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Population structure and marker-trait association study for kernel quality traits in sweet corn (*Zea mays* var. *saccharata*)

Anima Mahato, Jai Prakash Shahi, Pawan Kumar Singh, Monu Kumar, Ashok Singamsetti

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Authors:
A. Mahato ✉, M. Kumar
ICAR-Indian Agricultural Research Institute, Hazaribagh, Jharkhand, India

J. P. Shahi, P. K. Singh, A. Singamsetti
Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India

✉ anima.mahato87@gmail.com

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Abstract

The study utilized 63 microsatellite markers linked with QTLs affecting different seed quality traits *viz*., pericarp thickness, soluble sugar, dextrose, sucrose, and amylose content, for validation in 39 sweet corn inbred lines. Observations were recorded on two quality traits *viz*., total soluble solids (TSS) and reducing sugar. Out of 63 SSR markers, 30 showed polymorphism and were subjected to population structure study and, single marker analysis for detecting their association with TSS and reducing sugar. The estimate of the likelihood of ΔK (where K= 2), classified the 39 sweet corn inbreds into two genetically distinct groups. The single factor ANOVA revealed five SSR markers, having P-value ≤ 0.05, had a high association with each of the quality traits. The markers *viz*., *phi027*, *umc2056*, *umc1633*, *bnlg1265,* and *umc1031*, located on chromosome number 9, 6, 2, and 4, were found to have a significant association with TSS. Likewise, five markers *viz*., *umc1465*, *umc1633*, *umc1492*, *bnlg1079,* and *umc1320* had a significant association with reducing sugar. The marker, *umc1633*, had a significant association with both TSS and reducing sugar and can play a significant role in the selection of inbred lines superior in terms of TSS and reducing sugar for use in sweet corn breeding program.

Keywords marker trait association, reducing sugar, SSR markers, sweet corn

Introduction

Sweet corn is an attractive crop with a high market value in urban and semi-urban areas of the Indian subcontinent. This sweet textured corn is widely consumed as a fresh and processed vegetable throughout the world. The sweet taste of sweet corn kernels is featured by certain recessive mutations in endosperm that restricts the conversion of starch into polysaccharide. Several recessive endosperm mutant genes *viz*., *sugary1 (su1), shrunken2 (sh2), amylase extender (ae1), dull1 (du1), waxy1 (wx1), brittle1 (bt1), brittle2 (bt2), sugary enhancer1 (se1),* etc. have been reported to produce sweeter kernels, generally having three to eight folds higher sugar content than field corn [1-2]. The eating quality of sweet corn is determined by the combined effect of several factors *viz*., soluble sugar content, pericarp thickness, amylase, dextrose content, etc.

Many agriculturally significant characteristics such as yield, quality, etc. are regulated by several genes following a complex inheritance pattern. With the utilization of DNA (or molecular) markers, tracing the inheritance the pattern of these complex traits has become easier and more accurate as

compared to conventional breeding approaches. As far as eating quality in sweet corn is concerned, QTL analysis using microsatellite markers has been conducted in different sweet corn genetic backgrounds to identify QTLs affecting kernel chemical composition and other quality traits [3-5]. Although DNA markers are very effective and reliable in targeting a particular phenotype there is no guarantee that a marker-gene or marker-QTL linkage recognized in a population will be efficient for other distantly related germplasm populations [6]. Hence, the efficacy of the markers should be confirmed in finding the target phenotype of an independent population and dissimilar hereditary background [7-11].

Table 1. List of Sweet corn inbred lines

SN.	Inbred Line	SN.	Inbred Line
1	DMSC 1	21	su2su2o2o2Comp(Y)-BBB-28-BBB
2	DMSC 2	22	WNCDMRSC08R686(A)
3	DMSC 3	23	WNCDMRSC08R690
4	DMSC 4	24	WNCDMRSC08710
5	DMSC 6	25	WNCDMRSC08712
6	DMSC 8	26	WNCDMRSC08750
7	DMSC 9	27	WNCDMRSC08792
8	DMSC 19	28	WNCDMRSC08R753
9	DMSC 20	29	WNDMRSCY18R715
10	DMSC 24	30	WNDMRSCY18R716
11	DMSC 27	31	WNDMRSCY18R730
12	DMSC 35	32	WNDMRSCY18R736
13	DMSC 36	33	WNDMRSCY18R753
14	HKI-1827W-1	34	WNDMRSCY18R743
15	Dulce Amanillo	35	SC FEMALE
16	Win Sweet Corn	36	SCF
17	su2su2o2o2Comp(Y)-BBB-1-BBB4PI	37	NSS2W9301A
18	su2su2o2o2Comp(Y)-BBB-2-BBB	38	Phil Super Sweet
19	su2su2o2o2Comp(Y)-BBB-15-BBB	39	SC7-2-1-2-1(N)
20	su2su2o2o2Comp(Red)-BBB-40-BBB		

