Screening of Sweet Sorghum Genotypes for the Traits Related to Biomass and Sugar Content

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Abstract

The improvement of sweet sorghum [*Sorghum bicolor* (L.)] is getting more attention due to its sugar-rich stalk that can be used as a renewable energy product. The proper assessment of biofuel-related traits in sweet sorghum crop is an important step towards the development of superior cultivar. A study was conducted with the objective of evaluating 30 sweet sorghum genotypes for biomass and sugar related traits through identified sugar related markers. In the study, thirty genotypes along with two check varieties were screened by physiological evaluation and through molecular analysis. The experimental data was subjected to statistical analysis for elucidating the information on genetic variability. It was assessed for genotypic coefficient variation (GCV) and phenotypic coefficient of variation (PCV), heritability (H^2) and genetic advance over mean (GAM). The molecular diversity analysis was done by using 15 QTLs linked SSR markers. Genotypes possessing better TSS values were IS4835, IS5361 and IS14465, which can be preferred for the ethanol production also. These genotypes can be used in the breeding programs as parents for high TSS. The genotypes such as IS5361, IS14861 and IS4835 were found to be good biomass producers and these genotypes can be grown for fodder purpose. From the study, it is concluded that IS5361 is the most suitable genotype for the bioethanol production.

Keywords: Sweet sorghum, Biomass, TSS, QTLs and Ethanol

The rising cost of fossil fuels and associated increase in GHG emissions has recently resulted in an increased search for alternative renewable and sustainable energy sources such as bioenergy crops. Bioenergy crops are used to produce biofuels, that help in meeting the current growth in energy demand while reducing the emission of GHG (Lal *et al.*, 2008). There are several advantages of biofuels such as environmental friendliness, biodegradability and high potential for local production from various feedstocks. There is a renewed interest in using sugar rich agricultural crops as feedstock for the biofuel production. The new policies to blend petrol with up to 10 per cent ethanol are now widely adopted, which led to additional ethanol requirement to meet the demands. Existing feedstocks such as sugar beets or sugarcane has paved way to search for an alternative source for the ethanol production. Sweet sorghum is one such an alternative which has very good potential as a feedstock for

ethanol production and has emerged as a good supplementary crop to sugarcane (Reddy *et al.*, 2010).

Sorghum [*Sorghum bicolor* (L.) Moench] is grown as staple food throughout Asian and African regions and as a fodder and forage crop in the countries like America, Europe and Japan. Particular varieties of Sorghum known as 'Sweet Sorghums' or 'Sorgos', have sweet juicy stalks which accumulate 10-25 per cent sugar in the stalk juice at the time of grain maturity. These varieties can be used to produce Sorghum syrup or sugar although many of them are used for forage in China & sugar is being produced from Sweet sorghum, while in Brazil, it is grown as a source of Ethanol production. Sweet sorghum is self-pollinating, diploid (2n =20) with a genome size of 730 Mb, about 25 per cent the size of sugarcane or maize. It is a C4 plant with higher abiotic stress tolerance and higher photosynthetic efficiency. Its small genome (730 Mb) makes the Sorghum an attractive model for functional

genomics of C4 grasses. Drought tolerance makes sorghum a very important crop in dry regions such as India, northeast Africa, and the southern plains of the United States (Paterson *et al.*, 2009).

Sugar yield for which crop improvement is required, is a complex character, which is not only influenced by its associated characters but also by the environment. This requires the separation of genetic variability from total variability to make selections. Sugar yield is the result of numerous characters collectively working during the crop growth which are codependent in their development. It is hence desirable to study the association between yield influencing traits and yield since this would help effective selection for concurrent improvement of one or more yield influencing components.

Recent progress in sorghum genomic studies has generated a series of important tools and resources which can be used to find desirable alleles and genes for the genetic enrichment of traits of importance. By using the QTL mapping tools and molecular linkage maps, it is possible to approximate the number of loci governing the genetic variation in traits of importance. It is further possible to hasten the transfer the gene of interest between the varieties and to introgress novel genes from the wild species into the cultivated species. The SSR markers are the widely used and most prevalent molecular markers in crop improvement due to their abundance, multi allelic nature, high reproducibility and mono locus co-dominant nature. They are frequently used for the finger printing of cultivars, genetic diversity studies, mapping Quantitative Trait Loci (QTL) and marker assisted breeding of crop plants.

