The Pharma Innovation Journal 2024; 13(2): 193-199

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
TPI 2024; 13(2): 193-199

www.thepharmajournal.com
Received: 20-12-2023
Accepted: 23-01-2024

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Microsatellite diversity analysis in domestic gene pool of sweet corn inbreds and their implications on expression of heterosis

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Abstract
The narrow genetic base existing in the sweet corn limits its genetic improvement. This is associated with the fact that sweet corn does not have well defined heterotic groups as categorized in field corn. Therefore, application of marker assisted diversity analysis becomes a necessity to categorize the diverse genotypes for development of single cross hybrids or composites. Present molecular diversity analysis of sweet corn inbreds revealed that eight SSR primers were polymorphic out of fourteen SSR primers screened. The polymorphic information content (PIC) value obtained from the eight polymorphic primers ranged from 0.03 (umc2061) to 0.73 (umc1060 and umc1969). Present study revealed that umc1060 and umc1969 were the most informative SSR primers. Dendrogram constructed based on dissimilarity coefficient of sweet corn genotypes grouped the six domestic inbreds into three major clusters. Evaluation of sweet corn hybrids synthesized with diverse inbreds based on SSR markers polymorphism revealed maximum heterosis and desirable combining ability. It is evident from the best hybrid identified in the present study SC45508 (cluster II) x SC45679 (Cluster I) expressed superior heterosis over the check hybrid (Sugar75) and desirable specific combining ability for the green cob yield and quality traits.

Keywords: SSR markers, genetic diversity, heterosis, specific combining ability

Introduction
Sweet corn is a genetic mutant of field corn and was reportedly first grown in Pennsylvania in the mid -1700s. Sweet corn is produced for the fresh, frozen and canned markets. Yield and quality characters are the most important traits looked into sweet corn by the most of the sweet corn breeders. Preferable mode of exploitation of heterosis in sweet corn is development of single cross hybrids. But, the information about heterotic grouping is only little known (Yuwono *et al.,* 2017) [19] in this class of corn. The genetic narrowness of sweet corn is due to their origin from only few open pollinated varieties (Dickert and Tracy, 2002) [3]. Global spread, premium price, increasing demand of sweet corn leads to attraction among farmers for cultivation in recent years. Lack of adapted varieties, tall plants, lodging susceptibility and poor ear placement are the major problems experienced in sweet corn. Superior sweet corn genotypes are to be developed through heterosis breeding in order to address these issues.
The information about genetic diversity and heterotic grouping among sweet corn inbred lines has a significant impact on the utilization of germplasm to maximize the chances of obtaining hybrids expressing higher magnitude of heterosis (Laosatit *et al*., 2022) [8]. Identification of polymorphic molecular markers provide an useful tool for assessing the genetic diversity among available genotypes (Melchinger and Gumber, 1988) [11]. Knowledge about divergence of the sweet corn genotypes is of great importance for its breeding. Molecular marker studies in sweet corn became almost an important tool for sweet corn breeding. Since definite heterotic pattern is not known in sweet corn unlike in field maize, molecular marker might be used as a powerful tool to know the genetic diversity at DNA level. In particular, SSR markers show promising potential for large scale DNA fingerprinting of maize genotypes due to the high level of polymorphism detected (SMITH *et al*., 1997) [13]. SSR markers are co-dominant and are thus useful in detecting heterozygosity among the inbred lines. Hence, the present study was aimed for the identification of suitable markers to assess the genetic diversity of sweet corn genotypes in the domestic gene pool besides comparing implication of diverse cross combinations on exploitation of heterosis and specific combining ability effects.