Further, the study of population structure, arising due to local adaptation or selection, is important to prevent the declaration of false-positive associations. QTLs with the least effects and no false-positive associations are recognized through knowledge about population structure and similarities between individuals [12-13]. It reduces type I and type II errors due to unequal allele frequency distribution between subgroups in association mapping. Population structure should be determined to avoid false-positive associations between molecular markers and traits of interest [14-15]. In a previous report by Mahato et al., [16], genetic divergence among a similar set of sweet corn inbred lines has been studied but, both studies are conceptually different in the sense that the former utilizes a pair-wise distance matrix to classify the inbreds into different groups however model-based approach was applied in the later. Population structure research may be regarded as an essential step in marker-trait linked studies to avoid making spurious correlations or missing genuine correlations [17]. In this study, the structure of the population of the sweet corn inbred lines was evaluated by using the model-based method.

Microsatellite markers are one of the most important tools in assessing the amount of genetic variability which may further lead to new opportunities for conservation and utilization of that variability in the genetic improvement of crops. They support breeders to utilize genetic resources with fewer pre-breeding activities for efficient cultivar development by providing information on the structure of population, richness of alleles, and diversity parameters of germplasm [18-19]. These markers are being utilized on regular basis in the preparation of linkage map, QTL analysis, marker-assisted selection, etc. Although DNA markers are very effective and reliable in targeting a particular phenotype there is no guarantee that a marker-gene or marker-QTL linkage recognized in a population will be effective for another population coming from indistinctly connected germplasm [6]. Hence, markers should be confirmed for the efficacy of finding the target phenotype in independent populations and diverse genetic

background [7-11]. In maize, Quantitative trait loci responsible for protection against plant pathogens (rQTL) have been revealed and studied by several authors [20]. Likewise, the gene for acyl-CoA, diacylglycerol acyltransferase (DGAT1-2), was identified as an important QTL controlling content of oil and oleic acid in maize seeds. Chai et al., [21] re-sequenced the DGAT1-2 regions accountable to variation of oil in a maize landrace set and 155 inbred lines and validated in diverse hereditary backgrounds using both linkage mapping and association mapping analysis.

Table 2. Physicochemical parameters

SN.	SSR marker	Repeat	Trait	Reference
1.	*umc1165*	(TA)6	Soluble sugar content	Qi et al., [3]
	bnlg1297	(AG)32		
	umc1465	(ACACA)4		
	umc1259	(GCG)4		
	umc1669	(AGA)4		
	bnlg1126	(AG)20		
	umc1303	(CCG)4		
	phi093	AGCT		
	umc1586	(ATC)5		
	bnlg1714	(AG)25		
	umc1492	(GCY)4		
	bnlg1012	(AG)16		
	bnlg1079	(AG)14		
	bnlg1712	(AG)20		
	umc1605	(GGC)4		
	umc1633	(GCG)4		
	bnlg2323	(AG)25		
2.	*bnlg1265*	(AG)33	Amylose, sucrose and dextrose content	Park et al., [4]
	phi027	GCGCT	Amylose content	
	umc1634	(AG)7		
	bnlg1867	(AG)17	Dextrose content	
	umc2056	(ATC)5		
	umc2173	(CGT)5	Sucrose content	
	umc1130	(TAA)4		
3.	*umc1273*	(AAG)4	sh2 locus	Hossain et al., [22]
	umc1320	(GAAC)4		
	umc2276	(GGC)4		
	umc1896	(CA)8	su1 locus	
	umc1142	(TGGA)5		
	umc1031	(CT)6AT(CT)9		

In sweet corn, several reports on QTL analysis for kernel quality have reported many QTLs associated with quality parameters like sugar content, pericarp thickness, dextrose content, etc. in different sweet corn genetic backgrounds [3-5, 22]. To identify the robust QTLs and utilize them for transferring the trait of interest in a desirable genetic background, the mapping and validation of marker-trait association have to be followed for accelerating the sweet corn improvement program. In sweet corn, two specific genes involved in the metabolism of starch *su1* and *sh2*, have been widely utilized for the growth of improved cultivars. The above-mentioned genes as well as other loci affecting primary eating qualities of consumers' concern like kernel tenderness, sweetness, pericarp thickness, etc. have been mapped by several

workers to date. Hence, the present investigation has been designed to study the population structure and validate previously reported QTLs associated with sweetness and other quality parameters to determine consisted marker-QTL association in the local breeding population before its use in the hybrid breeding program.

