Unveiling the Sweetness: Evaluating Yield and Quality Attributes of Early Generation Sweet Corn (Zea mays L.var. saccharata) Inbred Lines through Morphological, Biochemical and Marker-based Approaches.

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Abstract

Background

Sweet corn is gaining tremendous demand worldwide due to urbanization and changing consumer preferences. However, genetic improvement in this crop is limited due to narrow genetic base and other undesirable agronomic traits that hinders the development of superior sweet corn genotypes.

Methods

A study was undertaken to carry out morphological and biochemical evaluation of 80 early generation inbred lines (S_2) of sweet corn that were developed from a cross between two sweet corn hybrids (Mithas and Sugar-75) at Mountain Research Centre for Field Crops, Khudwani, SKUAST Kashmir during *Kharif* 2020. Furthermore, validation for favourable recessive alleles for sugar content was also done using SSR markers. The 80 sweet corn inbreds evaluated for phenotypic characterization showed wide range of variability with respect to different traits studied. The highest content of total carotenoids was found in the inbred S27 (34μgg^{-1}) followed by the inbred S65 (31.1μgg^{-1}). The highest sugar content was found in the S60 (8.54%) followed by the S14 (8.34%). Comparing the results of scatter plot for biochemical traits with morphological traits, it was revealed that inbreds S9, S23, S27 and S36 contains high levels of total sugars and total carotenoids along with moderate values for yield contributing traits indicating that these inbred lines could be utilized as source of favourable alleles in sweet corn breeding programmes after further validation for yield attributes, disease resistance and other preferable agronomic traits.

Conclusion

The results of the present study, has identified several inbreds harbouring desirable biochemical and agro morphological attributes related to high yield. Consequently, the study will not only enhance the genetic base of sweet corn germplasm but will also lead to development of high-yielding hybrids with improved quality. Molecular characterization of 60 inbred lines led to the identification of seven inbreds *viz*., S21, S28, S47, S48, S49, S53, and S54, which were sugary at both the loci (umc2061 and bnlg1937) and were also found to possess high sugar content through biochemical analysis, indicating their potential as desirable candidates for breeding programs aimed at improving sweet corn yield and quality. These findings demonstrate the effectiveness of these molecular markers in facilitating marker-assisted selection for important traits in sweet corn breeding.

Introduction

Maize is one of the most versatile and promising crops having extensive adaptability over diverse range of environments globally [31]. With changing global food demands and consumer choices, maize is now becoming the wonder crop for many countries especially in developing countries [20]. In India, maize is the third important cereal crop after wheat and rice, cultivated over an area of 9.7 million hectares with a production of 30.2 million tonnes and a productivity of 3.1 tonnes ha^{-1} [1].

The production and cultivation of speciality corn like sweet corn is also the most effective strategy when facing climate changes as it is relatively drought-tolerant and it therefore adaptable to a wide range of climates [3]. Therefore, sweet corn (*Zea mays var. saccharata*), which is either consumed at immature stages of endosperm development at 20–24 days following pollination or sold as a highly desired fresh or canned vegetable globally [10], has become one of the preferred options in both domestic and foreign markets [18]. The total sugar content in case of sweet corn is in the range of 25 to 30% at the milky stage as compared to 2 to 5% in case of field corn [5].

The distinctive characteristics of sweet corn in comparison to field corn kernels are primarily due to various recessive endosperm mutants, of which *shrunken2* (*sh2*), *sugary1* (*su1*), *sugary enhancer* (*se) and brittle1* (*bt1*) are of primary importance in decreasing starch content and enhancing sugar content [13]. The *bt1and sh2* mutants, located on chromosome 5 and 3, respectively, lead to an accumulation of sugars at the expense of starch, whereas *su1* and *se*, positioned on the chromosome number 4 and 2, respectively, participate in later stages of starch biosynthetic pathway and influence the types and quantity of polysaccharides that are stored in the endosperm [28]. The *su1* mutants possess lustrous and creamy texture with excellent flavour and shows about three-fold increase in the concentration of reducing sugars in kernels at milky ripening stage in contrarily to field corn [5]. Similarly, *sh2* and *bt1* mutant accumulate about six-fold more sucrose than the field corn during milky ripening stage [5]. However, *sh2* based sweet corn types are preferred for long term storage compared to *su1* based mutants as slower depletion rate of sugar level is observed in the kernels of *sh2* mutants (Mehta et al. 2017). On the other hand, the *se1* gene acts a carbohydrate modifier and when combined with *su1* mutants accumulate sugar content comparable to *sh2* based sweet corn types [29].

The demand and popularity for sweet corn is rising nowadays due to increased consumption and the availability of more food-processing industries. However, sweet corn breeding is quite different practice from field corn breeding because of highly putrefiable nature of its end product. Although, sweet corn varieties with prolonged shelf life, improved consistency and better flavour have been developed through various breeding approaches. gains in yield potential have been observed to be lower in sweet corn compared to field corn, because the main objectives of breeders in sweet corn genetic improvement programme was to improve ear appearance and quality, rather than improving reproductive potential [20]. Additionally, several limitations *viz.*, presence of several undesirable phenotypic traits such as lodging susceptibility, high cob placement and poor plant stand, and lack of adapted varieties hamper the improvement of sweet corn genotypes [30]. Therefore, improvement for yield potential in sweet corn genotypes, while retaining quality is a significant challenge for sweet corn breeders [11]. To address this challenge, efforts have been undertaken to identify superior sweet corn genotypes through morphological and biochemical characterization that could be further used in the hybridization programmes to breed for high yielding varieties with added quality characteristics. However, relying solely on morphological characterization may result in disingenuous estimates due to environmental influences. Hence, a comprehensive strategy based on morphological and biochemical evaluation of sweet corn inbreds and further validation using molecular markers linked to favourable recessive alleles has been undertaken to strengthen breeding program for sweet corn.

