Theor Appl Genet (2010) 121:323–336
DOI 10.1007/s00122-010-1312-y

ORIGINAL PAPER

Genetic mapping of QTLs for sugar-related traits in a RIL population of *Sorghum bicolor* L. Moench

Amukelani Lacrecia Shiringani · Matthias Frisch · Wolfgang Friedt

Received: 18 December 2009 / Accepted: 22 February 2010 / Published online: 14 March 2010

Abstract The productivity of sorghum is mainly determined by quantitative traits such as grain yield and stem sugar-related characteristics. Substantial crop improvement has been achieved by breeding in the last decades. Today, genetic mapping and characterization of quantitative trait loci (QTLs) is considered a valuable tool for trait enhancement. We have investigated QTL associated with the sugar components (Brix, glucose, sucrose, and total sugar content) and sugar-related agronomic traits (flowering date, plant height, stem diameter, tiller number per plant, fresh panicle weight, and estimated juice weight) in four different environments (two locations) using a population of 188 recombinant inbred lines (RILs) from a cross between grain (M71) and sweet sorghum (SS79). A genetic map with 157 AFLP, SSR, and EST-SSR markers was constructed, and several QTLs were detected using composite interval mapping (CIM). Further, additive × additive interaction and QTL × environmental interaction were estimated. CIM identified more than five additive QTLs in most traits explaining a range of 6.0–26.1% of the phenotypic variation. A total of 24 digenic epistatic locus pairs were identified in seven traits, supporting the hypothesis that QTL analysis without considering epistasis can result in biased estimates. QTLs showing multiple effects were identified, where the major QTL on SBI-06 was significantly associated with most of the traits, i.e., flowering date, plant height, Brix, sucrose, and sugar content. Four out of ten traits studied showed a significant QTL × environmental interaction. Our results are an important step toward marker-assisted selection for sugar-related traits and biofuel yield in sorghum.

Communicated by X. Xia.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-010-1312-y) contains supplementary material, which is available to authorized users.

A. L. Shiringani · W. Friedt (✉)
Department of Plant Breeding, Research Centre for Biosystems, Land Use and Nutrition (IFZ), Justus-Liebig University of Giessen, Heinrich-Buff-Ring 26-32, 35392 Giessen, Germany
e-mail: wolfgang.friedt@agrar.uni-giessen.de

M. Frisch
Institute of Agronomy and Plant Breeding II, Justus-Liebig University of Giessen, Heinrich-Buff-Ring 26, 35392 Giessen, Germany

Introduction

Sorghum bicolor is a diploid species ($2n = 20$) that is closely related to sugarcane, a polyploid species. Cultivated sorghum can be subdivided into grain, fiber, forage, and sugar (sweet) types. Each of these groups represents a broad genetic diversity as has been shown, e.g., for grain types (Assar et al. 2005). Sweet sorghum stores a high amount of sugar in its stem, and produces plenty of juice. Sweet sorghum stalks have already been processed to syrup, sugar, and molasses for a long time. Recently, sorghum breeders worldwide are aiming at improving various characteristics of the crop that can result in commercialization. Although for many years the main trait of interest in sorghum has been grain yield, recently interest has also shifted from grain to stalk to manipulate biofuel-related traits: fiber, sugar, juice, and biomass. As in grain yield, biofuel-related traits cannot be manipulated directly, because they are quantitative, polygenically inherited traits. Therefore, to successfully improve these complex traits, they need to be dissected into smaller morphological,

physiological, and genetical components, which are then analyzed and evaluated.