Integration of marker assisted selection in conventional breeding programs aids to increase the pace and efficiency of the crop improvement schemes. A success in improvement of grain yield has been made in the grain sorghum by plant breeding efforts and now it is the time to bring improvement in the sweet sorghum for its stalk sugar content. Hence, it is highly imperative to exhaustively screen the sweet sorghum genotypes for bioenergy-related traits in combination with other desirable traits. Such type of study will have significant application in sweet sorghum improvement program.

MATERIAL AND METHODS

The study was carried out in the Department of Plant Biotechnology, UAS, GKVK, Bengaluru, which consists of thirty sweet sorghum genotypes with two standard check varieties (SSV84 and CSV19SS) obtained from Indian Institute of Millets Research (IIMR), Hyderabad. Experiment was carried out on sandy loamy soil, during Rabi 2017. The Randomized Complete Block Design (RCBD) was followed with three replications. All the recommended packages of practice were followed to raise the crop. All the genotypes were assessed for the physiological traits such as, plant height (PH), days to 50 per cent flowering (DF), stem length (SL), ear head length (EHL), total soluble sugar (TSS), biomass (BM), hundred seed, weight stem width and grain yield (observations were taken during the physiological maturity).

Statistical Analysis

The mean values of genotypes in each replication were used for analysis of variance. The analysis of variance for individual character was carried out using the mean values of replications following the method given by Panse and Sukhatme (1967). The significance of the differences among all the genotypes was tested by F-test using the error variance. Further, Phenotypic variance, genotypic variance, heritability and genetic advance were estimated as per the formulae suggested by Lush (1949), Hanson *et al.* (1956) and Johnson *et al.* (1955).

Molecular Analysis

The leaf samples were collected from 20-25 days old seedlings and were kept immediately at -80 °C till DNA was isolated. Cetyl Trimethyl Ammonium Bromide (CTAB) method of DNA isolation was followed to isolate the DNA from the samples. The quality and quantity of DNA was also analyzed by running the genomic DNA samples on agarose gels. Fifteen QTL linked SSR markers (microsatellites) were used for amplification by the PCR (Polymerase Chain reaction). Existing sugar and biomass related primers (Menz *et al.,* 2004) was used for this experiment. The genotype profiles produced by SSR markers were scored manually. Each SSR loci was scored as present

(1) or absent (0) for each of the SSR loci. A total of 15 QTL linked SSR markers giving consistent expected size products were used for genotyping of 32 genotypes.

RESULTS AND DISCUSSION

Analysis of variance for biomass and sugar related traits of sweet sorghum genotypes in field experiment was computed and mean sum of squares for all the characters is presented in Table 1, the analysis of variance revealed significant differences among the mean of different genotypes for days to 50 percent

TABLE 1

List of Sweet Sorghum genotypes used in the study

Sl. No.	Genotypes	Sl. No.	Genotypes
1	IS3240	17	E247
2	IS3940	18	E254
3	IS4835	19	ERN2
4	IS5361	20	EJN47
5	IS9705	21	EJN50
6	IS9767	22	EJN51
7	IS9775	23	EJN57
8	IS14465	24	EJN58
9	IS14861	25	EJN63
10	IS20503	26	ERN4
11	IS21813	27	ERN10
12	E159	28	ERN12
13	E167	29	ERN18
14	E232	30	ERN32
15	E233	31	SSV84
16	E241	32	CSV19SS

flowering, plant height, shoot length, ear head length, stem width, biomass, 100 seed weight, grain yield and total soluble sugars indicating the choice of material for the investigation was appropriate. This was further supported by the fact that, range has also been quite wider for all the characters pointing out extreme genotypes for selection (Table 2).

Days to 50 per cent flowering were higher in the genotypes IS14465 and E232 (102 days) while the genotype E254 recorded the lower value (66 days). The plant height was higher in genotype IS4835 (201.64cm) whereas, genotype IS3240 has showed the lower plant height (141.24cm). The genotype IS1813 has showed the higher shoot length (176.77cm), while genotype IS3240 showed the lowest shoot length (116.73cm). The genotype ERN4 recorded the higher ear head length (32.77 cm) whereas, E241 has showed the lower ear head length (16.55 cm).