Material and methods

The Experimental material comprised of 80 early generation (S_2) sweet corn inbred lines that were developed from a cross between two sweet corn hybrids (Mithas and Sugar-75). The trial was laid out during *Kharif* 2020-21 at Mountain Research Centre for Field Crops, Khudwani, SKUAST Kashmir in an augmented block design that consisted of eight blocks, each containing 13 genotypes including 10 test entries and three check entries *viz.*, two single cross hybrids of sweet corn namely Mithas and Sugar-75 and a composite variety suitable for high altitude agroclimatic conditions namely Shalimar Maize-Composite-3 (SMC-3). Each inbred line was sown in one row of 2m length and 20 cm spacing between the plants 20 cm row to row spacing of 20 cm was kept for better expression of trait. Recommended agronomic practices were followed to raise a good crop. Individual plants were evaluated for agro-morphological traits and maturity. The harvested cobs were subjected to evaluation for kernel characteristics and quality.

Morphological characterization

In order to assess morphological variability, 31 agro morphological traits following the IIMR maize descriptor list were used with each trait represented by class and code. The morphological traits studied were recorded at appropriate stages. The leaf traits were taken at full foliage stage, tassel traits were recorded at days to 50% pollen shed, cob traits were taken

after harvesting of cobs except for silk emergence recorded when 50% of plants showed emerged silks; cob placement and plant length were measured when plants reached maximum height.

Biochemical characterization

The 80 sweet corn inbreds along with three checks were evaluated for biochemical traits *viz*, total carotenoids, total sugar and amylose content.

The total carotenoid content in the methanolic and the aqueous extracts of the sweet corn genotypes was estimated by the procedure reported by [16]. The total sugars in the sweet corn kernels were estimated by the method reported by [25] with certain modifications. To prepare a solution of amylose-iodine with the maize flour, the procedure described by [12] was used. For estimating the apparent amylose content of the sweet corn inbred lines, a low-cost colorimetry method was employed, substituting the spectrophotometer with a simple color chart, as reported by [2]. The color chart used for the analysis consisted of five subclasses, including dark grayish blue, dark grayish greenish blue, dark greyish green, grayish olive, and reddish yellow. These subclasses corresponded to very high, high, intermediate, low, and very low levels of color, respectively.

Marker analysis

The genomic DNA extraction was done using CTAB (Cetyl- Tri Methyl Ammonium Bromide) protocol as described by [21] with a little amendment. n the present investigation, a set of five SSR markers linked to sweetness genes (Su1, Se1, and Sh2) were selected. These markers were obtained from MaizeGDB (http://www.maizegdb.org). The list of primers along with their sequences and position are given in (**Supplementary Table 1).** PCR amplification was carried out in a thermocycler (Eppendorf, Hamburg, Germany) using a template with an initial denaturation for 5 minutes at 94°C followed by 35 cycles of denaturation for 45 seconds at 94°C, primer annealing for 45 seconds at 55–57°C and extension for 1 minute at 72°C. An additional extension period at 72°C was provided for seven minutes for the final cycle. The amplified PCR products were identified and resolved in 3.5% agarose (MolBioHIMEDIA) gel. The PCR products were visualized by staining with ethidium bromide (10mg/ml of double distilled water) and photographed under gel documentation unit (Bio-Rad Laboratories Inc., USA) followed by scoring of gel using 50 bp DNA extension ladder (MolBioHimedia) for estimating the size of bands.

Statistical analysis

Analysis of variance for nine morphological quantitative traits *viz*., Tassel: length of main axis (TL), plant length (PL), Leaf: width of blade (LW), Ear length (EL), ear diameter (ED), Ear: number of rows of grains (KRN), 1000 grain weight, time of anthesis (TA) and time of silking (TS) was obtained by SAS Proc GLM software (SAS Institute USA). Furthermore, the collected data on 31 morphological traits for 80 inbreds were subjected to cluster analysis following unweighted neighbour- joining method using DARwin5.0 (Perrier et al. 2003). The descriptive statistics for various biochemical parameters were calculated using Excel 2010. Furthermore, cluster analysis and principal coordinate analysis (PCoA) for biochemical parameters and molecular analysis were also undertaken using DARwin5.0 [24]. Moreover, the scatter plots and histograms for biochemical parameters were constructed using NCSS statistical software, 2020.

Results

Morphological characterization

The evaluation of 80 inbred lines revealed substantial variation for various qualitative and quantitative morphological traits studied. The analysis of variance (ANOVA) for nine quantitative morphological traits viz., Number of rows of grains in ear, 1000 kernel weight (g), Time of anthesis, Time of silking, Tassel: length of main axis (cm), Plant length(cm), Leaf: width of blade, Ear length without husk (cm) and Ear diameter without husk (cm), revealed that mean sum of squares (MSS) for blocks were non-significant for most of the traits, indicating the low heterogeneity among the blocks (Table 1). However, PL (cm) and ED (cm) differed over blocks. The MSS across 80 sweet corn inbred lines were found significant for all the traits revealing substantial magnitude of variability in the inbred lines studied, hence paves a way for further improvement of these inbred lines.

Table 1
Analysis of variance for various phenotypic traits in 80 sweetcorn (Zea mays var. saccharata) inbreds used in this study.

Source of Variation	DF	Tassel: length of main axis (cm)	Plant length (cm)	Leaf: width of blade	Ear length without husk (cm)	Ear diameter without husk (cm)	Ear: number of rows of grains	1000 kernel weight (g)	Time of anthesis	Time of silking
Blocks (eliminating treatments)	7	2.0	16.6*	392.0	0.2	0.16*	0.3	68.2	0.8	0.5
Treatment (ignoring blocks)	82	31.5*	1131.0*	20.0*	4.0*	0.3*	3.2*	2787*	4.4*	5.4*
Checks	2	279.3*	21259.3*	620.0*	21.4*	1.5*	12.1*	64541*	38.4*	50.7*
Varieties	79	16.9*	441.8*	1.1*	3.2*	0.1*	2.8*	604.0*	3.0*	3.7*
C vs Variety	1	687.4*	15317*	312.4*	36.0*	13.4*	15.1*	51768*	44.4*	44.8*
Error	14	0.9	5.2	399.2	0.3	0.0	0.2	33.2	0.6	1.0
*, Significant at 5% level of significance.										