Sorghum like maize and sugarcane is a C_4 plant characterized with high photosynthetic efficiency. Sugar yield is the major breeding objective in sweet sorghum. Aitken et al. (2006) defined sugar yield as a function of cane yield and recoverable sucrose content from the harvested cane. Milligan et al. (1990) reported that genetic correlations of sugar traits are a good strategy of selection in sugarcane. After screening a population in 2 years at five locations, they found that the stalk number shows a high correlation with cane yield ($r = 0.77$) and concluded that selection for sucrose should emphasize cane yield and attention may be given to stalk number. A high correlation between stalk weight and stalk diameter ($r = 0.70$) was also observed, and brix was highly correlated to sucrose. In sugarcane, stalk diameter and stalk number are highly heritable with more than 80% reported (Aitken et al. 2008), and a number of QTLs influencing these traits have been identified (Ming et al. 2002). In sorghum, Murray et al. (2008) reported a weak but significant correlation between sugar in dry stem and stem diameter ($r = 0.11$) and tillers ($r = -0.09$). Total sugar yield had no significant correlation with diameter but a significant correlation with tillers ($r = 0.16$), while juice sucrose was not correlated with tillers number but had a negative, weak but significant correlation with stem diameter ($r = -0.06$). These weak correlations in sorghum may limit indirect selection for sugar traits or sucrose using morphological traits.

Ritter et al. (2008) reported QTLs for sugar-related traits in a population of 184 recombinant inbred lines derived from grain × sweet sorghum. They used 247 AFLP, as well as sorghum and sugarcane SSR markers. Using composite interval mapping at a LOD score of 3.00, they detected 29 QTLs distributed in five different linkage groups for sucrose content (11 QTLs), sucrose yield (8 QTLs), and sugar yield (10 QTLs). Natoli et al. (2002) reported several QTLs for sugar-related traits in a population of 129 F_2 progenies derived from unrelated sweet sorghum parents. Yun-Long et al. (2006) detected several QTLs in 207 F_2 individuals derived from a cross between grain and sweet sorghum. Natoli et al. (2002) detected QTLs on chromosomes SBI-01, SBI-03, and SBI-05, Yun-Long et al. (2006) found QTLs on SBI-04, SBI-10, Ritter et al. (2008) and Murray et al. (2008) detected QTLs for sugar components on SBI-01, SBI-03, SBI-05, SBI-06, SBI-07, SBI-10, but with an additional one on SBI-02 in Murray et al. (2008). The distribution of these QTLs among chromosome and the less phenotypic variation explained suggest that sugar traits are controlled by more loci with complicated interactions.

To further understand the genetics of these traits, further studies should be done in populations with different genetic background and aim at partitioning these complicated interactions through epistasis, QTL × environmental interaction, and pleiotropic effect. The objectives of the present study were to (1) identify additive QTLs linked to sugar-related traits in sorghum, (2) detect possible additive × additive interaction among these QTLs, and to (3) identify QTLs showing pleiotropic effect(s).

Materials and methods

Plant material

A population of 188 recombinant inbred lines derived from a cross between the inbreds SS79 (sweet sorghum) and Macia-SA denoted as M71 (grain sorghum), developed in the Agricultural Research Council of South Africa (ARC) by Dr. Willy Wenzel, was used for this study. The female parent SS79 was collected from a traditional farmer in Limpopo province of South Africa. It is approximately 300 cm tall with long internodes, has thin but sweet juicy stalks. It has open and loose panicles with light brown seeds. The male parent, M71, originated from ICRISAT-Bulawayo in Zimbabwe bred under a Sorghum and Millet improvement program. It is an early maturing and high grain yielding, with white grains. It has short internodes and is approximately 140 cm tall. The stems are juicy, but the juice is insipid. After the introduction of SS79 and M71 at the ARC, a single ear was selected from each variety and selfed until homozygosity was reached. The mapping population of our study was developed using conventional single seed descent method until $F_{5:6}$ generation (W. Wenzel, personal communication).