Materials and Methods

Six parental sweet corn inbred lines of domestic origin (Table 1) were utilized in this study. Genomic DNA was isolated from leaves of inbred genotypes by Mini CTAB method (Williams *et al.*, 1993) [20]. The DNA of six inbred lines were screened for parental polymorphism with fourteen SSR markers (Table 2). Eight out of fourteen SSR markers presented clear bands while other six bands produced weak bands or no amplification. The bold, scorable and unambiguous polymorphic bands were scored in six inbred lines, visually for the presence and absence of the specific alleles of each SSR markers. The scores were obtained in the form of a matrix with '1' (presence of band) and '0' (absence of band) for each genotype. To measure the in formativeness of the markers, the PIC value for each SSR locus was calculated according to the following formula.

$$PIC = 1 - \sum_{j=1}^{n} P_{ij}^2$$

Where,

P_{ij} is the frequency of the j^{th} allele of the i^{th} marker (Anderson *et al.,* 1993) [2].

The binary data score was used to construct a dendrogram using NTSYS-PC software based on Jaccard's Similarity coefficient (Jaccard (1908) [5] with unweighted Pair Group Method and Arithmetic Average (UPGMA).

The selected diverse inbred parents based on SSR marker were crossed using diallel mating design with reciprocal combinations. The six parental lines, thirty hybrids obtained from diallel mating and one check hybrid (Sugar75) were utilized for evaluation. In a randomized complete block design (RBD) all the genotypes were evaluated with two replications. The trait, Green cob weight were recorded for all the single cross hybrids, their parents and the check hybrid. Regarding quality trait, total sugar (Yemm and Willis, 1954a.) [18], reducing sugar (Somogyi, 1952) [14] and non reducing sugar (Sadasivam, 1996) [21] were estimated from freshly harvested cobs of all the genotypes evaluated. Specific combining ability was analyzed according to Griffing (1956) [22] method. The magnitude of standard heterosis was estimated using the formula suggested by Turner (1953) [17].

Results

Fourteen SSR markers were utilized to determine the molecular diversity among six sweet corn inbred lines. Eight markers out of fourteen used were found to be polymorphic (Plate 1). The PIC values were calculated for eight polymorphic markers and are presented in Table 3. The PIC value ranged from 0.03 to 0.73. The highest PIC value was observed for SSR marker umc1060 (0.73) and umc1969 (0.73) followed by umc1142 (0.615), umc1303 (0.615), umc1937 (0.615), umc1896 (0.5) and umc1413 (0.5). The lowest PIC value was registered by the SSR marker umc2061 (0.03).

The dissimilarity coefficients calculated for each pair among the six sweet corn inbred lines are presented in Table 4. The dissimilarity coefficient was observed in the range of 0.33 to 1.00. Among the parents, SC 45530 and SC 45508 expressed highest dissimilarity coefficient (1.00) with all other parents. Next highest dissimilarity coefficient was found between SC 45678 and SC 45684 (0.75) followed by SC 45678 and SC 45679 (0.71), SC 45679and SC 45683 (0.71), SC 45678 and SC 45683 (0.66). The lowest dissimilarity coefficient was observed between SC 45679 and SC 45684 (0.43), SC 45683 and SC 45684 (0.33).

The cluster analysis grouped six sweet corn inbred lines into three major clusters. The cluster number and the genotype names are given in Table 5. Cluster I was found to be the largest cluster with three genotypes (SC 45679, SC 45683, SC 45684). Cluster II had two genotypes (SC 45530, SC 45508) whereas cluster III had only one genotype (SC 45678).

In the present investigation hybridization was carried out with the diverse sweet corn inbred lines in full diallel mating design. The resultant sweet corn hybrids and their parents and one check hybrid (Sugar75) were evaluated in randomized complete block design for green cob weight besides evaluating the quality traits *viz.,* total sugar, reducing sugar and non reducing sugar. The statistical analyses were carried out using the mean value recorded (Table 6). The analysis of variance for the biometrical and quality traits showed significant differences among the genotypes studied. The analysis of variance for specific combining ability revealed that variance due to parents was highly significant for all the characters studied.

