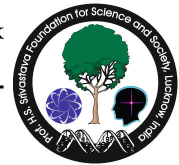
Physiol Mol Biol Plants (April–June 2017) 23(2):411–420
DOI 10.1007/s12298-017-0431-1



RESEARCH ARTICLE

Microsatellite-based genetic diversity analyses of *sugary1*-, *shrunken2*- and double mutant- sweet corn inbreds for their utilization in breeding programme

Brijesh Mehta[1] · Firoz Hossain[1] · Vignesh Muthusamy[1] · Aanchal Baveja[1] · Rajkumar Zunjare[1] · Shailendra K. Jha[1] · Hari S. Gupta[1,2]

Received: 5 August 2016 / Revised: 2 March 2017 / Accepted: 15 March 2017 / Published online: 27 March 2017
© Prof. H.S. Srivastava Foundation for Science and Society 2017

Abstract Sweet corn has recently experienced sharp rise in demand worldwide. Recessive *sugary1* (*su1*) and *shrunken2* (*sh2*) that enhances kernel sweetness have been abundantly used in sweet corn breeding. Analyses of genetic diversity among sweet corn inbreds assume great significance for their effective utilization in hybrid breeding. A set of 48 diverse sweet corn genotypes encompassing *su1su1*, *sh2sh2* and *su1su1/sh2sh2* types were analyzed using 56 microsatellite markers. A total of 213 alleles with mean of 3.8 alleles per locus were generated. Two unique- and 12 rare- alleles were identified. The average PIC and genetic dissimilarity was 0.50 and 0.73, respectively. Cluster analysis grouped the inbreds into three major clusters, with each of the *su1su1*-, *sh2sh2*- and *su1su1/sh2sh2*-types were broadly clustered together. Principal coordinate analyses also depicted the diverse origin of the genotypes. The study identified inbreds for synthesis of pools and pedigree populations to develop novel inbreds. The study led to the identification of prospective heterotic combinations in various genetic backgrounds (*sh2sh2* × *sh2sh2, su1su1* × *su1su1, su1su1/sh2sh2* × *su1su1/sh2sh2, sh2sh2* × *su1su1/sh2sh2* and *su1su1* × *su1su1/sh2sh2*).

Keywords Genetic diversity · Maize · SSR · *Shrunken2* · *Sugary1* · Sweet corn

✉ Firoz Hossain
fh_gpb@yahoo.com

[1] ICAR-Indian Agricultural Research Institute, New Delhi, 110012, India

[2] Present Address: Borlaug Institute for South Asia, New Delhi, India

Introduction

Sweet corn (*Z. mays* ssp. *mays* var. *saccharata*) among various specialty types has emerged as one of the popular choices in both domestic- and international- market (Lertrat and Pulum 2007; Hossain et al. 2013). It is generally consumed at immature stages of endosperm development at 20-24 days after pollination (DAP) (Khanduri et al. 2010, 2011). Sweet corn products like sweet corn milk and soups are gaining popularity in many countries, while kernels are sold as highly prized fresh- and canned- vegetables. Further, after harvest of the sweet corn cobs, green plants serve as source of fodder/forage to the cattle (Bian et al. 2015), and therefore provide extra sources of income to farmers. Global import of frozen sweet corn was valued US $393 million, while the same for preserved sweet corn was estimated as US $968 million during 2011 (FAOSTAT 2014). Countries like France, Hungary, Thailand and US are the leading exporters of sweet corn based products. Japan, UK, Germany, Belgium, China, Russian Federation and Spain are the leading importers of sweet corn, the demand of which has increased tremendously in the last few years.

In maize, several recessive mutants have been identified for high sugar content in the endosperm of immature kernels. Of these, *su1* and *sh2* have been extensively used for development of sweet corn cultivars worldwide. *Sugary1* (*su1*) located on chromosome 4S, codes for isoamylase-type debranching enzyme, mutant version of it enhances water-soluble, highly and randomly branched polysaccharide called water soluble phytoglycan (WSP) (Fisher and Boyer 1983). Sugary varieties (*su1su1*) at the milky ripening stage contain nearly three times more reducing sugar compared to normal maize (James et al. 1995; Feng et al. 2008). Besides, sugary kernels have creamy and

glossy texture with good flavor due to elevated level of WSP, that make the kernel more attractive to consumers over *sh2*-type (Creech 1965). *Shrunken2* located on chromosome 3L codes for large sub-unit of AGPase that involves in the synthesis of ADP-glucose from glucose-1-phosphate (Bhave et al.1990). The *sh2sh2*-based sweet corn types popularly called 'super sweet' or 'extra sweet corn' accumulate about six-fold more reducing sugars at milky ripening stage (Feng et al. 2008; Khanduri et al. 2011; Solomon et al. 2012). Moreover, the depletion of sugar level is much slower in *sh2sh2*-type even without refrigeration, thus varieties have extended shelf life, and are better suited for prolonged storage as compared to *su1su1*-type. Sweet corn genotypes with both *su1* and *sh2* (*su1su1/sh2sh2*) have also been developed and commercialized (Lertrat and Pulum 2007).