Experimental design and phenotypic evaluation

A total of four experiments were planted at 04 May 2007 and 14 May 2008 at two different locations: Gross Gerau (two field trials), and Rauischholzhausen (two pot trials). The characteristics of the locations are presented in Table 1. All experiments were planted in a randomized complete block design (RCBD) with three replications. At Gross Gerau (GG), a one-row plot of 5 m row length, and a three-row plot of 3 m row length were planted in 2007 and 2008, respectively. In both years, the inter-row space was 0.3 m, and seeds were sown at 0.03 m depth and at 0.15 m interval. In the pot experiment of Rauischholzhausen (RH), two seeds were sown per pot with a diameter of 0.20 m at 0.02 m depth. Best conditions for plant growth were implemented in both locations: In Gross Gerau, the plants were thinned to 100,000 plants per ha. Basal application of nitrogen (N) in the form urea was applied at a rate of 25 kgN/ha followed by irrigation.

Table 1 Biophysical information during field experiments in Gross Gerau (GG) and Rauischholzhausen (RH)

Location	Gross Gerau		Rauischholzhausen	
Year	2007	2008	2007	2008
Experiment no.	01	02	03	04
Averaged accumulative temperature (°C)[a]	85.7	84.6	82.0	81.9
Accumulative rainfall (mm)	311.1	445.9	275.6	481.2
Latitude	49°45′ N		50°45′ N	
Longitude	8°29′ E		8°52′ E	
Altitude (nn)	90.8		200.0	
Soil type	Sandy loam		Sandy loam	
Soil pH	6.8	6.4	5.2	5.0

Accumulative temperature and rainfall from planting to the last harvest (from May to end September)

[a] Averaged accumulated temperature was obtained by averaging the temperature per month and adding the averages

Regular monitoring of pest was done. Spider mites were identified and sprayed with proper insecticide in the RH 2009 pot experiment.

The trials were harvested manually by cutting the plant at the base by a scissor in both the two locations. In the two field experiments, three middle plants were selected at random for all data collection and later the average of the three plants was used. In the pot trials both plants per pot were harvested and averaged. Some lines were photoperiod sensitive, which resulted in differences in flowering dates of up to 2 months. To harvest the plots at the same physiological stage, each line was harvested at 40 days after anthesis (DAA), which estimated the hard dough stage (Zhao et al. 2009). At this stage, sugar accumulation has been reported to reach the optimum. Because the plants developed tillers at an early stage in both locations, the main stem and its tiller were treated as a single plant during harvest. Sampling was done in the following sequence: (1) Flowering date was recorded when 50% of the plot had flowered. (2) The length of the plant from the ground to the panicle tip was measured to estimate plant height. (3) Stem diameter was measured 20 cm above ground and the sum of tillers per plant was counted to estimate number of tillers per plant. (4) Panicles were cut at the flag leaf, weighed to estimate fresh panicle weight per plant. (5) The juice weight was estimated as the difference between fresh stripped stalk mass and dry stripped stalk mass. The fresh stripped stalks were milled and then dried in an oven at 105°C for dry mill. This difference gives a similar juice weight as that collected by roller miller in the same material (W. Wenzel, personal communication). (7) The fresh main stalk was pressed, and 2–3 droplets of juice were collected on a sucrose-sensitive refractometer to measure the Brix and another juice was squeezed to fill 2 ml tubes for future glucose and sucrose analysis.

Glucose and sucrose analysis

Carbohydrate quantification was carried only in the main stem. The samples were frozen at −20°C until the end of harvest. The three plants per replication were pooled to obtain an average sample extract. The pooled juice was diluted to 1:600 µl ml^{-1} ratios and then filtered. Analyses were carried out according to the manufacturer's recommendation using the enzymatic bioanalysis kit produced by Boehringer Mannheim/r-biopharm. 96-well microtitre plates were used for the analysis. Reagents were adjusted to make 200 µl total reaction volume. Measurements were taken at 340 nm wavelength using the Tecan, sunrise spectrophotometer (Tecan Trading AG, Switzerland). The final glucose and sucrose concentration was calculated based on the linear regression as reported by Henderson et al. (1998) and Viola and Davies (1992), and verified using the supplied manufacturer's formula. Sugar content was calculated according to Ritter et al. (2008). The concentration of fructose was replaced by concentration of glucose because fructose was not quantified in our study. Glucose and fructose can give similar content in sorghum (Godwin Ian, personal communication) and sugarcane (Robertson et al. 1996).