Methodology

Plant materials

The current study was carried out with 39 inbred lines of sweet corn, maintained and multiplied under AICRP on Maize undergoing at the Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, India (Table 1).

Molecular profiling of inbred lines

Healthy young leaves were selected for the extraction of genomic DNA using a modified CTAB extraction protocol [23]. A set of 63 SSRs, selected depending on their association with QTLs mapped for different kernel quality traits like soluble sugar content [3]; amylose, sucrose, and dextrose content [4], and, *sh2* and *su1* locus [23], etc., were used for molecular profiling of 39 sweet corn inbred lines. Primer sequence information of microsatellites was procured from the public domain, *viz.*, http://www.maizegdb.org. PCR amplification was done using 15 µl genomic DNA containing ~30-40 ng. The amplified products were resolved using 3.5% metaphor agarose at a constant voltage of 70 V for 4 h was used to run the gel. (till the tracking dye reached the other end of the gel). A UV Trans-illuminator gel doc system was used to visualize photograph and document the electrophoresed DNA samples. The DNA ladder containing 50 bp was used to measure the size of the allele (Bengaluru Genei, India). Thirty out of 63 SSR markers showed polymorphism and were further used for analyses. The detailed information of 30 microsatellite markers, QTLs and associated traits has been presented in Table 2.

Quality assessment

The inbred lines were self-pollinated and fresh kernels at 21-24 days after pollination were used for quality assessment.

(I). Total soluble solids (TSS): One or two drops of the juice, extracted from immature kernels, were poured on a hand refractometer and reading was recorded in terms of degrees Brix on a Brix scale mounted inside the refractometer.

(II). Reducing sugar: Fresh kernels were collected and preserved in absolute ethanol. The preserved samples were weighed and homogenized using 5ml of 80% hot ethanol with a hand blender. The supernatant was decanted and filtered into a beaker. This process was repeated thrice and the supernatant thus collected was evaporated in the hot water bath, so that the liquid does not dry completely. The sugar was then dissolved in 10ml of water. After that, a known volume (0.5 or 1.0 ml) of aliquots of the alcohol-free extract was transferred to different test tubes. At the same time, 0.2, 0.4, 0.6, 0.8, and 1.0 ml of the working standard solution were transferred to a series of test tubes. Both sample and standard tubes volume were made to 2ml using distilled water. For blank 2 ml, distilled water was used. To each test tube, alkaline copper tartrate reagent was mixed. The tubes were kept in the hot water bath for 10 mins and then cooled to room temperature. Arsenomolybdic acid reagent (1 ml) was mixed in all tubes and distilled water was used to make the volume to 10 ml. After 10 min the absorbance was recorded at 530 nm. A standard graph was plotted based on spectrophotometer reading by taking standard samples along X-axis and absorbance at 530 nm along Y-axis. Now sugar values for each sample were estimated by placing their absorbance values in the standard graph. The quantity of reducing sugars in the sample was measured using the following formula:

$$Reducing\ suagr\ in\ sample\ (\%) = \frac{Sugar\ value\ from\ graph\ (\mu g)}{Aliquot\ sample\ used\ (1.0\ ml)} \times \frac{Total\ volume\ of\ alcohol\ free\ extract\ (10\ ml)}{Weight\ of\ sample\ (g)} \times 100$$

Statistical analyses

The population structure of 39 inbred lines of sweet corn was investigated using the Bayesian model-based clustering programme STRUCTURE version 2.3.4 (Pritchard, 2000). The programme was set to run with a burn-in period of 50,000 steps, followed by a Monte Carlo Markov Chain of 50,000 steps (MCMC). The iteration for K was assigned from 1 to 10. A web-based STRUCTURE HARVESTER software [24] was applied to calculate the most likely number of populations (K). Inbred lines showing membership probability less than 0.80 were deemed as admixture, while those with membership probability more than 0.80 were allocated to the relevant sub-groups. The single factor analysis of variance was employed to estimate the significant marker-trait association. The P-value ≤ 0.05 represented a high association between SSR markers and quality traits.

Results and Discussion

The evaluation of the likelihood of ΔK extended its highest value when K= 2, organizing the 39 sweet corn inbreds into two genetically distinct groups (Figure 1). The red and green colors represent the percentage of the membership of sweet corn inbreds in group 1 and group 2, respectively. None of the inbreds were grouped in two subgroups with a 100% probability of belonging to one of the groups. 15 inbred lines were clustered in group 1, and the same number of inbred lines were clustered in group 2. With a probability of adherence of more than 0.80, nine individuals had mixed probability. This means the genome of a genotype has a portion of an allele from each of the two groups. This further suggests the hybrid nature of the genotype. Although the majority of the inbreds were allocated to the most reliable groups.

