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MICROSATELLITE MARKER BASED GENETIC DIVERSITY STUDY IN SWEET CORN INBREDS (*ZEA MAYS L.* SACCHARATA)

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ABSTRACT

A comprehensive polymorphism survey was conducted on a set of fifteen sweet corn inbreds using fifteen SSR primers. Among these, twelve primers showed polymorphism, revealing distinct differences between the inbreds. The analysis of Polymorphism Information Content (PIC) values highlighted the SSR primer umc2025 (PIC: 0.657) as the most informative, closely followed by umc2190 (PIC: 0.521), while the primer umc1827 exhibited the lowest PIC value (0.048). Utilizing UPGMA cluster analysis, the fifteen inbreds were effectively categorized into seven clusters. Remarkably, cluster I and VIII demonstrated the maximum genetic distance between them, making them prime candidates for selection in hybridization programs to exploit the fullest heterotic potential. Consequently, the hybridization of genotypes from these two clusters is strongly recommended to achieve the most substantial heterosis and improve sweet corn breeding efforts.
Keywords **:** Sweet corn, Polymorphism, inbred, PIC value, diversity.

Introduction

Sweet corn, also known as sugar corn, is a mutant variety of maize that originated as a recessive gene mutation in the 19th century. This mutation resulted in corn with higher sugar content, making it sweeter and more palatable than traditional field corn. It quickly gained popularity and is now one of the most popular vegetables in countries like the USA and Canada. Furthermore, sweet corn has been introduced to India from the United States of America, where it has also gained popularity. The fruit of the sweet corn plant is the corn kernel, which is the edible part of the plant. When mature, the kernels have a translucent, horny appearance, and they become wrinkled as they dry out (Suthar *et al*., 2012). Sweet corn is typically consumed in its immature stage when the kernels are tender, juicy, and sweetest. It is widely used for human consumption and is a versatile ingredient in various dishes.

Sweet corn is available in different types, including yellow, white, and bicoloured varieties. The cultivars vary in terms of days to maturity and are classified as early, mid, or late season. Late season cultivars are generally considered to be of better quality. Breeders have focused on developing new cultivars with high sweet content that retain their sweetness for longer periods, ensuring a more enjoyable eating experience (Lerner and Dana, 2001). Sweet corn differs from normal corn primarily due to specific genes that impact starch synthesis in the seed endosperm (Dinges *et al.,* 2001).

In the earlier history of sweet corn, lines that carried only the sugary (*su1*) allele on chromosome 4 were commonly referred to as sweet corn. However, modern sweet corn varieties utilize several endosperm genes that affect carbohydrate synthesis in the endosperm, either individually or in combination, to develop desired traits (Tracy, 1997). Four of the most valuable mutants used in sweet corn breeding are shrunken 2 (*sh2*), brittle (*bt*), sugary (*su1*), and sugary enhancer (*se*). The *sh2* gene is located on chromosome 3, the bt gene on chromosome 5, and both are classified as class 1 mutants. The *su1* and *se* genes are located on chromosomes 4 and 2, respectively, and are classified as class 2 mutants.

SSR markers are extensively employed in parental polymorphism studies due to their ubiquitous distribution across the genome, locus-specific nature, and ability to exhibit multiple alleles at each locus. These features make SSR markers highly informative and valuable for identifying and characterizing genetic polymorphisms within inbred lines (Lopes *et al.,* 2014). Such polymorphism studies of inbred lines play a crucial role in identifying diverse inbred lines. These diverse inbred lines can then be strategically used as parents in hybridization programs to exploit heterosis, also known as hybrid vigour.

Material and Methods

The present investigation was conducted using fifteen inbred lines (Table 1) obtained from the Department of Millets, Tamil Nadu Agricultural University (TNAU), Coimbatore. A set of fifteen sweet corn SSR markers were employed for the molecular analysis. The list of primers utilized in the study is presented in Table 2. DNA isolation

from the sweet corn inbreds was performed using the CTAB method.

Protocol for extraction of genomic DNA

The Genomic DNA was isolated using the CTAB method as described by Doyle and Doyle (1990)**.** Young fresh leaves of 10-15 days old were collected, cut into small pieces, and ground in a mortar using 600 µl of CTAB buffer. The samples were incubated in the water bath for 30 minutes at 65º C. After incubation, around 600 µl of chloroform: isoamyl alcohol (24:1) was added and inverted twice. Then it was kept in the centrifuge for 10 minutes at 10,000 rpm. The aqueous layer was taken and transferred to the new eppendorf tubes. Ice-cold isopropanol was added (twice the volume of supernatant) to eppendorf tubes containing the supernatant and it was allowed for an hour at -20º C and was again centrifuged at 5500 rpm for 20 minutes. The supernatant was discarded and the pellet settled in the bottom was allowed to air dry for 30 minutes. Pellet was dissolved by adding 200µl TE buffer and kept at -20 ºC for long term use.