Phenotypic data analysis

Descriptive statistics and analysis of variance was done using SAS® 9.1 software. Proc GLM procedure was used to test differences among the lines in each and across environments. Broad-sense heritability on RILs-mean basis was estimated for all traits as reported by Littell et al. (2006) and Fehr (1987)

$$h_B^2 = \sigma_G^2/\left(\sigma_G^2 + \sigma_{GY}^2/y + \sigma_{GL}^2/l + \sigma_{GYL}^2/yl + \sigma^2 E/ryl\right)$$

where σ^2_G is genetic variance, σ^2_{GL} is the variance of genetic × location interaction, σ^2_{GY} is the variance of genetic × year interaction, σ^2_{GYL} is the variance of genetic × year × location interaction, $\sigma^2 E$ is the error variance, and r is the replication.

Coefficients of determination (R^2) were obtained from the linear regression of individual yield in different environments on the mean yield of all the genotypes in each environment (Leon and Becker 1988):

$$R^2 = 1 - s_d^2/s_x^2$$

where s_d^2 is the deviation mean squares from regression and S_x^2 is the environmental variance.

Genotyping

The RIL population was planted in small pots in the greenhouse at Giessen in three replications with two pots per replication. Fresh leaf samples were collected 20 days after planting from two replications and bulked. Tissues were placed in Eppendorf tubes, frozen immediately in liquid nitrogen, and then stored in −80°C freezer. DNA extraction was performed according to Doyle and Doyle (1990). A total of 200 SSR primer pairs reported by Bhattramakki et al. (2000), Dean et al. (1999), Kong et al. (2000), and Schloss et al. (2002) were used to screen parents of the mapping population. A few EST-SSRs published by Srinivas et al. (2008, 2009a) were also included. The reaction protocol and thermal cycler touchdown protocol were performed according to Hasan et al. (2006) and Xu et al. (2005). Most of the SSR primers were labeled with M13 tailing as described by Berg and Olaisen (1994). The fluorescently labeled universal M13 primer 5′-AGGGTTTTCCCAGTCACGACGTT-3′ was added to the PCR reaction, and the forward primer of each SSR has been appended with the sequence 5′-TTTCCCAGTCAC GACGTT-3′. After the first cycle of PCR, fragments were successively amplified with the labeled primer (Hasan et al. 2008; Rygulla et al. 2007).

AFLP markers were amplified according to Vos et al. (1995) using Gibco AFLP core reagents. A total of 150 ng (6 µl × 25 ng) genomic DNA was restricted using 2 µl EcoRI/MseI in 5 µl × 5× reaction buffer and 12 µl AFLP-grade water. The solution was incubated for 2 h in 37°C followed by 15 min in 70°C in Perkin Elmer 9700 thermal cycler. After incubation, a ligation master mix consisting of 24 µl adapter ligation solution and 1 µl T4 DNA ligase was added to the solution, which was again incubated for 2 h at 20°C. Ligation was followed by a pre-amplification step (+0) using the 5 µl of 1:10 diluted ligation with non-selective primers in order to minimize background noise at electrophoresis and to receive more distinct bands. The +0 sequence is complementary to the adapters and to the enzyme cutting-sequence. The following +1 pre-amplification was carried out using 5 µl of 1:10 dilutions of +0 pre-amplification prior to amplification (+3) using selective primer combinations. The sequence of the primers +0 EcoR1, +0 Mse1, +1 EcoR1, and +1 Mse1 were 5′-GAC TGC GTA CCA ATT C-3′, 5′GAT GAG TCC TGA GTA A-3′, 5′-GAC TGC GTA CCA ATT CA-3′, and 5′GAT GAG TCC TGA GTA AC-3, respectively, as reported by Uptmoor et al. (2003).