The sweet corn inbreds evaluated for 31 morphological traits showed wide range of variability with respect to different traits studied (Fig. 1; **Supplementary Table 2**). For leaf traits, the angle between the stem and the blade showed maximum frequency for small angle (61.25%) and remaining inbred lines showed wide leaf angle (38.75%). The altitude of leaf blade showed a frequency of 60% for the drooping state. The anthocyanin coloration of the brace roots (87.5%), base of the glume of tassel (76.25%), glumes excluding the base of tassel (55%) showed maximum frequency for absent whereas anthocyanin coloration of the anthers was found to be present in most of the inbreds with a frequency of 72.5%. The time of anthesis and silk emergence was found to be late in all the inbreds with a value ranging from 75 to 80 days and from 77 to 84 days, respectively. The density of the spikelets of the tassel was mostly dense with a frequency of 80% indicating good pollen count on the tassels for maximum inbreds. The angle between the main axis and the lateral branches of tassel had a frequency of 82.5% for the wide leaf angle. The altitude of the lateral branches of the tassel showed three classes of expression and most of the inbreds expressed the curved altitude of the lateral branches with a frequency of 57.5%, followed by the strongly curved attitude with a frequency of 25% and the straight attitude with a frequency of 9%.

The absence of anthocyanin coloration was observed in the silks, leaf sheath, and glumes of the cob across all the evaluated inbreds. With regards to the length of the main axis of the tassel, all inbreds exhibited long lengths ranging from 31 to 46 cm, except for S13 which showed a medium length of 28 cm. The plant length was found long in all the inbreds which varied from 155 cm inbred S3 to 210 cm for S49 and S52. Ear placement on the plant was considered to be low,

medium and high, if the ear to plant height ratio is less than 0.5, equal to 0.5 and greater than 0.5, respectively. 50% of the inbreds showed a high placement of ears on the plant while as, medium placement of ears was observed in 45% inbreds. A low placement of ears was found in 5% inbreds. The width of the leaf blade was broad in maximum number of inbreds with a frequency of 83.75%. The length of the ear without husk showed a frequency of 63.75% for medium class ranging from 11.3cm to 14.8 cm and the frequency of 36.25% was found with long ear length with a value from 15.2 cm to 18.3 cm. The diameter of the ear without husk showed a frequency of 71.25% for medium ear diameter (4.2 to 4.9 cm) while as a frequency of 28.75% was found for the large ear diameter (5.1 to 5.5 cm). The shape of the ear of the inbreds was conico-cylindrical in all cases with a frequency of 100%. The number of rows of the grains on the ear was many in the maximum number of inbreds and showed a frequency of 95% while 5% was observed for the medium number of rows of the grain. The color of top of grain was yellow with cap in all the inbreds. The kernel row arrangement was scored into two classes, mostly the arrangement of the rows was straight with a frequency of 80% while as a frequency of 20% was observed for the spiral state. The kernel sweetness was present in all the inbreds while as kernel waxiness and opaqueness was absent in all the cases. The kernel shape was observed to be shrunken in all the cases.

The cluster analysis based on 31 agro-morphological traits of DUS descriptor resulted in grouping of the 80 sweet corn inbreds along with three checks into two major clusters (Fig. 2). SMC 3, a non-sugary genotype was clustered as a mono-cluster while as the rest of the inbreds were classified as a separate cluster along with the two parents. Similar studies were undertaken by Yuvaraja et al. (2017) and Mahato et al. (2018) in 50 and 39 sweet corn inbred lines, respectively.

Biochemical characterization

A comprehensive evaluation of carotenoid content and total sugar in 80 inbred lines revealed substantial variation among the inbred lines **(**Fig. 3**).** The 80 sweet corn inbreds along with the two parents Sugar 75 and Mithas and the non-sugary genotype SMC 3 varied significantly with respect to the total carotenoid content and showed a range of 9–34 $\mu g g^{-1}$ with a mean of 20.07 $\mu g g^{-1}$as shown in (Table 2**).** The highest content of total carotenoids was found in the inbred S27 (34µg/g) followed by the inbred S65 (31.1µg/g). The lowest amount of the total carotenoids was found in the inbred S56 (9µg/g) followed by the inbred S60 (11µg/g). In the 80 inbred lines, the total sugar was found to range from 1.3–8.5% with a mean value of 5.5% (Table 2). The highest content of total sugars was found in the inbred S60 (8.54%) followed by the S14 (8.34%), whereas, the lowest sugar content was found in S80 (1.3%) followed by S71 (2.1%).

Table 2
Total Carotenoid content (µg g− 1) and Total sugar (%) estimated in the 80 inbred lines during this study.

Genotype	Total Carotenoids (µg g^{-1})	Total sugars (%)
Sugar-75	25	10.87
Mithas	19.2	7.34
SMC-3	4.2	2.18
S1	14.4	3.34
S2	16	3.31
S3	14.9	3.78
S4	21	4.47
S5	20	5.78
S6	14	7.34
S7	15	4.52
S8	21	6.58
S9	28	6.82
S10	25	6.35
S11	23	4.48
S12	21	3.92
S13	19	2.98
S14	23.2	8.34
S15	22.5	7.45
S16	27	5.45
S17	16	6.28
S18	17	3.88
S19	16.1	4.76
S20	18.9	5.82
S21	12	5.98
S22	15.2	4.97
S23	31	5.58
S24	25	6.55
S25	26	7.32
S26	27	3.39
S27	34	6.21
S28	15	4.57

S29	12	7.15
S30	19.8	3.66
S31	17.6	5.58
S32	26.7	7.11
S33	21	6.11
S34	24.4	4.85
S35	25	7.71
S36	30	6.61
S37	27	5.55
S38	13.2	7.95
S39	17.7	4.28
S40	16	4.33
S41	25	7.36
S42	17.5	5.85
S43	19.6	4.66
S44	22.8	8.11
S45	27	6.28
S46	22.5	3.38
S47	13.3	4.45
S48	22	5.95
S49	14	4.55
S50	23.3	7.12
S51	14	8.1
S52	18	3.67
S53	22	6.21
S54	24.9	4.33
S55	13.3	3.58
S56	9	5.11
S57	18.2	6.66
S58	22	3.88
S59	16.7	7.77
S60	11	8.54
S61	16	7.75
S62	21.3	5.96