Breeding programme worldwide has emphasized in producing high yielding normal maize hybrids to cater to the demand of dried maize grains, and less effort has gone into the genetic improvement of sweet corn compared to normal maize. In view of the growing importance of sweet corn, and to accelerate the pace of progress of sweet corn cultivar development, it is therefore, important to strengthen the sweet corn breeding programme. Characterization of sweet corn inbreds assumes great significance for their objective utilization in the hybrid breeding programme (Lopes et al. 2014). Specialty corn breeding programme at ICAR-Indian Agricultural Research Institute (IARI), New Delhi has developed an array of diverse sweet corn inbreds in the genetic background of *su1su1*, *sh2sh2* and *su1su1/sh2sh2*. Only few promising sweet corn hybrids have been identified in the breeding programme (Hossain et al. 2013). However to develop more sweet corn hybrids adaptable to diverse ecologies characterization of the available inbreds holds significant promise. So far, only few studies on diversity of sweet corn inbreds (either in -*sh2sh2* or -*su1su1* background) worldwide have been undertaken, and to best of our knowledge, no comprehensive study encompassing *su1su1*-, *sh2sh2*- and *su1su1/sh2sh2*- types has been conducted so far. Keeping this in consideration, the present study was aimed to: (i) study the genetic relationships among of *su1su1*-, *sh2sh2*- and *su1su1/sh2sh2*-based sweet corn inbreds, and (ii) identify potential heterotic combinations for generation of promising sweet corn hybrids.

Materials and methods

Plant materials

A set of 48 inbreds comprising *su1su1* (16), *sh2sh2* (24) and *su1su1/sh2sh2* (8) inbreds were selected for characterization using microsatellite/SSR markers (Table 1). 39 inbreds were developed under the specialty corn breeding programme of ICAR-IARI, New Delhi; while eight inbreds were provided by WNC, ICAR-Indian Institute of Maize Research (IIMR), Hyderabad and one inbred was supplied by CIMMYT, Mexico. All these inbreds were grown in the field during 2014 rainy season at ICAR-IARI Experimental Farm, New Delhi.

SSR markers and PCR amplification

Total genomic DNA was extracted from young seedlings using modified CTAB procedure (Saghai-Maroof et al. 1984). A set of 56 SSR markers covering the whole genome were selected for molecular characterization of the sweet corn inbreds (Table 2). Primer sequence information of the different SSR loci was obtained from public domain (http://www.maizegdb.org), and custom synthesized. PCR amplifications were performed with a final reaction volume of 10 µl having ~30–40 ng of genomic DNA. The amplified products were resolved using 3.5% agarose (Amresco, USA) gel.

SSR data analysis

The allele size was determined by comparing the 100 bp DNA ladder (Bangalore Genei, India). Polymorphism information content (PIC), major allele frequency and heterozygosity were computed using Power Marker 3.25 (Liu and Muse 2005). The allele which occurred only in one genotype was counted as unique allele, and the allele with a frequency of <0.05 was considered as rare allele. Genetic dissimilarity was calculated using simple matching coefficient. Cluster analysis following unweighted neighbour-joining method and principal coordinate analysis (PCoA) were undertaken using DARwin5.0 (Perrier et al. 2003).

Results and discussion

Assessment of genetic variability

Molecular marker based genetic diversity analysis helps in the assessment of genetic relationship among the genotypes more effectively than the morphological markers (Lubberstedt et al. 2000). In the present study, a total of 56 SSRs with three to ten markers per linkage group, were used to characterize the sweet corn inbreds of different genetic constitution. The present study identified 213 alleles among the *su1su1*-, *sh2sh2*- and *sh2sh2/su1su1*-inbreds. In earlier studies, less number of alleles viz, 34 (Rupp et al. 2009), 39 (Lopes et al. 2014), 60 (Solomon

Table 1 Pedigree details and source of the sweet corn inbreds used for molecular characterization