For electrophoresis, a total of 10 µl fusion dyes was added to the PCR products; the samples were denatured at 94°C for 5 min, and then 2 μ1 samples was loaded in 8% polyacrylamide gel. Amplification products were separated and visualized using the LI-COR model 4200 (MWG Biotech, Ebersberg, Germany) automated DNA sequencing system. This system can detect amplification products that were labeled with two different dyes: IRD-700 nm and IRD-800 nm.

Linkage map construction and QTL analysis

A total of 215 scored markers and 188 RILs were used to construct linkage maps using the Joinmap® 4.0 software. The tentative linkage groups were grouped using the LOD score of 3.0, and the Kosambi mapping function was used to calculate map distances in cM from the recombination frequencies. The linkage maps were compared to the sorghum maps published by Bhattramakki et al. (2000) and Mace et al. (2009), and assigned to chromosomes according to a recent sorghum chromosome nomenclature (Kim et al. 2005).

QTLs were detected from the combined environmental means using composite interval mapping with the linear regression approach implemented in software PLABQTL version 1.2 (Utz and Melchinger 1994). Cofactors were selected on basis of stepwise regression using Akaike's information criterion. A LOD score of 3.0 was used for detection of QTLs. A QTL was considered suggestive if it had a LOD score between 2.5 and 3.0, but had significantly influenced the phenotype in a certain environment. When two peaks were detected for a single trait on the same chromosome, they were considered as two QTLs only if the distance between the QTLs was greater than 20 cM, otherwise only the higher peak was considered for better estimation of the QTL position (Parth et al. 2008; Ungerer et al. 2002).

Digenic epistasis (*aa*) was estimated from the detected set of QTLs in a stepwise regression based on f-to-enter value. Only additive × additive epistasis was estimated because no reliable dominance effect is expected in the lines after F_5 lines of self pollinating crops. For QTL × environment analysis, the detected QTLs undergo a

simultaneous fit for each environment, and the results are presented in an ANOVA. The difference between the fits of the data from individual environments and the means across environments give the mean squares of QTL × environment. The significance of QTL × environment has been tested using an F test with Bonferroni adjustment.

Results

Trait performance

The phenotypic performance of parents and recombinant inbred lines are presented in Table 2. The parents performed as expected for sugar-related traits, where SS79 was taller than M71 in all locations. Besides, SS79 was late flowering, had high sugar content, and low fresh panicle weight. Trait means of the recombinant inbred lines were intermediate, although transgressive segregation was observed.

The effect of the genotypes was significant ($P > 0.0001$) for all the traits studied except for estimated juice weight (Table 3). Although the estimated juice weight showed segregation among the RILs, both parents are known to have juicy stems. Environments were significantly different in all traits and high genotype by environment interaction was observed in all traits except for estimated stem juice weight. Most of the lines were photoperiod sensitive resulting in differences in harvest dates; therefore, harvest dates were used to estimate the same physiological state of the lines.

The heritability of the traits ranged from 0.18 to 0.81 (Table 3). Estimated juice weight showed a lower heritability ($h^2_B = 0.18$), indicating the impact of the environment in this trait. The moderate–high heritability in these sugar-related traits shows that breeding for these traits is possible. Repeatability of the traits ranged from 0.39 to 0.97 (Table 3). As in heritability, estimated juice weight showed a lower repeatability ($R^2 = 0.39$). As repeatability is used to estimate predictable performance of the genotypes, genotypes showed moderate–high stable performance across environments.

Correlation estimates

Pearson correlation coefficients (r) of sugar-related traits are shown in Table 4. Flowering date correlated significantly with all the traits. The strongest positive correlation was found between flowering date and estimated stem juice ($r = 0.445$), followed by stem diameter ($r = 0.369$) and Brix ($r = 0.251$), and the closest negative correlation was observed between flowering date and fresh panicle weight ($r = -0.626$). Fresh panicle weight has negatively and significantly correlated with all traits, except for a positive, non-significant correlation to number of tillers and a moderate significant positive correlation to sugar content. Number of tillers was only significantly but weakly correlated to estimated juice content ($r = 0.195$).