S63	26.8	6.66
S64	26.2	4.58
S65	31.1	6.67
S66	22.7	3.45
S67	13.4	3.88
S68	18.4	8.01
S69	22.2	7.06
S70	26	5.55
S71	23.5	2.1
S72	22	7.67
S73	24.5	4.35
S74	22	6.34
S75	17.7	3.88
S76	16.4	4.54
S77	18	5.34
S78	24	3.55
S79	24.6	5.55
S80	13	1.3
	Mean = 20.07	Mean = 5.5
	Standard deviation = 5.81	Standard deviation = 1.78
	Standard error = 0.63	Standard error = 0.19

Based on the results of total carotenoid and total sugar content, we selected set of 25 inbred lines which had high total carotenoid and total sugar content. These inbred lines were subsequently evaluated for total amylose content. The apparent amylose content for the 25 sweet corn inbreds along with the parents *viz*, Sugar75 and Mithas and for a non-sugary genotype SMC3 which was estimated on the basis of color chart showed that the nine inbreds had an intermediate amylose content, two inbreds *viz*, S59 and S5 had a high amylose content and six inbreds showed a low amylose content. In contrast to this, five inbreds were found to have very low amylose content (Table 3). Furthermore, the scatter plots of total carotenoids in relation to total sugars and for amylose content in relation to total carotenoids and total sugars are presented in (Fig. 4)

Table 3
Total Carotenoid content (µg g− 1), Total sugar (%) and Amylose content estimated in the selected set of 25 inbred lines during this study.

Genotype	Total carotenoids (µg/g)	Total sugar (%)	Amylose content
Sugar 75	25	10.87	High
Mithas	19.2	8.66	intermediate
SMC 3	4.2	2.18	intermediate
S1	14.4	3.34	intermediate
S2	16	3.31	low
S4	21	4.47	very low
S5	20	5.78	High
S6	14	7.34	intermediate
S8	21	6.58	intermediate
S10	25	6.35	low
S16	27	5.45	low
S17	16	6.28	intermediate
S18	17	3.88	very low
S19	16.1	4.76	low
S20	18.9	5.82	very low
S22	15.2	4.97	very low
S24	25	6.55	intermediate
S26	27	3.39	intermediate
S29	12	7.15	low
S44	22.88	8.1	intermediate
S47	13.3	4.45	intermdiate
S51	14	8.1	low
S54	24.9	4.33	intermediate
S58	22	3.88	very low
S59	16.7	7.77	High

Molecular marker-based validation of sweet kernel trait

Polymorphism survey was done between the two sugary parental hybrids and a non-sugary parent SMC-3 using five molecular markers. Out of these five markers, only two markers *viz*, umc2061 and bnlg1937, which were linked to the sugary gene (*su1*) were found polymorphic (Fig. 5a), and were subsequently used in the genotyping of rest of the inbred

lines. The results of genotyping revealed that 33 inbreds amplified the sugary (Fig. 5b) specific allele of 230 bp for umc2061, while 20 inbreds carried only the bnlg1937 allele linked to the sugary gene. These markers flank the sugary gene on the proximal and distal ends of chromosome 4, suggesting a low probability of recombination between the marker and the gene. Frequency of the inbred lines carrying both the markers or particular marker of the sugary gene is presented in (**Table 4**). The two markers, umc2061 and bnlg1937, linked to the sugary locus, were used to genotype the inbred lines. Out of the 80 inbred lines, 46 (57.5%) were found to carry the sugary gene. Among the sugary lines, 7 inbreds were homozygous for both the markers, 26 inbreds carried only the umc2061 marker, and 33 inbreds carried only the bnlg1937 marker. Among the non-sugary lines, 13 inbreds carried both the markers, 20 inbreds carried only the bnlg1937 marker, and 32 inbreds carried only the umc2061 marker. The remaining inbreds either did not carry either of the markers. These findings confirm the presence of the sugary gene in a subset of inbred lines and provide valuable information for further breeding programs aimed at improving the carbohydrate quality of maize.

Cluster analysis and PCA based on molecular and biochemical traits

The dendrogram representing the cluster analysis of the inbreds is shown in (Fig. 2). The cluster analysis performed on the basis of the molecular markers and the biochemical traits helped to classify the genotypes into two clusters. The parents *viz*, Mithas and Sugar75 were classified into the cluster I and were present in the same subcluster. The non-sugary genotype, SMC 3 was also classified in the cluster I but in a different sub cluster.

The principal component analysis was jointly performed on the allelic profile and the biochemical traits. The PCA 1 explained 38% of the total variation while as the PCA 2 explained 24% of the total variation with the cumulative value of 62%. The markers umc2061 and bnlg1937 explained high variability. The two biochemical traits were also comparable to one another in explaining the total variability among the inbred population.

Discussion

Sweet corn (*Zea mays var. saccharata*) is an important cereal crop worldwide. Its popularity is mainly due to its sweetness, tenderness, and unique flavor, which are all determined by the presence of specific genes and their expression. Understanding the genetic and biochemical basis of these traits helps in the development of improved sweet corn cultivars with higher yield, better nutritional value, and desirable agronomic traits. In this study, we conducted a comprehensive characterization of sweet corn using morphological, molecular, and biochemical approaches to gain insights into the genetic and biochemical basis of its desirable traits, such as sugar and carotenoid content.

Morphological characterization of crop plants is essential for understanding the genetic diversity and variation that exists within a population. In this study, the analysis of variance (ANOVA) inferred significant genetic differences among the 80 sweet corn inbred lines and hence, the possibility of selection of superior genotypes with improved yield potential and high levels of total sugars.

The evaluation of the 80 inbreds for 31 morphological traits showed that all the traits were quite informative with respect to the trait expression cum characterization. Phenotypic characterization of maize inbred lines using DUS traits is well documented previously by many researchers [7, 8]. Similar to our findings, Madhukeshwar and Sajjan [15] also observed enough magnitude of variation for various morphological traits like tassel angle, tassel attitude of lateral branches, tassel density of spikelet's, plant height, ear shape and 1000 grain weight in a study comprised of two maize hybrids and five parents. In this study, leaf angle between stem and leaf blade for maximum number of inbred lines was small, which is a crucial trait for obtaining higher yield due to its substantial role in light interception by the canopy and hence photosynthetic efficiency, specifically under high density planting stress. However, inbreds with wide leaf angle are thought to be good water harvesters and could be best used under rainfed conditions. The time of anthesis and silk

emergence was found to be late in all the inbreds, indicating that more days are available for the lines for photosynthesis, leading to higher biomass production and thus higher crop yield. Most of the inbred lines have dense tassel making them ideal male parent for quality seed production. However, a dense tassel could also cause redundant assimilation, consumption and resource competition with female reproductive organ. Therefore, lines with light tassel with more partitioning of assimilates towards ear could serve as ideal female parents. Furthermore, lines found with medium ear placement are believed to be ideal for making harvesting easy and improving lodging resistance. Plant height, number of rows of grains per ear, ear diameter, ear length and 1000 kernel weight are important secondary traits for the identification of genotypes from this study for higher productivity.