Higher stem width was seen in IS14465 (2.16cm) whereas, the lower value of stem width was recorded in IS9775 (1.26cm). The higher biomass was recorded in IS5361 (328.69g) whereas, the lower biomass was recorded in E233 (139.67g). The hundred seed weight was higher in IS4835 (9.87g), while it was low in IS21813 (4.36g). The genotype IS4835 recorded significantly the higher grain yield (18.29g) whereas; CSV19SS has recorded the lower grain yield (12.8g). The genotype IS5361 recorded significantly higher TSS (14.67 %), however, the genotype IS3940 has showed the lower TSS value (9.33 %).

To understand the extent to which the observed variation was due to genetic factors, the range, mean, Genotypic co-efficient of variation (GCV), Phenotypic co-efficient of variation (PCV), heritability (h^2) and

TABLE 2

Analysis of variance of sweet sorghum genotypes for morphological traits under study

Source of variance	Mean Sum of Squares								
	DF	PH	SL	EHL	SW	BM	HSW	GY	TSS
Replication	2.16	5.11	8.47	23.15	0.016	26.07	0.28	7.5	0.21
Treatment	436.93	645.70	578.26	66.39	0.22	8,769.86	10.09	8.01	9.48
Error	1.18	18.53	9.10	7.52	0.09	78.57	0.17	2.32	0.39

TABLE 3

Estimation of Genetic variability parameters

Traits	Range			GCV%	PCV%	H^2%	GA as % of mean
	Max	Min	Mean				
DF	102	66	85.59	17.24	17.29	99.46	36.78
PH	201.64	141.24	181.33	9.77	10.05	94.42	21.22
SL	176.77	116.73	154.86	10.89	11.07	96.90	23.42
EHL	32.77	16.55	26.46	20.50	22.98	79.64	50.34
SW	2.16	1.26	1.68	15.46	23.92	41.80	74.16
BM	328.69	139.67	208.75	31.58	31.86	98.22	66.11
HSW	9.87	4.36	6.84	32.38	32.94	96.65	81.90
GY	18.29	12.8	15.50	10.88	14.67	55.06	33.76
TSS	14.67	9.33	11.69	17.77	18.52	92.08	45.82

DF- Days to 50 per cent flowering, PH- Plant Height (cm), SL- Shoot Length(cm), EHL- Earhead length(cm), SW-Stem width(cm), BM- Biomass (g), HSW-Hundred seed weight (g), GY- Grain yield (g), TSS- Total soluble sugars (%), PCV- Phenotypic co-efficient of variation, GCV- Genotypic co-efficient of variation, GAM- Genetic advance as percent mean, H^2- Heritability (Broad sense).

genetic advance as percent of mean (GAM) were computed for all the characters under consideration. The results indicated narrow difference between the GCV and PCV values indicating less interference of environmental effect on genotypes. PCV and GCV were found to be high for stem width, ear head length, biomass and hundred seed weight. These traits offer scope for direct selection. Days to 50 per cent flowering, plant height and stem length recorded lower PCV and GCV indicating limited scope for these characters. The estimates of high heritability have been observed for days to 50 per cent flowering, plant height, stem length, ear head length, biomass, hundred seed weight and TSS indicating that selection for these characters will be rewarding, as they were least influenced by environment. High heritability estimates coupled with high genetic advance as percent of mean was noticed for days to 50 per cent flowering, ear head length, stem width, biomass, hundred seed weight, grain yield and TSS, suggesting that, these characters are under the control of additive genes and phenotypic selection for these characters may be effective.

In the study, PCV and GCV estimates were relatively high for stem width, ear head length, biomass and hundred seed weight. High heritability estimates coupled with high genetic advance as percent mean was recorded for most of the traits under the study, indicating the predominance of additive gene action and scope for improving these traits through simple selection process.

Screening of Sweet Sorghum Genotypes using QTL Linked SSR Markers

IS5361 and the check variety SSV84 confirmed the presence of eleven QTLs for sugar content and it was the highest score among 32 genotypes used in this experiment. Followed by E167 with ten, E159 with nine, and IS4835, IS14465, IS20503 each possessing eight QTLs respectively. Genotypes like IS 3940, IS9705, IS2181, E233 and E241 possess the least number of QTLs (three).

The genotypes such as IS5361 and SSV84 showed the highest number of QTLs (score-11) showed the highest and moderate TSS (14.67 and 13.67 % respectively). Interestingly, the genotypes with next higher scores E167 and E159 (10 and 9 respectively), showed 13.67 per cent TSS and the genotypes with 8 QTLs (IS4835, IS14465, IS20503) showed the higher TSS (14.33%) than the genotypes with 9 and 10 QTLs.