Out of the 63 SSR markers used in the study, 27 markers did not produce clear scorable bands. Thirty-six markers produced distinct and sharp scorable bands of which six microsatellites produced monomorphic bands and did not use for further analyses. However, 30 out of 36 showed polymorphism among all genotypes [16] and were subjected to single-marker analysis for detecting their association with TSS and reducing sugar. In this study single factor analysis of variance has been carried out to study the marker-trait association. The single factor ANOVA as presented in Table 3, revealed five SSR markers, having P-value ≤ 0.05, had a high association with each of the quality traits. Three out of five markers viz., *phi027*, *umc2056*, and *umc1633*, having significant association with TSS, were located on chromosome number 9, 6, and 2 respectively however, two (*bnlg1265 and umc1031*) were present in chromosome number 4. Among five microsatellites significantly associated with reducing sugar, two markers, *viz.*, *umc1465* and *umc1633*, were present on chromosome number 2 however, the rest three, *umc1492*, *bnlg1079*, and *umc1320*, were located on chromosome 9, 10 and 3, respectively. It was observed that marker, *umc1633*, had a significant association with both TSS and reducing sugar hence; this marker could serve a significant role in selecting inbred lines superior in terms of TSS and reducing sugar.

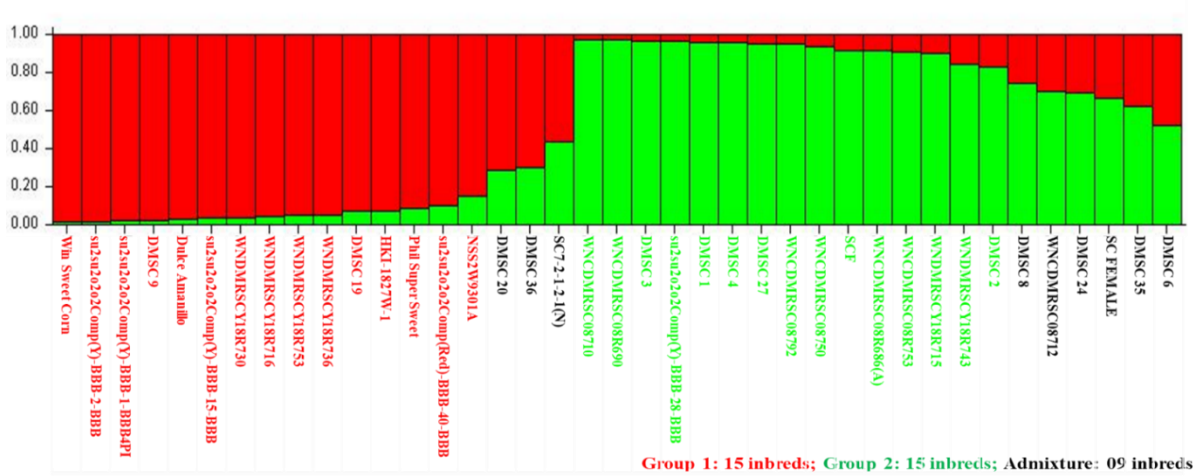

Figure 1. Population structure analysis of the 39 sweet corn inbred lines at K = 2x

Microsatellite marker, *phi027*, produced the favorable QTL allele of 140 bp in 28 inbred lines and showed significant association with TSS. This marker was reported to be a fragment of the gene responsible for granule-bound starch synthase, *Wx1*, by Park et al., [4]. Also, the role of *Wx1* in estimating the amount of amylose present in the kernel had been concluded by Senior et al., [25]. As reported by Park et al., [4] marker, *umc2056*, had a tight association with QTL affecting dextrose content in sweet corn kernels and this marker produced 140 bp allele in 28 out of 39 inbred lines.

Marker *bnlg1265* produced four alleles of size 180 bp, 200bp, 220 bp, and 330 bp in eleven, twenty, nine, and two genotypes, respectively, of which the allele of 220 bp size had a P-value of 0.04 and showed significant association with TSS. Park et al., [4] have already shown a close association of bnlg1265 with three QTLs *viz.*, qAMY4, qDEX4, and qSUC4. Hossain et al., [19] mapped *umc1031* at a distance of 0.6 cm from su1 on chromosome 4. In the present study, one of the three alleles produced by *umc1031* showed significant association with TSS and was found in four genotypes *viz.*, DMSC19, DMSC20, su2su2o2o2Comp(Y)-BBB-15-BBB, WNCDMRSC08R753.