S. No.	Inbreds	Source population	Institution
sugary1 (*su1su1*)			
1.	MGUSC-701	MGU-5454	ICAR-IARI, New Delhi
2.	MGUSC-702	CM137/DMR-2322	
3.	MGUSC-703	BLSB-RIL-106/SCH	
4.	MGUSC-704	V400/SCH	
5.	MGUSC-705	V401/SCH	
6.	MGUSC-706	V354/SCH	
7.	MGUSC-707	V360/(CM135/MGU-RIL62)	
8.	MGUSC-708	DMRIL66/ALMSC	
9.	MGUSC-709	(CM145/MGU-5454)-BC	
10.	MGUSC-710	CM138/DMR-2323	
11.	MGUSC-711	V354/SCH	
12.	MGUSC-712	V364/SCH	
13.	MGUSC-713	MGU-138/ALMSC	
14.	MGUSC-714	DM-RIL-60/ALMSC	
15.	MGUSC-715	CM138/DMR-2323	
16.	MGUSC-716	DMR-2322	ICAR-IIMR, New Delhi
shrunken2 (*sh2sh2*)			
17.	MGUSC-301	MGU-RIL91	ICAR-IARI, New Delhi
18.	MGUSC-302	CM140/DMR-2317	
19.	MGUSC-303	CM140/DMR-2317	
20.	MGUSC-306	(V25/MGU-RIL91)-BC	
21.	MGUSC-308	CM140/DMR-2317	
22.	MGUSC-311	DQPM-03-102/Priya	
23.	MGUSC-314	CM140/DMR-2317	
24.	MGUSC-318	BLSB-RIL-107/Madhuri	
25.	MGUSC-319	DMRIL-47/Priya	
26.	MGUSC-320	DMRIL-48/Madhuri	
27.	MGUSC-321	CM140/DMR-2317	
28.	MGUSC-322	CM140/DMR-2317	
29.	MGUSC-323	CM140/DMR-2317	
30.	MGUSC-324	DQPM-03-102/Priya	
31.	MGUSC-325	Hawaii & Thailand Super Sweet/ Pool 21- C12-HGB	CIMMYT, Mexico
32.	MGUSC-326	CM140/DMR-2317	ICAR-IARI, New Delhi
33.	MGUSC-327	HKI1105/MGU-RIL91	
34.	MGUSC-328	WIN Sweet Corn 2	ICAR-IIMR, New Delhi
35.	MGUSC-329	WOSC	
36.	MGUSC-330	Mus Madhu	
37.	MGUSC-331	DMR-EC619315	
38.	MGUSC-332	DMR-EC619339	
39.	MGUSC-333	DMR-EC619461	
40.	MGUSC-335	DMR-EC619466	
sugary1/shrunken2 (*su1su1/sh2sh2*)			
41.	MGUSC-501	(CM143/WOSC)/(LM16/SCH)	ICAR-IARI, New Delhi
42.	MGUSC-502	(BLSB-RIL15/WOSC)/(DM-RIL48/ALMSC)	
43.	MGUSC-511	(LM17/SCH)/(BAJIM-06-17/WOSC)	
44.	MGUSC-512	MGU-41833-SU/SH Bulk	
45.	MGUSC-513	MGU-41833-SU/SH Bulk	
46.	MGUSC-507	(CM143/WOSC)/(DQPM-58/ALMSC)	
47.	MGUSC-514	(CM143/WOSC)/(DQPM-58/ALMSC)	
48.	MGUSC-509	MGU-41843-SU/SH Bulk	

et al. 2012), 84 (Srdic et al. 2011), 86 (Lopes et al. 2015) and 155 (Jun et al. 2012) were detected among various sweet corn inbreds. It is important to mention here that these studies were conducted on a limited number of genotypes employing less number of markers in the analysis. Number of genotypes used and the number of marker loci analysed were 15 & 13 (Rupp et al. 2009), 6 & 40 (Srdic et al. 2011), 10 & 20 (Solomon et al. 2012), 15 & 15 (Lopes et al. 2014) and 22 & 30 (Lopes et al. 2015), respectively. Only Jun et al. (2012) has used 56 markers to analyse the genetic diversity in 54 genotypes. Number of alleles per SSR marker ranged from two to seven with a mean of 3.8 (Table 2), which was also higher as compared to earlier reports viz. 2.10 (Srdic et al. 2011), 2.60 (Lopes et al. 2014), 2.61 (Rupp et al. 2009), 2.77 (Jun et al. 2012), 2.86 (Lopes et al. 2015), and 3.40 (Solomon et al. 2012). High number of alleles per marker locus observed in the present study is due to utilization of higher number (56) of genome wide SSRs and inbreds (48) compared to less number of sweet corn genotypes (6–22) and SSRs (13–32) used in majority of the earlier studies. Further, higher number of alleles also confirms the availability of wide genetic variation among the sweet corn inbreds analyzed in the present study.

The allele size ranged from 60 bp (*bnlg1413*) to 800 bp (*bnlg1057*) across 56 markers. Of the SSR loci screened, 12 loci revealed two alleles, 19 loci produced three alleles; while four, five and six alleles were generated by six, 10 and five loci, respectively. Four loci (*bnlg1621*, *bnlg1523*, *bnlg1162*, *bnlg1740*) produced seven alleles - the maximum number of alleles amplified by a marker in the study (Table 2). All the markers that produced seven alleles had di-nucleotide AG repeats viz, $(AG)_{18}$, $(AG)_{17}$, $(AG)_{20}$ and $(AG)_{21}$ of *bnlg1621*, *bnlg1523*, *bnlg1162* and *bnlg1740*, respectively (Table 2). The amplification profiles of the selected SSR markers are presented in Fig. 1. Among the markers, *phi118* and *bnlg1729* had the major allele frequency of 0.92 for the allele 100 and 160 bp, respectively. SSR loci, *bnlg1162* (130 and 155 bp) and *bnlg1191* (230 bp) had the major allele frequency of 0.31. Lesser the frequency of the major allele is indicative of diverse nature of the locus. Nearly one-third of the SSR loci analysed in the present study showed major allele frequency of <0.5, indicating the wide diversity in the panel of inbreds. The mean PIC value was 0.50 suggesting the presence of enough polymorphism in the selected panel, and the efficiency to group the genotypes into different clusters. The PIC value ranged from 0.14 (*bnlg1729*) to 0.74 (*bnlg1621*) with a mean value of 0.50. Eight (*bnlg1621*, *bnlg1162*, *phi021*, *bnlg1154*, *bnlg1740, bnlg1191, phi051* and *phi118*) markers showed PIC value of 0.70 and above (Table 2). The PIC value in fact, depends upon the germplasm that was under investigation. Closely related lines would express lower PIC, whereas genetically diverse lines exhibit higher PIC values (Choudhary et al. 2015; Muthusamy et al. 2015). The mean PIC obtained in the present study is higher than earlier findings (Jun et al. 2012; Lopes et al. 2015).