Fig. 1:   Gel picture showing the Sugar related QTL profile of all the genotypes using QTL linked SSR marker 1 (mSbCIR223)

Fig. 2:   Gel picture showing the Sugar related QTL profile of all the genotypes using QTL linked SSR marker 2 (mSbCIR238)

Fig. 3: Gel picture showing the Sugar related QTL profile of all the genotypes using QTL linked SSR marker 3 (mSbCIR240)

L- 100 bp Ladder, 1-IS3240, 2- IS3940, 3-IS4835, 4-IS5361, 5-IS9705, 6-IS9767, 7-IS9775, 8-IS14465, 9-IS14861, 10-IS20503, 11-IS21813, 12-E159, 13-E167, 14-E232, 15-E233, 16-E241, 17-E247, 18-E254, 19-ERN2, 20-EJN47, 21-EJN50, 22-EJN51, 23-EJN57, 24-EJN58, 25-EJN63, 26-ERN4, 27-ERN10, 28-ERN12, 29-ERN18, 30-ERN32, 31-SSV84, 32-CSV19SS.

Even though, some genotypes possessed more number of QTLs, they showed less TSS. Till today, there are more than 800 QTLs have been identified for biofuel relates QTLs in sweet sorghum and only fifteen QTLs were used in the present study. Hence, the variation observed in TSS in majority of the varieties in the current study could be explained by the fact that the presence of QTLs other than those included in this study might be contributing for the total sugar accumulation in the stalk. Conversely, some genotypes showed more TSS in spite of them harboring less number of QTLs. This suggested that it is not only the number of QTLs present, but also the effective combination of these QTLs which is important in governing the TSS in the genotypes.

Similar results were found in earlier study in a crossing between two unrelated sweet sorghum lines, they identified QTL having upto 18.8 per cent of the phenotypic variation for sucrose and and sugar yield on chromosomes SBI-02, SBI-03 and SBI-05. Later, Yun-long *et al.* (2006) identified a QTL explaining up to 25 per cent of phenotypic variance on chromosomes SBI-04 and SBI-10 for Brix content. But they inferred

TABLE 4

Comparison of Number of QTLs with TSS and biomass

Genotypes	No. of QTLs	TSS	Biomass	Genotypes	No. of QTLs	TSS	Biomass
IS3240	7	10.67	245.73	E247	4	12.67	205.32
IS3940	3	9.33	216.00	E254	5	11.33	168.49
IS4835	8	14.33	311.85	ERN2	4	11.67	155.78
IS5361	11	14.67	328.69	EJN47	5	9.33	163.93
IS9705	3	10.67	150.91	EJN50	4	10.67	234.13
IS9767	6	9.33	197.85	EJN51	4	9.67	170.64
IS9775	4	11.67	163.12	EJN57	5	11.67	174.24
IS14465	8	14.33	184.35	EJN58	4	12.67	188.49
IS14861	6	10.33	295.82	EJN63	3	11.67	215.10
IS20503	8	14.33	290.27	ERN4	5	11.67	185.10
IS21813	3	11.67	211.91	ERN10	5	10.33	186.22
E159	9	13.67	226.38	ERN12	4	11.67	155.02
E167	10	13.67	271.88	ERN18	4	10.33	195.59
E232	5	13.67	162.19	ERN32	5	12.67	173.56
E233	3	12.67	139.67	SSV84	11	13.67	312.47
E241	3	10.33	254.77	CSV19SS	6	7.33	144.36

that, combination of several QTLs was responsible for Sugar content which is a quantitative trait.

From the present study, it can be concluded that, Genotypes possessing better TSS values are IS4835, IS5361, IS14465 and IS20503 which can be preferred for the ethanol production also these genotypes can be used in the breeding programs as parents for high TSS. The genotypes such as IS5361, IS14861, IS4835 and E167 are found to be good biomass producers and these genotypes can be grown for fodder purpose, also can be used in breeding programs as a parent for high biomass. The best genotype among the 30 genotypes is IS5361 for the bioethanol production. The genotypes where early flowering was observed, (ERN32, EJN57, IS9767 and IS20503) can be designated as early maturing and short duration genotypes which has a further scope of being used in the breeding programs to combine other desirable traits into these short duration genotypes.

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