The highest positive significant combining ability for the trait green cob weight and total sugar was reported in the hybrid SC 45508 x SC 45679 (Table 7). The same hybrid recorded highest negative significant combining ability for the trait reducing sugar which is desirable. For the trait non reducing sugar the hybrid SC 45508 x SC 45678 recorded the highest positive significant specific combining ability. Heterosis was estimated as a per cent deviation from standard check (Sugar75) mean. The highest positive significant standard heterosis for the trait green cob weight was noticed at the cross SC 45508 x SC 45678. For the entire quality trait SC 45508 x SC 45678 and SC 45508 x SC 45679 hybrid recorded highest desirable significant standard heterosis. (Table 8)

Discussion

Improvement of crops possessing very less genetic variability would be a difficult task for the crop breeders. At times breeders would attempt to create variability artificially so as to practice selection. Hybridization is one of the breeding method had helped the breeders to create genetic variability and to select superior recombinants in the segregating generations for past decades. One of the prerequisite for generating array of variants would be attempting the hybridization between distantly related individuals. With the advent of molecular marker technology, identification of diverse genotypes in a crop with very narrow genetic diversity has become relatively easier than through classical plant breeding approaches. The molecular analysis provides information about genetic diversity at DNA level among genotypes which helps to predict the genetic variation existing in a particular crop. In the present study, the SSR markers were employed in sweet corn inbred lines in order to obtain information on genetic diversity among them. It had facilitated identification of genetically diverse parental lines for developing single cross sweet corn hybrids through exploitation of heterosis appropriately.

In the present study, eight SSR primers showed polymorphism out of fourteen SSR primers amplified in six parental sweet corn inbreds. Kashiani *et al.,* (2012) [7] found

that 95 SSR markers were polymorphic out of 99 SSR marker used. Polymorphic information content indicates the amount of information present in particular SSR primers. It provides an estimate for the discriminatory power of that SSR marker by taking into account both number of alleles and relative frequencies of those alleles. The PIC value obtained from the eight polymorphic markers ranged from 0.03 (umc2061) to 0.73 (umc1060 and umc1969). However, reported PIC value of 0.45 with 10 SSR markers in field corn diversity analysis. Similar higher PIC values were reported earlier in maize by Madhav *et al*., 2016 [9]. The highest PIC value of 0.657 was observed with the SSR primer umc2025 out of 15 markers used by Suhasini *et al*., 2016 [23]. Laosatit *et al*., (2022) [8] assessed 268 sweet corn inbreds and three commercial hybrids using 20 SSR markers and they found relatively high genetic diversity among the inbreds. All the lines evaluated by them were clustered into two major cluster which were suggested to be included in hybridization programme in the future. In the present study revealed that umc1060 and umc1969 were the most informative SSR markers.

The highest dissimilarity co-efficients recorded in the study indicates that the sweet corn inbreds utilized for the study were genetically diverse. Dendrogram constructed based on dissimilarity coefficient of parents, which grouped the six parents into three clusters (Fig.1). This also indicates that the genotypes could be selected from different clusters in order to harness the heterosis fruitfully for the development of single cross sweet corn hybrids. Similar work was carried out by Mehta *et al*., 2017 [10] in 48 inbreds. They analyzed the genotypes with 56 SSR markers and they reported the average PIC value of 0.50 and genetic dissimilarity of 0.73. The 48 inbreds were grouped into three major clusters through cluster analysis.

The sweet corn hybrids synthesized based on genetic diversity of studied inbreds as revealed by the SSR markers exhibited maximum heterosis and desirable combining ability. This is evident from the parents involved in the best hybrid identified *i.e.* SC 45508 x SC 45679 with superior heterosis and desirable specific combining ability for green cob yield and quality traits as these two inbreds were located in two different clusters (SC 45508 in cluster II and SC 45679 in cluster I) and considered to be genetically diverse. Similarly, Srdic *et al*., 2011 [24] evaluated six sweet corn inbred lines for its genetic diversity using 47 SSR markers out of which 40 SSR markers produced clear band and they reported that hybrid combinations with higher estimates of specific combining ability and heterosis expressed less genetic similarity with each other, while inbreds that were genetically most similar expressed low heterosis and specific combining ability in their hybrid combination. In fodder maize, Palaniyappan *et al.,* (2023) [12] reported the association between Genetic distance and heterosis. In their study parents of greater GD_{MOL} exhibited higher green fodder yield and superior standard heterosis.