Of the 213 alleles, two unique alleles (0.94%) were identified among 56 markers assayed. *umc1335* generated a unique allele (150 bp) in MGUSC-705, and *phi314704* revealed a unique allele (120 bp) in MGUSC-703. The markers that generated unique allele may be used for identification of lines. Further exploration of these unique alleles is very important since they would often associate with trait expression and can be effectively used in DNA based fingerprinting of the inbreds (Muthusamy et al. 2015). Also a total of 12 (5.63%) rare alleles were also identified, of which *bnlg1113*, *bnlg1162*, *bnlg1621*, *phi077*, *bnlg1740*, *bnlg1191*, *umc1296*, *bnlg2191*, *umc1335* and *phi314704* produced one rare allele each, and *dupssr34* generated two rare alleles. Occurrence of unique- and rare-alleles depends upon the number of genotypes analysed and type of SSR markers used in the study, and earlier studies have reported the occurrence of unique and rare alleles in maize inbreds differing for various quality traits (Sivaranjani et al. 2014; Choudhary et al. 2015; Muthusamy et al. 2015).

SSR markers being co-dominant in nature can be used in genetic diversity analysis and population genetics studies as they can differentiate heterozygotes from the homozygotes. Estimation of heterozygosity among the inbred lines at molecular level has been a useful tool to assess the cycle of inbred development besides understanding the purity of the seed lot (Muthusamy et al. 2015). Mean heterozygosity (0.08) observed in the present study revealed high degree of homozygosity among the inbreds studied, and signified stringent maintenance of these inbreds in the breeding programme (Table 2). Though some of the markers such as *bnlg1209* showed a heterozygosity value of 0.35, it could be due to the residual heterozygosity present in the inbreds. Maize is a highly cross pollinated crop and some heterozygosity remains inherent as breeders look for morphological uniformity while developing the inbred lines. Some of the cryptic genetic variation at DNA levels may continue to exist, which segregates even after repeated generations of selfing and maintains the heterozygosity in the inbreds (Muthusamy et al. 2015). This could also be due to mutation at specific SSR loci or non-specific amplification, where similar sequences in different genomic region get amplified due to duplications in the genome (Choudhary et al. 2015). Inbreds developed through doubled haploid (DH) technology are completely homozygous for all the loci and this system is getting popular as it requires only one to two seasons.

Table 2 Details of SSR markers used in molecular characterization

S. No.	Primer	Bin	Repeat	No. of alleles	PIC*	Major allele frequency	Heterozygosity
1.	*umc2226*	1.02	$(TGG)_6$	3	0.53	0.48	0.08
2.	*bnlg1057*	1.06	$(AG)_{17}$	5	0.63	0.38	0.13
3.	*umc1335*	1.06	$(AG)_{24}$	6	0.64	0.40	0.13
4.	*umc1838*	1.08	$(TA)_8$	3	0.45	0.73	0.19
5.	*umc1082*	1.09	$(GA)_{16}$	4	0.61	0.50	0.00
6.	*bnlg1297*	2.02	$(AG)_{32}$	4	0.49	0.54	0.21
7.	*bnlg125*	2.02	–	6	0.66	0.52	0.06
8.	*bnlg1621*	2.03	$(AG)_{18}$	7	0.74	0.33	0.08
9.	*bnlg1396*	2.06	$(AG)_{15}$	5	0.63	0.42	0.15
10.	*bnlg1413*	2.07	$(AG)_{26}$	2	0.22	0.85	0.04
11.	*bnlg1316*	2.08	$(AG)_{13}$	3	0.60	0.50	0.00
12.	*bnlg1520*	2.09	$(AG)_{22}$	4	0.55	0.56	0.08
13.	*umc2101*	3.00	$(AG)_7$	5	0.66	0.44	0.08
14.	*phi404206*	3.01	–	2	0.39	0.71	0.02
15.	*bnlg1523*	3.03	$(AG)_{17}$	7	0.59	0.52	0.08
16.	*bnlg1113*	3.04	$(AG)_{23}$	6	0.65	0.38	0.10
17.	*bnlg1452*	3.04	$(AG)_{22}$	2	0.26	0.75	0.00
18.	*umc1539*	3.05	$(GGC)_5$	2	0.18	0.90	0.00
19.	*bnlg1035*	3.05	$(AG)_{13}$	3	0.45	0.52	0.08
20.	*umc1102*	3.05	GGAT	2	0.18	0.88	0.06
21.	*bnlg1951*	3.06	$(AG)_{11}$	3	0.58	0.46	0.00
22.	*bnlg1182*	3.09	$(AG)_{19}$	3	0.21	0.85	0.10
23.	*phi074*	4.00	AG	2	0.40	0.69	0.00
24.	*bnlg1126*	4.02	CAA	3	0.27	0.83	0.02
25.	*bnlg1162*	4.03	$(AG)_{20}$	7	0.71	0.31	0.15
26.	*phi021*	4.03	$(AG)_{21}$	5	0.72	0.29	0.04
27.	*bnlg1729*	4.05	$(AG)_{21}$	2	0.14	0.92	0.04
28.	*dupssr34*	4.07	$(TTG)_{14}$	5	0.56	0.58	0.02
29.	*umc2360*	4.08	$(GCC)_4$	3	0.47	0.63	0.04
30.	*phi314704*	4.09	–	4	0.46	0.58	0.08
31.	*nc130*	5.00	AGC	2	0.39	0.71	0.00
32.	*bnlg1879*	5.03	–	5	0.61	0.42	0.17
33.	*bnlg278*	5.05	$(AG)_{14}$	5	0.64	0.48	0.15
34.	*umc1296*	6.00	$(GGT)_7$	3	0.50	0.58	0.06
35.	*phi077*	6.01	AG	5	0.63	0.40	0.10
36.	*umc1186*	6.01	$(GCT)_5$	2	0.46	0.54	0.00
37.	*bnlg2191*	6.02	$(AG)_{33}$	5	0.67	0.40	0.00
38.	*bnlg1617*	6.04	$(AG)_{16}$	3	0.53	0.52	0.08
39.	*bnlg1154*	6.05	$(AG)_{27}$	6	0.70	0.33	0.13
40.	*bnlg1740*	6.07	$(AG)_{21}$	7	0.71	0.33	0.10
41.	*bnlg1136*	6.07	$(AG)_{14}$	5	0.67	0.31	0.15
42.	*phi089*	6.08	ATGC	2	0.30	0.81	0.13
43.	*umc1929*	7.02	$(GA)_{10}$	3	0.52	0.58	0.00
44.	*phi051*	7.05	$(GGA)_4$	4	0.70	0.33	0.02
45.	*phi072*	7.05	AGG	3	0.39	0.67	0.17
46.	*umc2334*	7.05	AAAC	3	0.46	0.54	0.23
47.	*umc2190*	7.06	(CCT)4	3	0.37	0.67	0.17
48.	*umc1304*	8.02	$(TCGA)_4$	3	0.51	0.52	0.08
49.	*phi115*	8.03	AT	3	0.41	0.67	0.06
50.	*bnlg2046*	8.04	$(AG)_{15}$	3	0.46	0.69	0.04