Carotenoids are comparatively found to be abundant in sweet corn; thus, their quantification is vital from the nutritional point of view [14]. The total carotenoids of the 80 early generation sweet corn inbreds (S_2 stage) showed a continuous distribution pattern, as expected for a quantitative trait. However, carotenoid content in both parental hybrids was found to be in the intermediate range. The results of the present research are in similar direction with the earlier reports of Song [16] where the total carotenoids were in the range 8.42 to 39.71 µg/g. Similarly, analysis for total sugar content also revealed continuous pattern, however, in case of the parental hybrids from which the 80 inbreds were derived, the mean value for the total sugars was found to be highest. The results of this research are comparable with the previous reports of Ghada and Ibrahim [6]. However, Tosun [27] and Hemavathy and Priyadarshani [9] revealed slightly higher range for total sugars in 49 inbreds (0.66–16.84%) and 26 sweet corn lines (2.05 to 17.14%), respectively.

The scatter plot of total carotenoids and total sugars showed that the non- sugary genotype SMC-3 had low levels of total carotenoids as well as total sugars. The parents viz., Sugar-75 and Mithas showed intermediate levels of total carotenoids with high levels total sugars while as the inbred S9, S23, S27 and S36 showed high levels of total carotenoids and total sugars confirming that these inbred lines could be utilized in sweet corn breeding programmes after further validation for yield attributes, disease resistance and desirable agronomic traits.

In addition to better nutritional profile, the food must have good digestibility in order to have widespread acceptability. High amylose maize is a source of resistant starch which is a type of starch that resists digestion and therefore confers with low glycemic index, thereby can prevent diabetes, obesity and colon cancer [19]. Amylose concentration is found to be high in the *su*1 kernels [10]. The study has also confirmed that the 80 sweet corn inbreds have increased amounts of amylose in them. The parent sugar 75 was found to contain high content of total carotenoids, total sugars and high amylose. Among the inbreds, the inbreds S5 and S59 were found to have high level of amylose in addition to having high amounts of total carotenoids and total sugars. The results obtained are similar to the results of that of [19, 30].

The results of molecular marker-based validation of sweet kernel trait confirmed that out of the 60inbreds, seven inbreds *viz.*, S21, S28, S47, S48, S49, S53, and S54 carried the alleles specific to sugary trait with respect to the markers umc2061 and bnlg1937. These lines were also found to contain high sugar content, thus confirming the utility of these markers *viz, umc*2061 and bnlg1937 in marker assisted selection. The other lines which carry the sugar specific alleles for only one of the markers may undergo some recombination between the marker and the gene. Though these lines were also tested for the phenotypic appearance of the grain and with respect to the biochemical traits, the chance of these lines belonging to the non-sugary class is very low. However, these lines are required to be analysed further and validated with more closely linked markers to *su-1*mutants. Hossain [10] also identified and validated *umc* 2061 as the most effective and closest marker for *su1*in sweet corn. Similar studies were carried out by Tosun [27] in 49 sweet corn inbred lines and it was confirmed that the primers which were found to be immensely correlated with the sugar content were phi44 marker for the gene *sh1*, phi328175 for the *ea1* gene, umc1031 for the *su* gene and umc2276 for the *sh2* gene. They also observed that for *su1*, umc 2061 marker were found useful to determine higher sugar content lines. However, the lines carrying *su1* allele with respect to bnlg 1937 had lower sugar content.

The cluster analysis performed on the basis of the phenotypic traits, molecular markers and the biochemical traits revealed that the study have implications in effectively developing improved lines of sweet corn using the information from the clustering pattern which is necessary for successful continuation of any breeding programme. The crosses involving inbred lines from diverse clusters were expected to show maximum heterosis and create wide variability in genetic architecture and could be used as parental source for breeding programmes with selective objective.

Conclusion

In case of sweet corn, a relatively narrow genetic base with limited sources of germplasm and poorly defined heterotic groups are the reasons for slow improvement in sweet corn breeding as compared to field corn. The results of the present study, has identified several inbreds harbouring desirable biochemical and agro morphological attributes related to high yield. Consequently, the study will not only enhance the genetic base of sweet corn germplasm but will also lead to development of high-yielding hybrids with improved quality. The inbreds *viz*, S9, S23, S27, S25 and S36 with high levels of total carotenoids and total sugars could be utilized in sweet corn breeding programmes after further validation for yield attributes, disease resistance and desirable agronomic traits. Furthermore, the narrow range of diversity particularly for agro-morphological traits could be explained from the fact that the 80 inbred lines used in the study were derived from selfing the double cross hybrid that was originally obtained from only two single cross hybrids. Thus, in order to capture maximum amount of variability, it could be recommended that inspite of using only single cross hybrids, it is better to begin with the very diverse source population which may provide diverse base material for further improvement in sweetcorn breeding.

Declarations

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Conflict of interest

The authors declare that there is no conflict of interest.

Data Availability Statement

All the data is available in the manuscript and as supplementary material (Supplementary Table 1 and 2).

Compliance with Ethical Approval Standards

This article does not contain any studies with animals performed by any of the authors.

