Cob length, CG- Cob girth, NKRPE- Number of kernel rows per ear, NKPR- Number of kernels per row, TSS- Total soluble sugars, CWWOH- Cob weight without husk, CWWH- Cob weight with husk

Table 3 : Relative contribution of individual traits towards diversity

S. No.	Character	Times ranked first	Percent contribution
1.	Days to 50% tasseling	219	21.16
2.	Days to 50% Silking	0	0.00
3.	Plant height	8	0.77
4.	Ear height	33	3.20
5.	Cob length	162	15.65
6.	Cob girth	176	17.00
7.	Number of kernel rows per ear	8	0.77
8.	Number of kernels per row	177	17.10
9.	Total soluble sugars (%)	234	22.61
10.	Cob weight without husk	16	1.55
11.	Cob weight with husk	2	0.19

Table 4 : List of markers along with sequence, PIC, number of alleles and heterozygosity values

Marker	Sequence (F=Forward, R= Reverse)	No. of alleles	PIC	H_0
bnlg 1137	ATGAGCTCAGTCACACTGTAGTG-F ACTGATGACTGGTCCATGCA-R	4	0.4836	0.5369
umc 2232	CATTCATCCACCATAAATATCCTGC-F CTAGATTGCCTCGGACCTGTAAGA-R	3	0.5324	0.6049
phi015	GCAACGTACCGTACCTTTCCGA-F ACGCTGCATTCAATTACCGGGAAG-R	2	0.3429	0.4395
bnlg 2241	GTGCACACTCTCTTGCATCG-F TAGTCAGCATCTGCCGTGTC-R	3	0.3180	0.3733
umc 1582	GTGCGTGTGAGAGTGATATCGAG-F AGATTACGTAGCCACGCTTATTCG-R	2	0.3146	0.3911

umc 2309	CTGTGTTTTGTGTATTAGCGCCAG-F GTCGAAATTCCTGACACAAAAAGG-R	2	0.3155	0.3926
bnlg 1159	GTGTGCCTATCCTTCCGAGA-F AAGGACGTCAACAACGAACC-R	3	0.4615	0.5416
bnlg 1305	GCACGGGCATCAGAGAGAG-F CATGGGTAAGTTGCTGAAAGTTT-R	2	0.2896	0.3512
umc 1077	CAGCCACAGTGAGGCACATC-F CAGAGACTCTCCATTATCCCTCCA-R	3	0.3947	0.4546
bnlg 1175	ACTTGCACGGTCTCGCTTAT-F GCACTCCATCGCTATCTTCC-R	3	0.4592	0.5132
umc 1066	ATGGAGCACGTCATCTCAATGG-F AGCAGCAGCAACGTCTATGACACT-R	2	0.2011	0.2268
bnlg 1185	CGGTCCAGGCAGGTTAATTA- F GACTCGAGGACACCGATTTC- R	6	0.7661	0.7960
Average		2.916	0.4061	0.4680