Table 2 continued

S. No.	Primer	Bin	Repeat	No. of alleles	PIC*	Major allele frequency	Heterozygosity
51.	*bnlg1272*	9.00	$(AG)_{16}$	4	0.62	0.50	0.02
52.	*bnlg1209*	9.04	$(AG)_{12}$	3	0.31	0.90	0.35
53.	*bnlg1191*	9.06	$(AG)_{39}$	6	0.71	0.31	0.02
54.	*phi118*	10.0	AGG	2	0.14	0.92	0.00
55.	*phi059*	10.0	ACC	2	0.34	0.75	0.04
56.	*phi050*	10.0	AAGC	3	0.50	0.63	0.15
Mean	3.80	0.50	0.57	0.08			

* *PIC* polymorphism information content

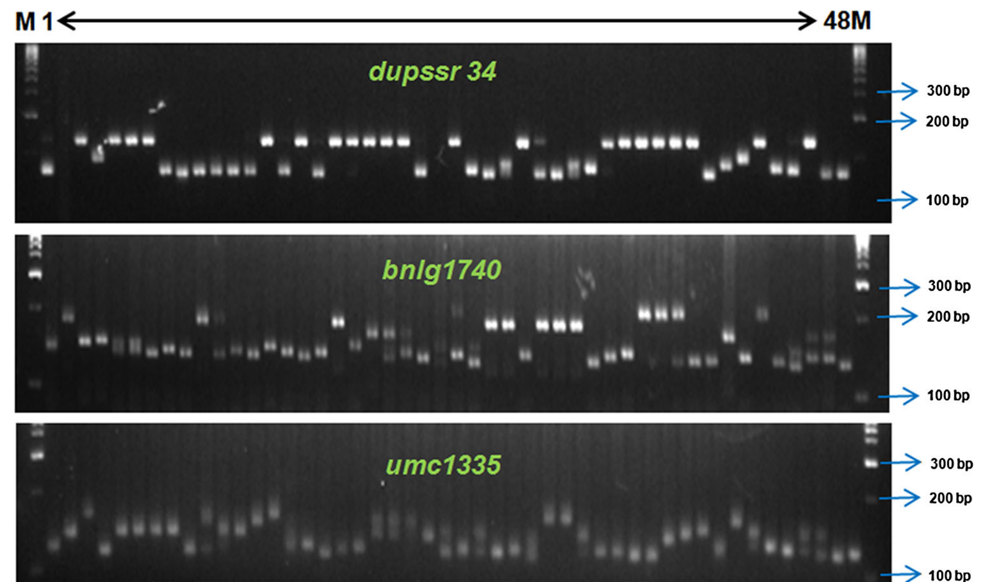


Fig. 1 SSR polymorphism among sweet corn inbreds. M: 100 bp ladder

Genetic relationships

Among the inbreds, the pair-wise genetic dissimilarity ranged from 0.33 to 0.87 with a mean of 0.73. Wide range of genetic dissimilarity (0.33–0.87) observed among the genotypes depicts highly diverse nature of the inbreds. The mean genetic dissimilarity (0.73) was relatively high indicating that the sweet corn inbreds are developed from diverse sources. Mean genetic similarity of 0.56 among the sweet corn inbreds was reported by Solomon et al. (2012) and Srdic et al. (2011). 48 genotypes were grouped into three major clusters (Fig. 2). Group A and B had 18 genotypes each, while group C possessed 12 genotypes. In general, *sh2*-inbreds were grouped together in group B and C, while most of the *su1su1*-inbreds (14) belonged to group A and two *su1su1*-inbreds clustering to group B. The *su1su1/sh2sh2*-inbreds were distributed in group A and C. Group A consisted of three subgroups (A1, A2 and A3), of which A2 had maximum of eight genotypes, all being *su1*-based sweet corn inbreds viz, MGUSC-702, MGUSC-708, MGUSC-710, MGUSC-711, MGUSC-712, MGUSC-713, MGUSC-714 and MGUSC-716. The subgroup A1 had five *su1su1*-inbreds (MGUSC-703, MGUSC-704, MGUSC-705, MGUSC-709 and MGUSC-715) and one *sh2sh2*-inbred (MGUSC-325), whereas A3 had one *su1su1*-(MGUSC-701) and three *sh2sh2/su1su1*- based inbreds. Group B had four sub-groups, with B1 possessing six genotypes with four *sh2sh2*- (MGUSC-302, MGUSC-303, MGUSC-308 and MGUSC-323) and two *su1su1*-(MGUSC-706 and MGUSC-707) inbreds. B2, B3 and B4 comprised of five (MGUSC-314, MGUSC-321, MGUSC-322, MGUSC-326 and MGUSC-332), four (MGUSC-311, MGUSC-324, MGUSC-329 and MGUSC-333) and three (MGUSC328, MGUSC330 and MGUSC331) genotypes respectively, all being *sh2sh2*-inbreds. The group C also had three sub-groups viz., C1, C2 and C3, of which C1 had five genotypes (four *sh2sh2*: MGUSC-318, MGUSC-319, MGUSC-320 and MGUSC-325; and one *sh2sh2/su1su1*: MGUSC-511). Sub-group C2 had three *sh2sh2*- genotypes (MGUSC-327, MGUSC-301 and MGUSC-306), and C3 had four *sh2sh2/su1su1*-inbreds (MGUSC-507, MGUSC-509, MGUSC-512 and, MGUSC-514).