Hence, molecular screening of inbreds of sweet corn by employing SSR primers not only reveals the precise genetic diversity but also helps the breeders in developing highly heterotic single cross hybrids and composites.

Table 1: List of domestic sweet corn inbred used in the study

S. No.	Name of the inbred	Pedigree	Origin
1	SC 45530	WNDMRSC 19R 773	Indian Institute of Maize Research, Winter Nursery Centre, Hyderabad
2	SC 45508	MRCSC2	
3	SC 45678	DMSC 20	
4	SC 45679	951-7	
5	SC 45683	DMSC 36	
6	SC 45684	DMSC 37-3	

Table 2: List of SSR markers used for parental diversity analysis

S. No.	Name of the primer	Sequence
1	umc1031	F:TTTGTGCCGAATATAAATGTGACG R:AATAATATCAAATGGCGCCAAGC
2	umc2061	F:GTCTGGAGAACTCCCTACCCATTC R:TAGCTTGAGAGACCGGAACAGC
3	umc1142	F:AGACAGGATCATCGAAAACACACA R:ACCTCAGCCTCCTCGTCAACTACT
4	umc1303	F:AGCTCTACCAAACACGAGCTTCAT R:CAAATGCAGAAAGATAACGCGAAT
5	umc1060	F:ACAGGATTTGAGCTTCTGGACATT R:GGCCTCTCCTTCATCCTATTCAA
6	umc1969	F: GTATGCGTCGCTAGTCGTGA R: TGTTGTCTATTGGCAACCGA
7	bnlg1937	F:AATGCTCGGTCCACAGAATC R:AACTGGAGCCAAAAGTGGTG
8	umc1827	F:GCAAGTCAGGGAGTCCAAGAGAG R:CCACCTCACAGGTGTTCTACGAC
9	umc1896	F:CATACACCAAGAGTGCAGCAAGAG R:GGAGGTCTGGAATTCTCCTCTGTT
10	bnlg1803	F:GTATGCGTCGCTAGTCGTGA R:TGTTGTCTATTGGCAACCGA
11	umc2190	F:GATCCGTTGAGGTCGATCCTTT R:GAGGAGTTCCTGCAGTTTCTTGAC
12	umc1525	F:TTTGTGCCGAATATAAATGTGACG R:AATAATATCAAATGGCGCCAAGC
13	umc1066	F:ATGGAGCACGTCATCTCAATGG R:AGCAGCAGCAACGTCTATGACACT
14	umc1413	F:CATACACCAAGAGTGCAGCAAGAG R:GGAGGTCTGGAATTCTCCTCTGTT

Table 3: List of primers used in molecular diversity analysis of parents with polymorphic information content

S. No.	Name of the primers	PIC value
1	umc2061	0.03
2	umc1142	0.615
3	umc1303	0.615
4	umc1060	0.73
5	umc1969	0.73
6	umc1937	0.615
7	umc1896	0.5
8	umc1413	0.5

Table 4: Dissimilarity index matrix

	SC 45530	SC 45508	SC 45678	SC 45679	SC 45683	SC 45684
SC 45530	0.00					
SC 45508	1.00	0.00				
SC 45678	1.00	1.00	0.00			
SC 45679	1.00	1.00	0.71	0.00		
SC 45683	1.00	1.00	0.66	0.71	0.00	
SC 45684	1.00	1.00	0.75	0.43	0.33	0.00

Table 5: Cluster of six parents based on SSR markers

Cluster number	Number of parents	Name of the parents
I	3	SC 45679, SC 45683, SC 45684
II	2	SC45530, SC 45508
III	1	SC 45678