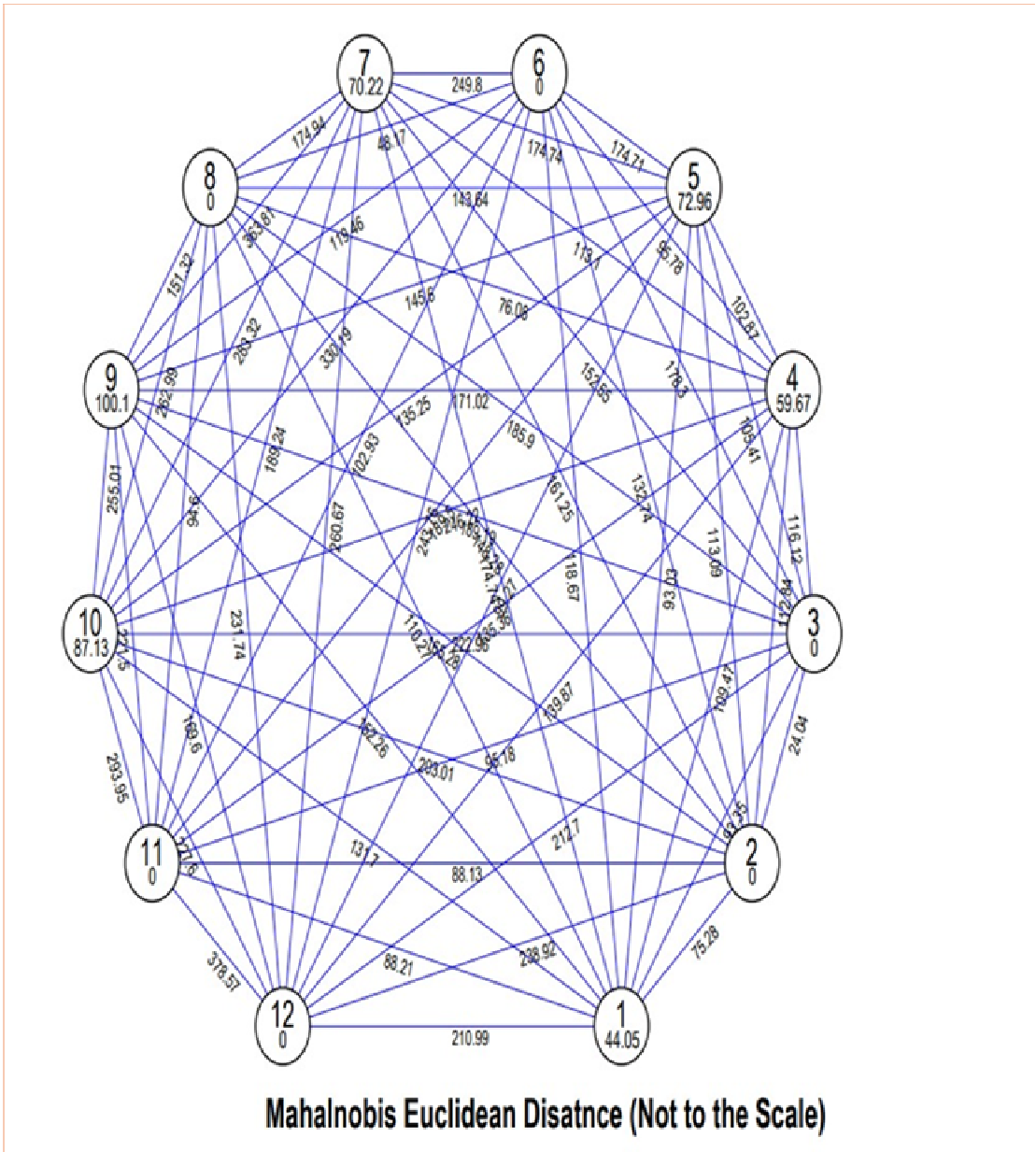

Fig. 2 : Intra and inter-cluster distances (D^2) among twelve clusters of sweet corn genotypes