Clustering of genotypes based on markers was closely related with their pedigree information. The inbreds

Fig. 2 Cluster analyses depicting genetic relationships among 48 inbreds. Bootstrap value of ≥30 is presented. Inbreds in *red sh2sh2*, *black su1su1, green sh2sh2/su1su1*

developed from the same source population have been found to cluster in the same group (Fig. 2). The *sh2sh2*-inbreds viz., MGUSC-311 and MGUSC-324 are the segregants of DQPM 03-102 × Priya, and are clustered together in the sub-group B3. Similarly, MGUSC-302, MGUSC-323, MGUSC-303, MGUSC-308, MGUSC-314, MGUSC-321, MGUSC-322 and MGUSC-326 were together in B1 and B2, and they share the common ancestry. Though these inbreds are present in the same group, they had genetic dissimilarity value ranging from 0.33 to 0.68. This is due to the recombination and fixation of different alleles during the process of selfing, and this diversity aided in selection of different phenotypes during the inbred development. Same is the case with *su1su1/sh2sh2* inbreds, where the inbreds MGUSC-507 and MGUSC-514 were grouped together in the C3 as they were derived from the common pedigree. Molecular marker-based heterotic grouping among maize inbred lines simplifies the choice of parental lines for the production of high-yielding hybrids (Reif et al. 2003). The reliability of genetic distance on heterosis/specific combining ability in sweet corn was reported by Srdic et al. (2008, 2011). In the present study, MGUSC-301 and MGUSC-302 belonged to group C and B, respectively; and they were found to be heterotic in our breeding programme. Similarly, hybrid of MGUSC-301 and MGUSC-308 was also heterotic due to their distant genetic relationship. These inbreds validated the robustness of clustering pattern used in the present study.

The result of PCoA also reveals the diverse nature of the materials used in the study and agreed well with the clustering pattern of the inbreds (Figs. 2 and 3). All the *su1su1*-inbreds were distributed in the left quadrangle and most (20

out of 24) of the *sh2sh2*-inbreds were distributed in the right quadrangle indicating the highly diverse nature of *su1su1*- and *sh2sh2*- group of mutants (Fig. 3). On the other hand, *sh2sh2/su1su1* genotypes were present mostly in the left lower quadrangle except for one each in left upper (MGUSC-502) and right lower (MGUSC-509) quadrangles. These diverse nature of inbreds developed in the breeding programme would be highly helpful in developing heterotic sweet corn hybrids in both *su1su1*- and *sh2sh2*- type.