Table 6: Mean performance of parents and sweet corn hybrids and checks for Green cob weight (GCW), Total sugar (TS), Reducing sugar (RS), Non reducing sugar (NRS)

Crosses	GCW	TS	RS	NRS
SC 45530 X SC 45530	201.24	16.75	1.43	15.33
SC 45530 X SC 45508	253.7	15.65	1.63	14.03
SC 45530 X SC 45678	270.71	14.83	1.55	13.28
SC 45530 X SC 45679	278.76	14.55	1.23	13.32
SC 45530 X SC 45683	311.35	12.65	1.34	11.32
SC 45530 X SC 45684	279.38	14.65	1.63	13.03
SC 45508 X SC 45530	202.2	16.75	1.36	15.2
SC 45508 X SC 45508	213.38	15.65	1.83	12.73
SC 45508 X SC 45678	459.98	19.13	1.18	18.13
SC 45508 X SC 45679	459.29	19.4	1.15	18.25
SC 45508 X SC 45683	272.15	12.65	1.33	14.18
SC 45508 X SC 45684	267.15	14.65	1.73	13.78
SC 45678 X SC 45530	439.52	13.4	1.11	12.3
SC 45678 X SC 45508	204.1	16.6	1.46	15.15
SC 45678 X SC 45678	122.95	16.75	1.45	15.3
SC 45678 X SC 45679	282.2	14.65	1.57	13.08
SC 45678 X SC 45683	220.66	16.45	1.17	15.28
SC 45678 X SC 45684	282.1	14.55	1.53	13.03
SC 45679 X SC 45530	267.26	14.4	1.43	12.98
SC 45679 X SC 45508	312.3	15.75	1.46	14.29
SC 45679 X SC 45678	295.92	13.3	1.34	11.97
SC 45679 X SC 45679	169.88	13.55	1.83	11.73
SC 45679 X SC 45683	257.65	14.65	1.25	13.4
SC 45679 X SC 45684	271.65	15.5	1.55	13.95
SC 45683 X SC 45530	279.3	15.5	1.25	14.25
SC 45683 X SC 45508	205.74	16.55	1.33	15.23
SC 45683 X SC 45678	317.27	14.7	1.23	13.48
SC 45683 X SC 45679	282.43	15.75	1.3	14.45
SC 45683 X SC 45683	115.9	16.5	1.1	15.4
SC 45683 X SC 45684	241.29	18.95	1.55	17.4
SC 45684 X SC 45530	248.26	15.75	1.23	14.53
SC 45684 X SC 45508	263	15.65	1.25	14.4
SC 45684 X SC 45678	232.35	14.5	1.26	13.24
SC 45684 X SC 45679	134.7	17.65	1.33	16.32
SC 45684 X SC 45683	266.8	15.3	1.83	13.48
SC 45684 X SC 45684	137.38	14.55	1.55	13
Grand mean	265.80	15.43	1.41	14.03
Mean of hybrids	278.63	15.18	1.38	14.22
Mean of parents	160.11	15.63	1.53	13.91
Sugar75	391.16	18.43	1.12	17.31
CD at 5%	57.67	1.10	0.15	1.12

Table 7: Specific combining ability effects of hybrids for Green cob weight (GCW), Total sugar (TS), Reducing sugar (RS), Non reducing sugar (NRS)