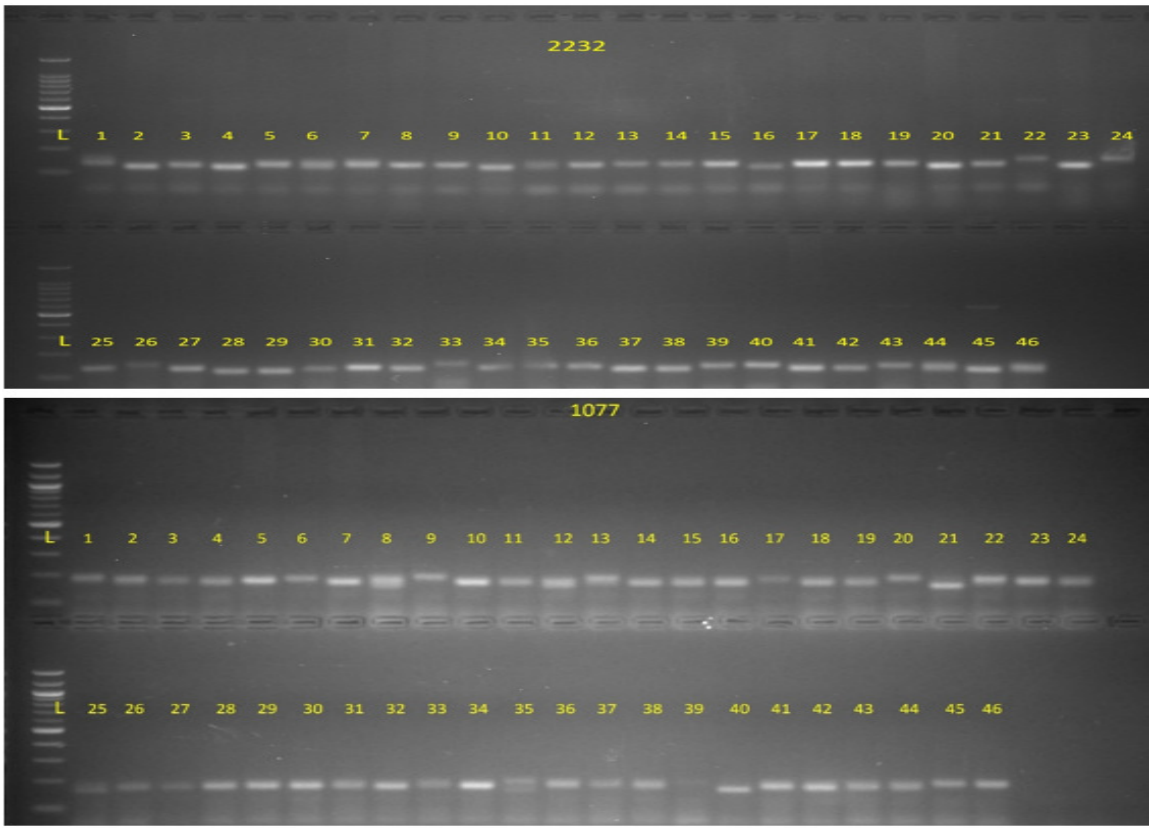

Fig. 3 : Amplification profile of genotypes with umc 2232 & umc 1077 and Ladder (L)- 100 bp

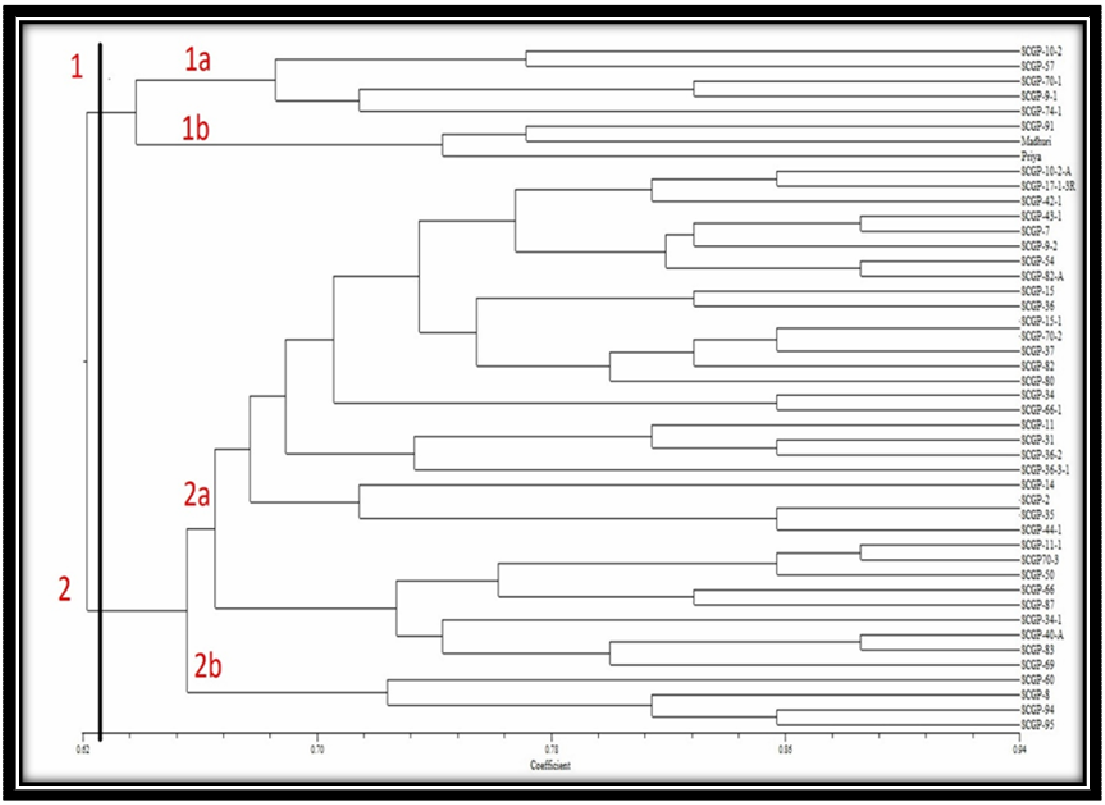

Fig. 4 : Clustering of genotypes using NTSYS software

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Conflict of Interest: Authors declare no conflict of interest

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