Qi et al., [3] mapped *umc1465* and *umc1633* on chromosome number 2 which were closely linked to the QTLs affecting soluble sugar content. These two markers, *umc1465* and *umc1633*, produced alleles of size 130 bp and 150 bp in 26 and 12 inbred lines, respectively. Further, *umc1492* and *bnlg1079* were reported to have a tight association with QTLs for soluble sugar content by the same group of researchers which produced favorable QTL alleles in fourteen and six genotypes, respectively in the genetic background used for this study. The marker, *umc1320* was mapped as a common marker, linked to the *Sh2* gene, on the long arm of chromosome 3 by Hossain et al., [19] whose distance from the target gene varied from 1.8-2.8 cM. This marker produced a favorable QTL allele of 100 bp in 32 out of 39 inbred lines used in this study.

Discussion

To understand genetic diversity, distinguish the population, and perform association mapping studies, population structure and genetic relatedness are used [26]. Two genetically differentiated sub-groups were recognized; both included 15 different inbreds using population structure analysis. The presence of a sub-group in sweet corn lines can be justified by selection and genetic drift [27-28]. Close examination of the population structure bar graph revealed that nine of the inbreds were admixture, indicating that they were derived from the same population or same crosses. This study truly revealed the genetic relationship between studied sweet corn inbreds. The result of structure analysis is similar to the study of Mahato et al. (16) who also classified the same set of inbreds into three clusters with some differences in the genotypes representing different clusters. Diversity and relatedness of inbred lines are crucial in determining the appropriate breeding tactics to be used in a breeding program to optimize their potential [29]. Additionally, the integration of pedigree information might be extremely useful for grouping inbred lines into diverse heterotic groups as well as it reduces cross-pollination between closely related lines. Understanding the population structure of these sweet corn inbreds will help pick excellent inbred line combinations for hybrid development programmers. It will be further useful in designing a suitable product development approach for the effective harnessing of heterosis in sweet corn.

The study presented 30 polymorphic markers that have been subjected to single-marker analysis for detecting their association with TSS and reducing sugar. The highly informative nature of these markers in estimating genetic diversity and creating heterotic groups for hybrid development have already been described by Mahato et al., [16] and Mahato et al., [30], respectively. Four markers, *phi027*, *umc2056*, *bnlg1265*, and *umc1031* were found to have a tight association with TSS, and another four markers *viz.*, *umc1465*, *umc1633*, *umc1492*, and *umc1320*, showed high association with reducing. However, one marker, *umc1633*, showed a good association with both the traits under study. The marker-trait association study here will help determine the markers in the breeding population to be used in breeding for improved quality of sweet corn. The relevance of marker-trait association study through validation of previously reported markers for genetic improvement of the stay-green trait in sorghum and multiple agronomic traits in maize was well described by Edema and Amoding [31] and, Mikic et al., [12], respectively. Likewise,

Langade et al., [32] have described the use of SSR markers in validating maize inbred lines with markers associated with QTLs governing high oil content and importance in marker-assisted breeding. As quality is one of the most important aspects, apart from yield, in sweet corn, this study has identified many elite inbreds with good quality traits. Genetic markers are linked with capitalistically significant parameters can be a useful tool that not only supplements conventional plant breeding in the selection of elite genotypes but also accelerate the process of selection to a great extent. The validation of already reported microsatellites associated to important quality traits such as TSS and reducing sugar carried out in the sweet corn genetic background has generated new opportunities for utilization of these inbreds in the future breeding program.

Table 3. Single marker analysis representing marker-trait association

Trait	Marker	P-value	Chromosome number
TSS	*phi027*	0.04	9
	umc2056	0.05	6
	bnlg1265	0.04	4
	umc1633	0.05	2
	umc1031	0.04	4
Reducing sugar	*umc1465*	0.03	2
	umc1633	0.04	2
	umc1492	0.04	9
	bnlg1079	0.04	10
	umc1320	0.02	3

P-value ≤ 0.05 significant

Conclusion

Close examination of the population structure bar graph revealed that 39 inbred lines can be categorized into two sub-groups, and nine inbreds were admixture, indicating that they were derived from the same population or same crosses. The diversity and relatedness of inbred lines are crucial in determining the appropriate breeding tactics to be used in a breeding program to optimize their potential. This study has also identified four robust SSR markers to be tightly associated with each of the quality traits, reducing sugar and TSS, respectively. One SSR marker *umc1633* has shown a tight association with both the quality traits. Such marker-trait association information will be highly helpful in identifying and selecting elite inbred lines for use in a hybrid breeding program regarding improved yield and quality.

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