A relatively close positive correlation was found between Brix and sucrose ($r = 0.606$), even though a higher correlation estimate was expected. Glucose correlated negatively and significantly to sucrose ($r = -0.334$) but positively to Brix ($r = 0.122$). The estimated sugar content correlated with all traits except estimated juice weight. The strongest but correlation was observed between sugar content and sucrose ($r = 0.988$); estimated correlations to glucose ($r = 0.556$), Brix ($r = 0.386$), and stem diameter ($r = 0.320$) were moderate.

Table 2 Mean values, standard deviations, and range for sugar components and sugar-related agronomic traits in recombinant inbred lines across environments

Trait	Parental lines		Recombinant inbred lines (RILs)			
	SS79	M71	Mean	Standard deviation	Minimum	Maximum
Flowering date	112.00	95.00	105.85	6.78	91.69	142.73
Plant height (cm)	238.27	118.23	152.61	38.89	69.25	251.63
Stem diameter (cm)	1.66	1.76	1.77	0.16	1.33	2.26
Number of tillers per plant	3.00	2.00	2.23	0.35	1.22	5.40
Fresh panicle weight (g/plant)	43.96	105.43	57.97	19.00	2.57	124.53
Estimated juice weight (g/plant)	488.19	192.35	296.8	93.28	148.63	623.08
Brix	16.98	10.71	14.89	1.59	10.02	17.93
Glucose content (g/L)	12.38	5.49	7.88	0.99	5.02	19.97
Sucrose content (g/L)	112.82	48.89	115.92	15.83	42.87	156.12
Sugar content (g/L)	134.40	59.15	131.68	16.49	44.39	176.87

Table 3 Mean squares, heritabilities, repeatabilities, and coefficient of variation for sugar components and sugar-related agronomic traits in recombinant inbred lines across environments

Trait	Mean squares						
	Genotype	Environment	G × E	Harvest date	CV	h_B^2	R^2
Flowering dates	***	***	***	***	2.81	0.33	0.97
Plant height (cm)	***	***	***	NS	21.31	0.81	0.84
Stem diameter (cm)	***	***	***	NS	11.19	0.60	0.88
Number of tillers per plant	***	***	***	NS	26.44	0.68	0.75
Fresh panicle weight (g/plant)	***	***	***	NS	37.37	0.69	0.81
Estimated juice weight (g/plant)	NS	***	NS	NS	261.67	0.18	0.39
Brix	***	***	***	NS	14.39	0.59	0.72
Glucose content (g/L)	***	***	***	NS	29.34	0.47	0.82
Sucrose content (g/L)	***	***	***	NS	28.82	0.52	0.65
Sugar content (g/L)	***	***	***	NS	24.97	0.50	0.71

G genotype; *E* environment; *CV* coefficient of variation; h_B^2 broad-sense heritability on an entry-mean basis; R^2 repeatability

*** Significant at 0.001 probability level; *NS* non-significant level at $P < 0.05$

Table 4 Pearson's correlation coefficient of sugar-related traits

	FD	PH	Diam.	PW	Tillers	Juice	Brix	Glucose	Sucrose
PH	0.183**	1							
Diam.	0.369***	−0.277***	1						
PW	−0.626***	−0.128	−0.141*	1					
Tillers	−0.148*	−0.003	−0.111	0.024	1				
Juice	0.445***	0.741***	0.016	−0.239***	0.195***	1			
Brix	0.251***	0.626***	−0.056	−0.290***	0.080	0.636***	1		
Glucose	0.147*	0.088	−0.173*	−0.133***	0.051	0.239***	0.122**	1	
Sucrose	0.152*	0.427***	−0.051	−0.141***	0.046	0.363***	0.606***	−0.334***	1
Sugar	−0.142**	0.214***	0.320***	0.256***	−0.052*	0.011	0.386***	0.556***	0.988***

FD flowering date; *PH* plant height; *Diam.* stem diameter; *PW* fresh panicle weight; *Tillers* number of tillers per plant; *Juice* estimated juice weight; *Glucose* glucose content; *Sucrose* sucrose content; *Sugar* estimated sugar content