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Figures

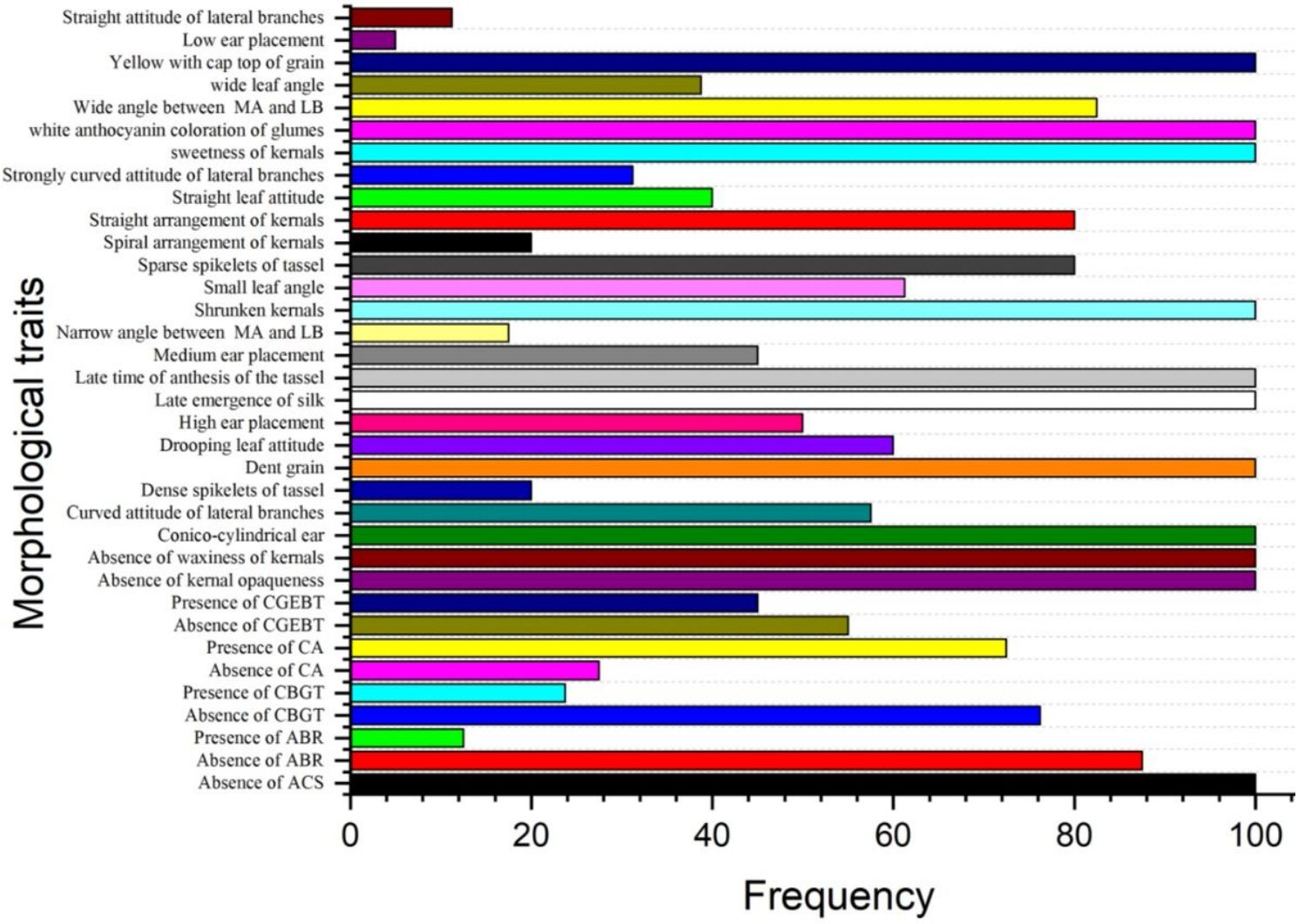


Figure 1

Bar graphs showing frequency distribution of qualitative morphological traits in the 80 inbred lines used in this study. ACS= Anthocyanin coloration of sheath; ACS'= Anthocyanin coloration of sheath; ABR= Anthocyanin coloration of the brace roots; CBGT= Coloration at base of glume of tassel; CA= Coloration of anthers.