PCR amplification

The DNA was quantified, and its concentration was adjusted to 40 ng/µl for the SSR analysis. PCR amplifications were conducted in 10 µl reaction volumes containing 1 × PCR buffer, 0.2 mM dNTPs, 0.2 µM each of forward and reverse primers, 0.5 U of Taq DNA Polymerase and 40 ng of template DNA. The amplified PCR products, along with a 1000 bp DNA ladder serving as a molecular marker, were separated on a 3% agarose gel stained with ethidium bromide (0.5µg/ml). Gel visualization and documentation were performed using a gel documentation system under UV light.

Analysis of polymorphism

The clearly resolved, unambiguous polymorphic bands were scored visually for their presence or absence. The scores were obtained in the form of a matrix '1' and '0', which indicate the presence or absence of bands in each species, respectively. The binary data scored was used to construct a dendrogram using NTYSYSpc 2.02i.

Polymorphic information content

Polymorphic Information Content (PIC) values were calculated for SSR markers to characterise each primer's capacity to reveal or detect polymorphic loci among the genotypes. It is the sum total of polymorphism information content values of all the markers produced by a particular primer.

Cluster analysis

The binary data score was used to construct a dendrogram. The genetic association between accessions were evaluated by calculating Jaccard's similarity coefficient for pair wise comparisons based on the proportions of shared bands produced by the primers (Jaccard, 1908). The similarity matrix was generated using the SIMQUAL programme of NTYSIS-pc software version 2.02i (Rohlf, 1998). The similarity coefficients were used for cluster analysis and the dendrogram was constructed by the Unweighted Pair-Group method (UPGMA) (Sneath and Sokal, 1973).

Results and Discussion

Polymorphic information Content

Polymorphic Information Content (PIC) reveals the amount of information that can be obtained from a particular primer. The PIC value of SSR marker provides an estimate for the discriminatory power of that SSR marker by taking into account not only number of alleles that are detected but also the relative frequencies of those alleles (Smith *et al*., 2000). The result obtained indicated that out of fifteen SSR markers used, twelve markers were found to be polymorphic (Table 3). The Polymorphic Information Content (PIC) was calculated for twelve markers for fifteen inbreds of sweet corn. The PIC value was highest for SSR primer umc2025 (0.657), followed by umc 2190 (0.521). The lowest PIC value is recorded for the primer umc1827 (0.048), indicating the presence of a potential difference between the sweet corn inbreds (Sserumaga *et al.,* 2014). Pandit *et al.* (2016) reported an average PIC value of 0.65 using eighteen markers in maize. Lopes *et al.* (2015) reported a maximum PIC of 0.71 for the marker umc2205.

Clusters based on the dendrogram

The cluster analysis grouped fifteen parents into seven major clusters (Table 5). Jacquard's similarity coefficient ranged from 0.41 to 0.88, with an average similarity index of 0.72 (Table 4). Only one genotype USC-10-3 was present in cluster I. Two genotypes, *viz.,* WNC 12084-1 and WNC 12068-2, were grouped under cluster II and two genotypes *viz.,* USC 1378-5-1 and WNC 12069-2 were grouped in cluster III. Cluster IV and V had single genotype 1421-5-2-1 and USC 1421-2-2-2, respectively. Cluster VI had three genotypes *viz.,* SC 1107, 1413-6-2-2 and SC 11-2 and cluster VII had five genotypes *viz.,* USC 1-2-3, WNC 12012-2, USC 11-2, USC 8324-3 and12039-1. A similar study was conducted by Kumar *et al*. (2016), who grouped 13 genotypes into five clusters. Patel *et al.* (2017) divided eight inbreds into three clusters. Kumari *et al.* (2018) divided eight genotypes in three clusters in maize by using UPGMA cluster analysis. In the present investigation, cluster VII was the largest among the seven clusters with five genotypes. Cluster I and VIII exhibited maximum distance and the selection of genotypes from these two clusters in the hybridization programme is recommended to exploit the maximum heterosis.

Conclusion

This study revealed the presence of molecular diversity among the fifteen inbred lines of sweet corn. The SSR markers, umc2025 (PIC;0.657) and umc 2190 (PIC:0.521) were identified as the most effective markers for genotype identification. In the cluster analysis using SSR markers, the genotypes viz., USC 10-3,1421-5-2-1 and USC 1421-2-2-2 were distinctly separated from the other genotypes. Hence selection of genotypes from the two farthest clusters, viz., cluster I and cluster VII, could be advantageous in harnessing their heterotic potential. Thus, SSR markers offer a precise molecular- level characterization of lines, aiding corn breeders in accurately assigning lines to heterotic groups and selecting suitable parents for developing new hybrids.