* Significant at 0.05 probability leve1

** Significant at 0.01 probability leve1

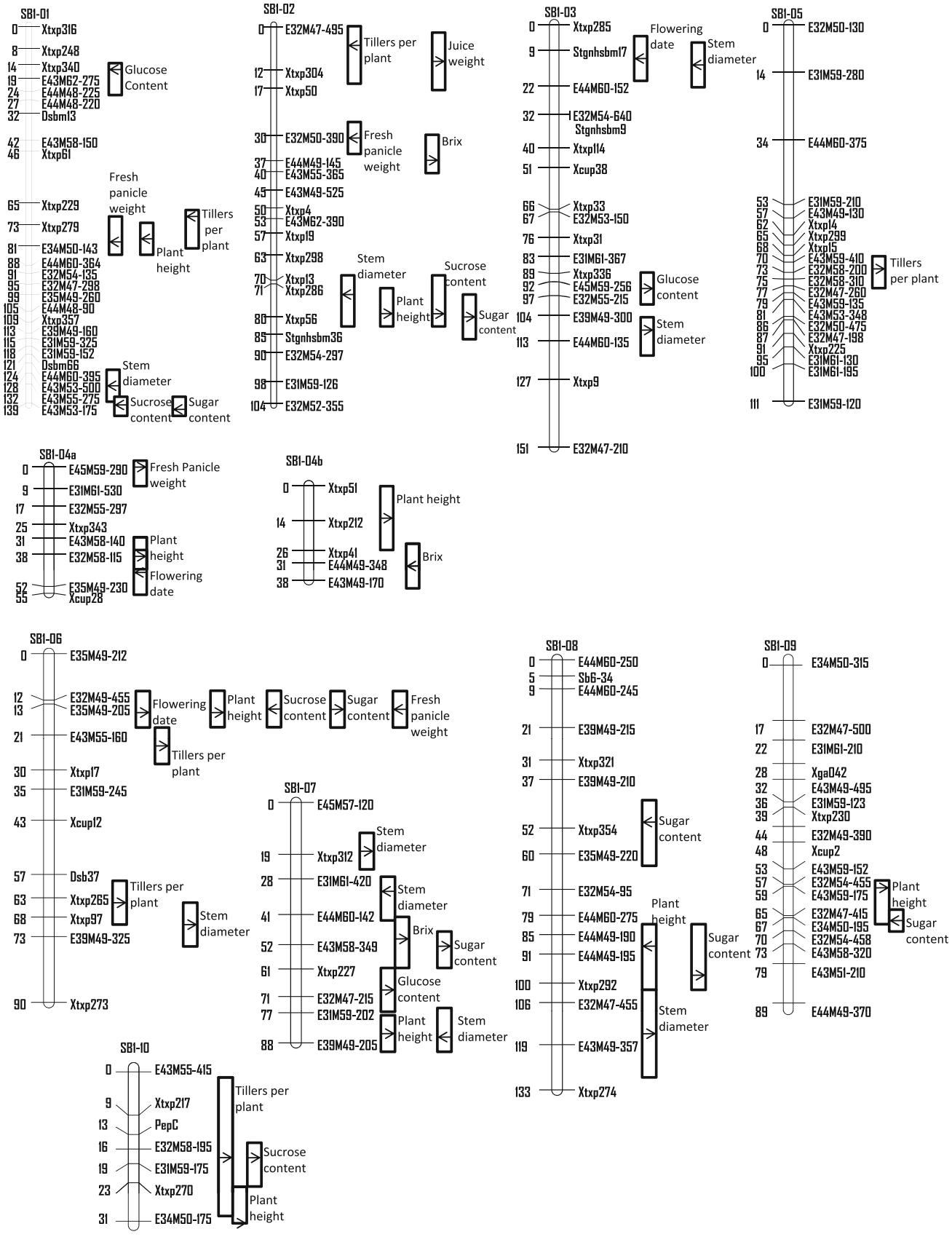
*** Significant at the 0.001 probability leve1

Linkage map construction and QTL detection

The genetic map was constructed from 188 lines and consisted of 102 AFLP, 49 SSR, and 6 EST-SSR markers, where 59 markers were excluded because of missing values or divergence from the expected segregation ratio using a chi-square test with one degree of freedom at $\alpha = 0.05$. Eleven linkage groups were formed with an average distance of 6.55 cM between markers. The longest distance between markers was 20 cM and the shortest between markers was 1 cM.