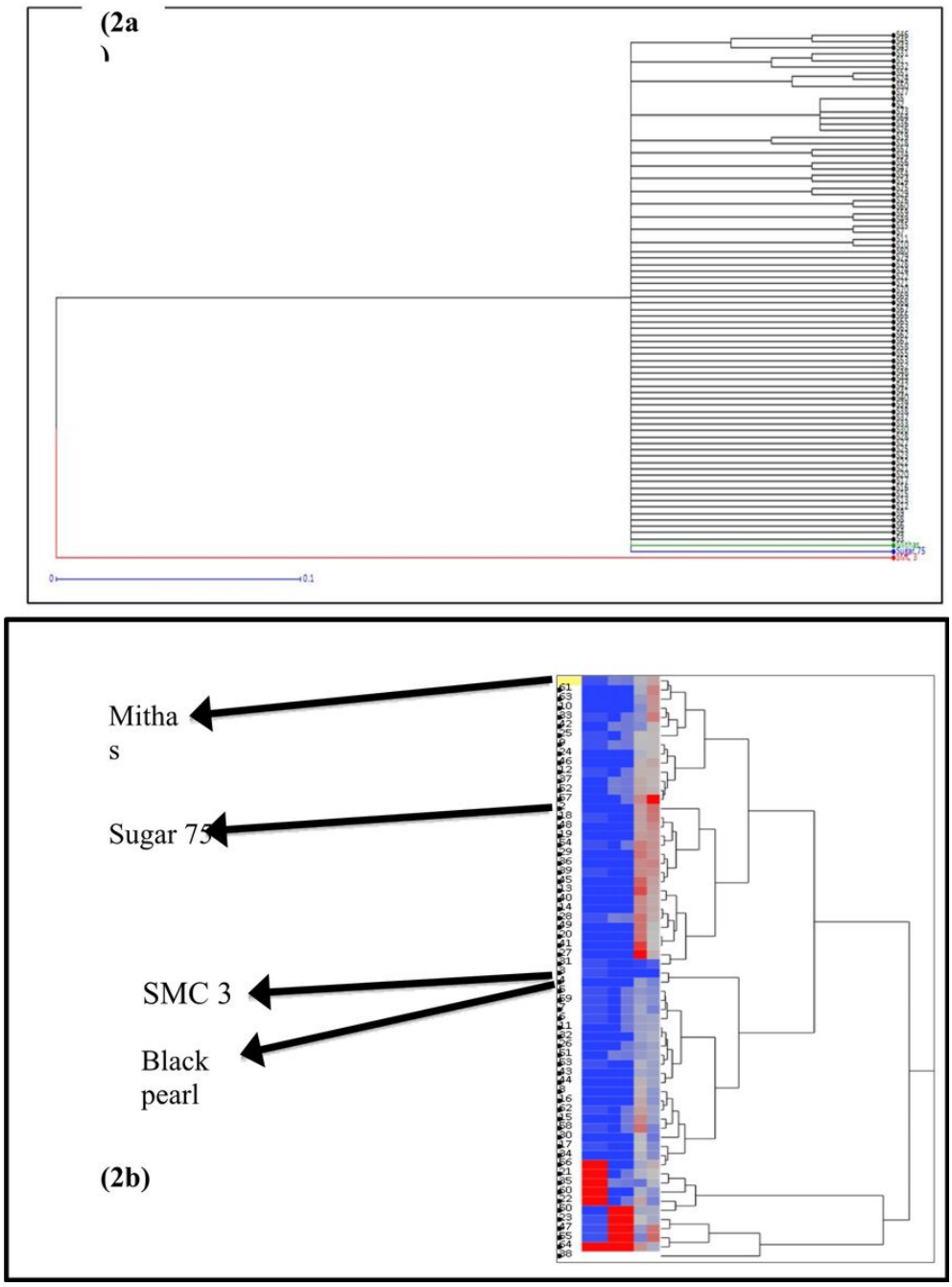
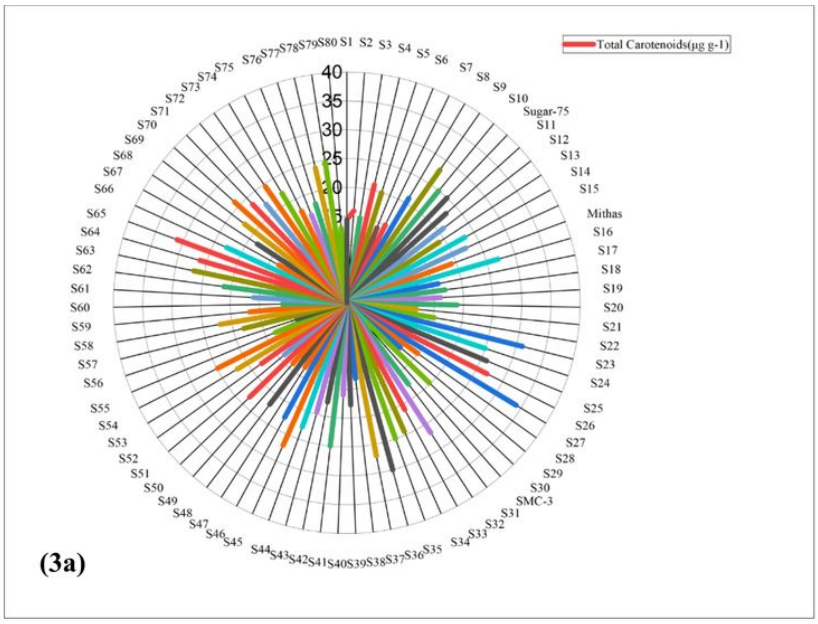


Figure 2

Dendrogram of 80 sweet corn inbred lines generated through DARwin software using unweighted neighbor joining method based on morphological traits (2**a**). Cluster analysis performed on the basis of the molecular markers and the biochemical traits using DARwin software (2**b**).



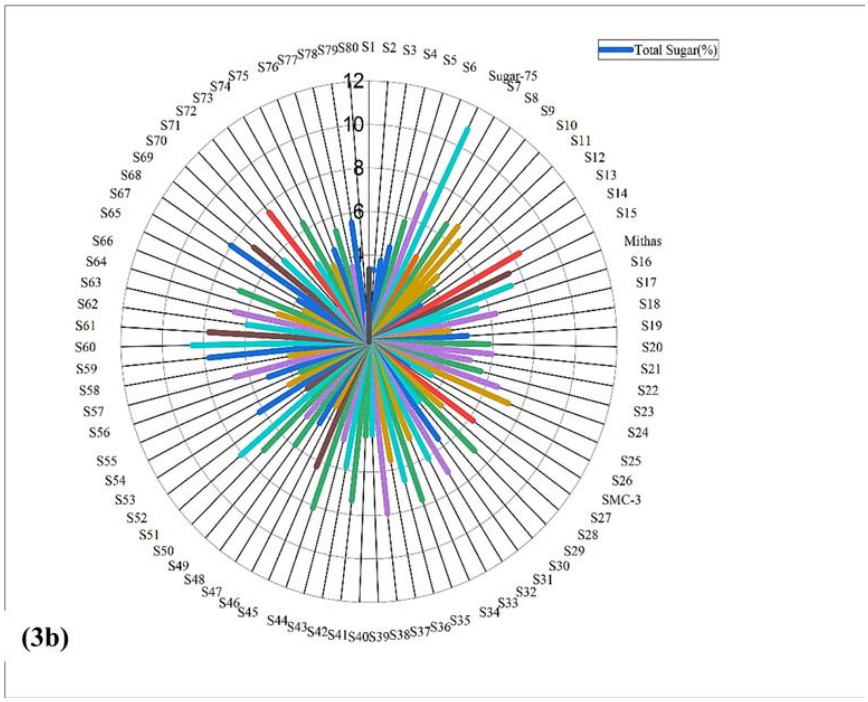
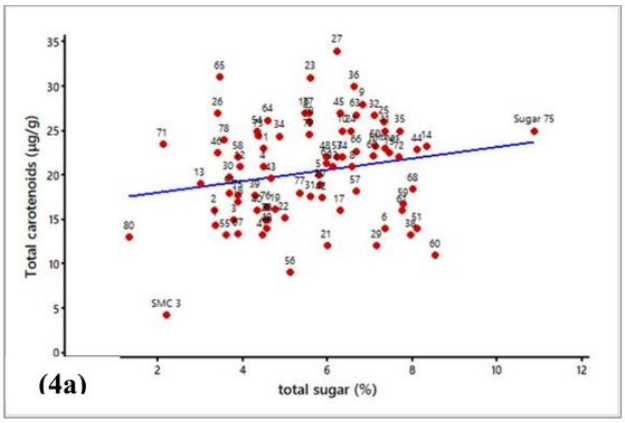
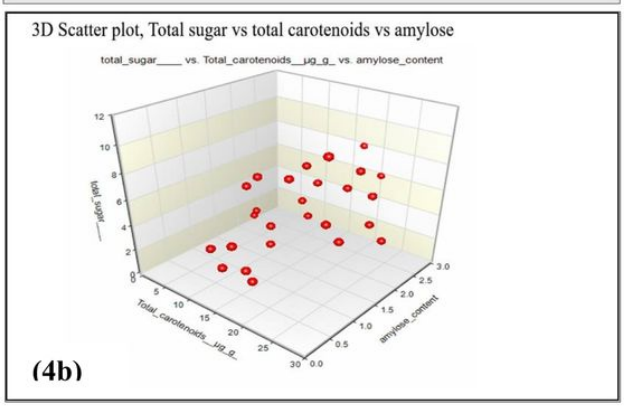