Potential utilization in breeding programme

A number of putative sweet corn hybrid combinations can therefore be explored for their exploitation in the breeding programme using specific sweet corn inbreds. For developing *sh2sh2*-based hybrids, inbreds from group B can be crossed with inbreds of group C (MGUSC-301, MGUSC-306, MGUSC-318, MGUSC-319, MGUSC-320, MGUSC-327 and MGUSC-335). Further, MGUSC-325 is an exotic inbreds and belong to cluster A, and can be crossed with inbreds of cluster B and C (Fig. 2). Further, inbreds of B1 and B2 can also be crossed with inbreds of B3 (MGUSC-311, MGUSC-324, MGUSC-329, MGUSC-332, MGUSC-333) and B4 (MGUSC-328, MGUSC-330 and MGUSC-331). Sugary-based sweet corn hybrids are also popular due to its desirable aroma and appearance of kernels. Inbreds of cluster A can be crossed with inbreds of B1 (MGSCU-706 and MGUSC-707). Crosses between inbreds of A1 (MGUSC-703, MGUSC-704, MGUSC-705, MGUSC-709 and MGUSC-715), A2 (MGUSC-702, MGUSC-708, MGUSC-710, MGUSC-711, MGUSC-712, MGUSC-713, MGUSC-714 and MGUSC-716) and A3 (MGUSC-701) can also be attempted, with an exception of crossing

Fig. 3 Principal Coordinate Analyses depicting genetic relationships among 48 inbreds. Inbreds in *red sh2sh2*, *black su1su1*, *green sh2sh2/su1su1*

MGUSC-715 and MGUSC-710, as they have been derived from same pedigree (Fig. 2). Apart from conventional sweet corn hybrids, some novel combinations viz., *su1su1/sh2sh2* × *su1su1/sh2sh2*, *sh2sh2* × *su1su1/sh2sh2* and *su1su1* × *su1su1/sh2sh2* are also available (Lertrat and Pulum 2007). In order to develop, heterotic *su1su1/sh2sh2*-based hybrids, inbreds of C3 (MGUSC-507, MGUSC-509, MGUSC-512 and MGUSC-514) can be crossed with inbreds of A3 (MGUSC-501, MGUSC-502 and MGUSC-513). MGUSC-511 is present in C1, and can therefore be also crossed with inbreds of C3 and A1 (Fig. 2). Further, to generate *sh2sh2* × *su1su1/sh2sh2* combinations, *sh2sh2*-inbreds of B and A2 can be crossed with *su1su1/sh2sh2*-inbreds present in cluster C1, C3 and A3. Crosses can also be attempted between inbreds (*sh2sh2*-) of C1 (except MGUSC-511) and C2 with inbreds (*su1su1/sh2sh2*-) of C3. In case of *su1su1* × *su1su1/sh2sh2*, *su1su1*-inbreds of A1, A2 and B1 can be crossed with *su1su1/sh2sh2*-based inbreds present in C1, C3 and A3.

Development of inbred lines is necessary for successful continuation of any breeding programme, and derivation of new lines would augment and sustain the development of new hybrids. Inbreds of similar genetic nature can be crossed to develop new and better inbreds. For example, five *su1su1*-inbreds of A1 (MGUSC-703, MGUSC-704, MGUSC-705, MGUSC-79 and MGUSC-715) can be crossed among themselves to generate a pool. Further, a pedigree population can also be developed by crossing two selected inbreds. These sources can lead to the development of promising inbreds through repeated selfing (Fig. 2). Similarly, improved lines of *sh2sh2* and *su1su1/sh2sh2* can also be effectively developed using the information from the cluster pattern (Fig. 2).

So far, diversity analyses have been undertaken among few sweet corn inbreds, and majority of them were done with only one type of sweet corn genotypes. Among them, Amorim et al. (2003) analyzed F_2 plants of 13 populations, of which three were of *sh2sh2*-type, and seven belonged to *bt2bt2* (*brittle2*)-type. Ten *sh2sh2*-based sweet corn inbreds were used by Solomon et al. (2012). 15 *su1su1*-based inbreds were used by Lopes et al. (2014) and Rupp et al. (2009), while six *su1su1*-based inbreds were used by Srdic et al. (2011). Genetic variation of one *bt2bt2*- and two *su1su1*-based sweet corn populations were analysed using molecular markers by Bered et al. (2005). In the present study, comprehensive analyses of genetic diversity of 48 sweet corn inbreds encompassing *su1su1*-, *sh2sh2*- and *su1su1/sh2sh2*-types were undertaken. These inbreds are indigenously developed as a part of our breeding programme and they are of different origin compared to the inbreds used in the earlier studies. Thus, the novel information generated can be effectively utilized in the development of promising sweet corn hybrids in *su1su1*-, *sh2sh2*- and *sh2sh2/su1su1*-genetic backgrounds, which can cater to the ever increasing market demand.

Acknowledgements The financial support from ICAR-Indian Agricultural Research Institute, New Delhi is gratefully acknowledged. The authors thank ICAR-Indian Institute of Maize Research (formerly DMR), New Delhi and CIMMYT, Mexico for providing sweet corn lines used in the study. The help of Dr. J.C. Sekhar, WNC-IIMR, Hyderabad is duly acknowledged.