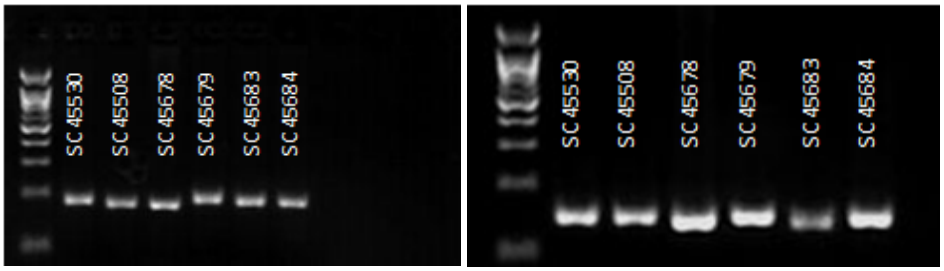
Crosses	Sca			
	GCW	TS	RS	NRS
SC 45530 X SC 45508	-59.7710 **	1.0275 **	0.0500 ns	0.9689 **
SC 45530 X SC 45678	73.6986 **	-0.9392 **	-0.0125 ns	-0.9344 **
SC 45530 X SC 45679	-2.6735 ns	-0.3233 ns	-0.0750 *	-0.2394 ns
SC 45530 X SC 45683	44.2644 **	-1.3679 **	-0.0000 ns	-1.3736 **
SC 45530 X SC 45684	23.1761 ns	-0.2575 ns	-0.0375 ns	-0.2115 ns
SC 45508 X SC 45678	42.8373 **	1.5463 **	-0.0875 **	1.8625 **
SC 45508 X SC 45679	102.3228 **	1.7250 **	-0.2000 **	1.6339 **
SC 45508 X SC 45683	-19.9018 ns	-1.1783 **	-0.0250 ns	-1.1399 **
SC 45508 X SC 45684	16.6449 ns	-0.5304 *	-0.0625 ns	-0.4703 ns
SC 45678 X SC 45679	11.8949 ns	-0.7254 **	0.0625 ns	-0.7940 **
SC 45678 X SC 45683	16.4228 ns	0.1750 ns	-0.0625 ns	0.2393 ns
SC 45678 X SC 45684	15.0994 ns	-0.8396 **	-0.0500 ns	-0.7861 **
SC 45679 X SC 45683	23.2307 ns	0.2458 ns	-0.0750 *	0.3143 ns
SC 45679 X SC 45684	-33.2177 *	0.4029 ns	-0.0875 **	0.4943 ns
SC 45683 X SC 45684	42.2753 **	1.3167 **	0.2875 **	1.0322 **

**-significant at 1% level. *-significant at 5% level. ns-non significant

Table 8: Magnitude of standard heterosis for Green cob weight (GCW), Total sugar (TS), Reducing sugar (RS) and Non reducing sugar (NRS)

Crosses	Standard Heterosis			
	GCW	TS	RS	NRS
SC 45530 X SC 45508	-35.14 **	-15.18 **	50.00 **	-18.79 **
SC 45530 X SC 45678	-30.79 **	-19.51 **	40.91 **	-23.12 **
SC 45530 X SC 45679	-28.73 **	-21.14 **	13.64 *	-23.12 **
SC 45530 X SC 45683	-20.40 **	-31.44 **	22.73 **	-34.68 **
SC 45530 X SC 45684	-28.58 **	-20.60 **	50.00 **	-24.57 **
SC 45508 X SC 45530	-48.31 **	-10.30 **	22.73 **	-12.14 **
SC 45508 X SC 45678	17.60 *	4.61 **	9.09 ns	4.91 **
SC 45508 X SC 45679	17.42 *	5.15 **	4.55 ns	5.49 **
SC 45508 X SC 45683	-30.42 **	-15.99 **	22.73 **	-17.92 **
SC 45508 X SC 45684	-31.70 **	-15.99 **	59.09 **	-20.23 **
SC 45678 X SC 45530	12.36 ns	-27.37 **	0.00 ns	-28.90 **
SC 45678 X SC 45508	-47.82 **	-10.03 **	31.82 **	-12.43 **
SC 45678 X SC 45679	-27.85 **	-20.60 **	40.91 **	-24.28 **
SC 45678 X SC 45683	-43.59 **	-10.84 **	4.55 ns	-11.56 **
SC 45678 X SC 45684	-27.88 **	-21.14 **	40.91 **	-24.57 **
SC 45679 X SC 45530	-31.67 **	-21.95 **	31.82 **	-24.86 **
SC 45679 X SC 45508	-20.16 **	-14.63 **	31.82 **	-17.34 **
SC 45679 X SC 45678	-24.35 **	-27.91 **	22.73 **	-30.92 **
SC 45679 X SC 45683	-34.13 **	-20.60 **	13.64 *	-22.54 **
SC 45679 X SC 45684	-30.55 **	-15.99 **	40.91 **	-19.36 **
SC 45683 X SC 45530	-28.60 **	-15.99 **	13.64 *	-17.63 **
SC 45683 X SC 45508	-47.40 **	-10.30 **	22.73 **	-11.85 **
SC 45683 X SC 45678	-18.89 *	-20.33 **	13.64 *	-21.97 **
SC 45683 X SC 45679	-27.80 **	-14.63 **	18.18 **	-16.47 **
SC 45683 X SC 45684	-38.31 **	2.71 **	40.91 **	0.58 ns
SC 45684 X SC 45530	-36.53 **	-14.63 **	13.64 *	-15.90 **
SC 45684 X SC 45508	-32.76 **	-15.18 **	13.64 *	-16.76 **
SC 45684 X SC 45678	-40.60 **	-21.41 **	13.64 *	-23.41 **
SC 45684 X SC 45679	-65.56 **	-4.34 **	22.73 **	-5.78 **
SC 45684 X SC 45683	-31.79 **	-17.07 **	68.18 **	-21.97 **