Table 1: List of Sweet corn inbreds used in present investigation

S. No.	Name of the lines		
1.	USC 1-2-3	9.	WNC 12084-1
2.	USC -10-3	10.	USC 8324
3.	USC 1378-5-1	11.	12039-1
4.	USC 1421-5-2-2	12.	SC 11-2
5.	USC 11-2	13.	1413-6-2-2
7.	WNC 12069-2	15.	SC 1107
8.	WNC 12068-2		

Table 2 : List of microsatellite markers used in the study

S. No.	Name of the primers	Sequence
1.	umc1704	F:TTCACCGGGTAGTCCTTCTTACTG R:AAGTACGCTGTACGCAGGCAG
2.	umc2025	F:CGCCGTAGTATTTGGTAGCAGAAG R:TCTACCGCTCCTTCGTCCAGTA
3.	umc1525	F:TTTGTGCCGAATATAAATGTGACG R:AATAATATCAAATGGCGCCAAGC
4.	umc2190	F:GATCCGTTGAGGTCGATCCTTT R:GAGGAGTTCCTGCAGTTTCTTGAC
5.	umc1353	F:AGACAGGATCATCGAAAACACACA R:ACCTCAGCCTCCTCGTCAACTACT
6.	umc2112	F:AGCTCTACCAAACACGAGCTTCAT R:CAAATGCAGAAAGATAACGCGAAT
7.	umc1827	F:GCAAGTCAGGGAGTCCAAGAGAG R:CCACCTCACAGGTGTTCTACGAC
8.	umc1066	F:ATGGAGCACGTCATCTCAATGG R:AGCAGCAGCAACGTCTATGACACT
9.	umc1060	F:ACAGGATTTGAGCTTCTGGACATT R:GGCCTCTCCTTCATCCTATTCAA
10.	umc2100	F:AAAGGCATTATGCTCACGTTGATT R:TGACGTGCAAACAACCTTCATTAC
11.	umc1142	F:CCGAAAACCCATTCTTCTAGCATC R:GTGCGGTGTTCTCTCTTTCACTCT
12.	umc1413	F:CATACACCAAGAGTGCAGCAAGAG R:GGAGGTCTGGAATTCTCCTCTGTT
13.	bnlg1803	F:GTATGCGTCGCTAGTCGTGA R:TGTTGTCTATTGGCAACCGA
14.	umc1676	F:AGTCGTACGATGACGGAGGC R:GCACCACCGACTGATCAAGA
15.	umc1698	F:CTAGCACCAACACCACTTTACAGC R:AAACTACTGTAATTGCGAGGCTGC

Table 3: SSR Primers with the level of polymorphism detected

S. No.	Name of the primers	PIC value
1.	umc 1704	0.133
2.	umc2025	0.657
3.	umc2190	0.521
4.	umc1353	0.497
5.	umc1827	0.048
6.	umc2112	0.231
7.	umc1066	0.391
8.	umc1060	0.297
9.	Umc1142	0.12
10.	umc2100	0.497
11.	bnlg1803	0.057
12.	umc2650	0.137

Table 4 : Similarity index matrix

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	1.00														
2	0.56	1.00													
3	0.53	0.47	1.00												
4	0.69	0.56	0.59	1.00											
5	0.72	0.53	0.50	0.66	1.00										
6	0.72	0.47	0.50	0.66	0.75	1.00									
7	0.53	0.59	0.75	0.66	0.50	0.50	1.00								
8	0.66	0.53	0.56	0.59	0.69	0.63	0.75	1.00							
9	0.69	0.63	0.66	0.69	0.59	0.59	0.72	0.78	1.00						
10	0.78	0.53	0.50	0.66	0.88	0.81	0.56	0.69	0.66	1.00					
11	0.69	0.56	0.41	0.63	0.84	0.78	0.59	0.72	0.63	0.84	1.00				
12	0.59	0.59	0.63	0.66	0.75	0.63	0.56	0.56	0.66	0.81	0.66	1.00			
13	0.56	0.69	0.59	0.69	0.78	0.72	0.66	0.66	0.69	0.78	0.69	0.84	1.00		
14	0.53	0.59	0.44	0.53	0.63	0.69	0.50	0.50	0.41	0.63	0.72	0.50	0.59	1.00	
15	0.59	0.66	0.50	0.59	0.63	0.63	0.56	0.56	0.72	0.75	0.66	0.75	0.78	0.69	1.00

Table 5 : Cluster of fifteen inbreds based on SSR analysis

Cluster number	Number of parents	Name of the parents
I	1	USC 10-3 (L2)
II	2	WNC 12084-1 (L9), WNC 12068-2 (L8)
III	2	WNC12069-2 (L7), USC 1378-5-1(L3)
IV	1	1421-5-2-1(T4)
V	1	USC 1421-2-2-2(L4)
VI	3	SC 1107(T5),1413-6-2-2 (T3), SC 11-2(T2)

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