References

Amorim EP, Almeida MJC, Sereno M, Bered F, Neto JFB (2003) Genetic variability in sweet corn using molecular markers. Maydica 48:177–181

Bered F, Terra TF, Spellmeier M, Neto JFB (2005) Genetic variation among and within sweet corn populations detected by RAPD and SSR markers. Crop Breed Appl Biotechnol 5:418–425

Bhave MR, Lawrence S, Barton C, Hannah LC (1990) Identification and molecular characterization of *Shrunken-2* cDNA clones of maize. Plant Cell 2:581–588

Bian Y, Gu X, Sun D, Wang Y, Yin Z, Deng D, Wang Y, Li G (2015) Mapping dynamic QTL of stalk sugar content at different growth stages in maize. Euphytica 205:85–94

Choudhary M, Hossain F, Muthusamy V, Thirunavukkarasu N, Saha S, Pandey N, Jha SK, Gupta HS (2015) Microsatellite marker-based genetic diversity analyses of novel maize inbreds possessing rare allele of *β-carotene hydroxylase* (*crtRB1*) for their utilization in β-carotene enrichment. J Plant Biochem Biotechnol. doi:10.1007/s13562-015-0300-3

Creech RG (1965) Genetic control of carbohydrate synthesis in maize. Genetics 52:1175–1186

FAOSTAT (2014) http://faostat.fao.org

Feng ZL, Liu J, Fu FL, Li WC (2008) Molecular mechanism of sweet and waxy in maize. Intl J Plant Breed Genet 2:93–100

Fisher MB, Boyer C (1983) Immunological characterization of maize starch branching enzymes. Plant Physiol 72:813–816

Hossain F, Nepolean T, Vishwakarma AK, Pandey N, Prasanna BM, Gupta HS (2013) Mapping and validation of microsatellite markers linked to *sugary1* and *shrunken2* genes in maize. J Plant Biochem Biotechnol. doi:10.1007/s13562-013-0245-3

James MG, Robertson DS, Myers AM (1995) Characterization of the maize gene *sugary1*, a determinant of starch composition in kernels. Plant Cell 7:417–429

Jun H, Fa QF, Qing FF, Feng WQ, Jing Z, Peng Z, Qin XL (2012) Study on genetic diversity of 54 sweet corn inbred lines by SSR markers. J South China Agric Univ 33:1–4

Khanduri A, Prasanna BM, Hossain F, Lakhera PC (2010) Genetic analysis and association studies of yield components and kernel sugar concentration in sweet corn. Indian J Genet 70:257–263

Khanduri A, Hossain F, Lakhera PC, Prasanna BM (2011) Effect of harvest time on kernel sugar concentration in sweet corn. Indian J Genet 71:231–234

Lertrat K, Pulum T (2007) Breeding for increased sweetness in sweet corn. Intl J Plant Breed 1:27–30

Liu KJ, Muse SV (2005) Power Marker: an integrated analysis environment for genetic marker analysis. Bioinformatics 21:2128–2129

Lopes AD, Scapim CA, Mangolin CA, Machado MFPS (2014) Genetic divergence among sweet corn lines estimated by microsatellite markers. Genet Mol Res 13:10415–10426

Lopes AD, Scapim CA, Machado MFPS, Mangolin CA, Silva TA, Cantagali LB, Teixeira FF, Mora F (2015) Genetic diversity

assessed by microsatellite markers in sweet corn cultivars. Sci Agric 72:513–519

Lubberstedt T, Melchinger AE, Duble C, Vuylsteke M, Kuiper M (2000) Relationships among early European maize inbreds: IV. Genetic diversity revealed with AFLP markers and comparison with RFLP, RAPD, and pedigree data. Crop Sci 40:783–791

Muthusamy V, Hossain F, ThirunavukkarasuN Pandey N, Vishwakarma AK, Saha S, Gupta HS (2015) Molecular characterization of exotic and indigenous maize inbreds for biofortification with kernel carotenoids. Food Biotechnol 29:276–295

Perrier X, Flori A, Bonnot F (2003) Data analysis methods. In: Hamon P, Seguin M, Perrier X, Glaszmann JC (eds) Genetic diversity of cultivated tropical plants. Science Publishers Montpellier, Enfield, pp 43–76

Reif JC, Melchinger AE, Xia XC, Warburton ML (2003) Use of SSRs for establishing heterotic groups in subtropical maize. Theor Appl Genet 107:947–957

Rupp JV, Mangolin CA, Scapim CA, Silva P, Machado MF (2009) Genetic structure and diversity among sweet corn (*su1*-germplasm) progenies using SSR markers. Maydica 54:125–132

Saghai-Maroof MA, Soliman KM, Jorgenson R, Allard RW (1984) Ribosomal DNA space length polymorphisms in barley: mendelian inheritance, chromosomal locations and population dynamics. Proc Natl Acad Sci USA 81:8014–8018

Sivaranjani R, Santha IM, Pandey N, Vishwakarma AK, Nepolean T, Hossain F (2014) Microsatellite-based genetic diversity in selected exotic and indigenous maize (*Zea mays* L.) inbred lines differing in total kernel carotenoids. Indian J Genet 74:34–41

Solomon KF, Martin I, Zeppa A (2012) Genetic effects and genetic relationship among *shrunken2* (*sh2*) sweet corn lines and F_1 hybrids. Euphytica 385:385–394

Srdic J, Nikolic A, Pajic Z (2008) SSR markers in characterization of sweet corn inbred lines. Genetika 40:169–177

Srdic J, Nikolic A, Pajic Z, Drinic SM, Filipovic M (2011) Genetic similarity of sweet corn inbred lines in correlation with heterosis. Maydica 56:251–256