**-significant at 1% level. *-significant at 5% level. ns-non significant



Umc1969 **Umc1937**

Plate 1: Polymorphic SSR marker profile of six inbreds

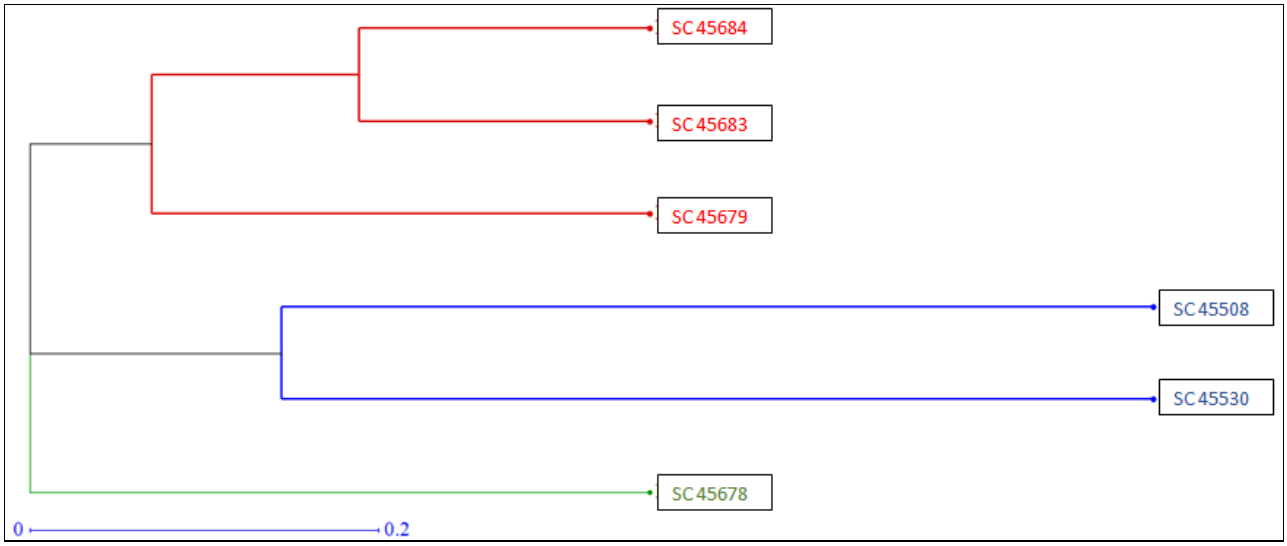


Fig 1: Dendrogram of sweet corn inbreds based on SSR marker profile.

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