Figure 3

Spider graph depicting the substantial variation for total carotenoid content (3**a**) and total sugar content (3**b**) in the 80 inbred lines used in this study.





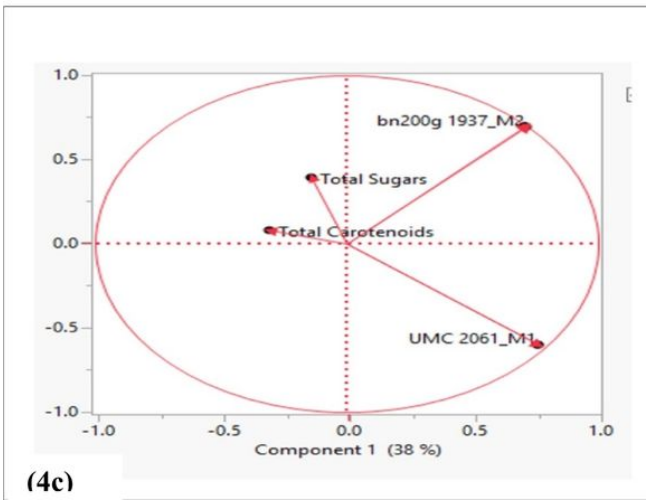
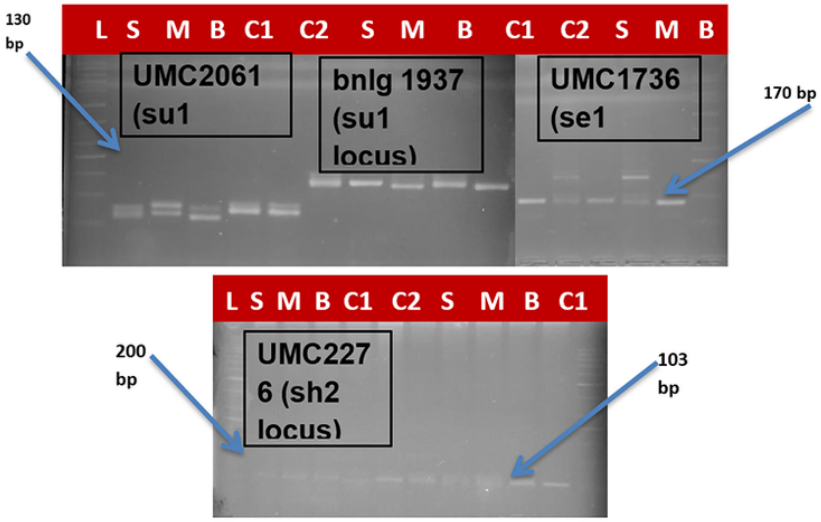


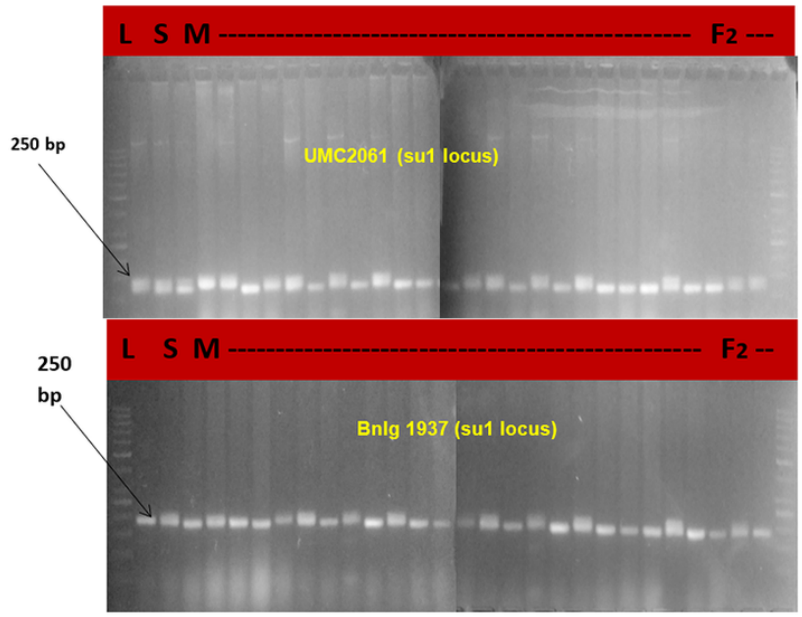
Figure 4

Scatter plots of total carotenoids in relation to total sugars (4**a**) and for amylose content in relation to total carotenoids (4**b**). Principle component analysis (PCA) performed on molecular markers (umc2061 and bnlg1937) and biochemical traits *viz,*total sugars and total Caratenoids (4**c**).



L: 50bp ladder; S: Sugar 75; M: Mithas ; B: F2 bulk; C1: SMC-3 ;C2: Black pearl.

Fig. 5a



L: 50bp ladder; S: Sugar 75; M: Mithas

Fig. 5b

Figure 5

a Gel image showing polymorphism between the two sugary parental hybrids and a non-sugary parent SMC-3 detected only by two markers “, umc2061 and bnlg1937” out of 5 markers used in this study.

b Represenative gel image showing polymorphism in the 80 inbred lines detected by two markers *viz*, umc2061 and bnlg1937used in this study.

Supplementary Files

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- SupplementaryTables.docx