Figure 1 presents the results of the identified significant QTLs either in each environment or from the mean of the combined environments or both. Supplementary Table S1 shows the details of the identified additive QTLs. The QTLs that significantly affected the traits using combined environmental means or mean of each single environment are reported here as significant. Environments 01, 02, 03,

Fig. 1 Genetic linkage map of the M71 × SS79 RILs consisting of 102 AFLP, 49 SSR, and 6 EST-SSR. Markers starting with *E* are AFLPs; *X* and *Sb* are SSRs; *Stg* and *Dsb* are EST-SSRs. All QTL shown were detected by composite interval mapping (CIM), using the combined environments (four) for each trait, and also found significant in one or many environments, respectively. The *top* and *bottom borders* of the QTL *boxes* represent support intervals of the detected QTLs associated with the respective trait on the *right side* of the *box*. The peak of each QTL is shown by the position of the *arrow* inside the *box*, and the direction indicates the allele contribution by parent M71 (*pointing left*) or by parent SS79 (*pointing right*)


SBI-01
0 Xtxp316
8 Xtxp248
14 Xtxp340
19 E43M62-275
24 E44M48-225
27 E44M48-220
32 Dsbm13
42 E43M58-150
46 Xtxp61
65 Xtxp229
73 Xtxp279
81 E34M50-143
88 E44M60-364
91 E32M54-135
95 E32M47-298
99 E35M49-260
105 E44M48-90
109 Xtxp357
113 E39M49-160
115 E31M59-325
118 E31M59-152
121 Dsbm86
124 E44M60-395
128 E43M53-500
132 E43M55-275
139 E43M53-175
Glucose Content
Fresh panicle weight
Plant height
Tillers per plant
Stem diameter
Sucrose content
Sugar content
SBI-02
0 E32M47-495
12 Xtxp304
17 Xtxp50
30 E32M50-390
37 E44M49-145
40 E43M55-385
45 E43M49-525
50 Xtxp4
53 E43M62-390
57 Xtxp19
63 Xtxp298
70 Xtxp13
71 Xtxp286
80 Xtxp56
85 Stgnhsbm36
90 E32M54-297
98 E31M59-126
104 E32M52-355
Tillers per plant
Juice weight
Fresh panicle weight
Brix
Stem diameter
Plant height
Sucrose content
Sugar content
SBI-03
0 Xtxp285
9 Stgnhsbm17
22 E44M60-152
32 E32M54-640
Stgnhsbm9
40 Xtxp114
51 Xcup38
66 Xtxp33
67 E32M53-150
76 Xtxp31
83 E31M61-367
89 Xtxp336
92 E45M59-256
97 E32M55-215
104 E39M49-300
113 E44M60-135
127 Xtxp9
151 E32M47-210
Flowering date
Stem diameter
Glucose content
Stem diameter
SBI-05
0 E32M50-130
14 E31M59-280
34 E44M60-375
53 E31M59-210
57 E43M49-130
62 Xtxp14
65 Xtxp299
68 Xtxp15
70 E43M59-410
73 E32M58-200
75 E32M58-310
77 E32M47-260
79 E43M59-135
81 E43M53-348
86 E32M50-475
87 E32M47-198
91 Xtxp225
95 E31M61-130
100 E31M61-195
111 E31M59-120
Tillers per plant
SBI-04a
0 E45M59-290
9 E31M61-530
17 E32M55-297
25 Xtxp343
31 E43M58-140
38 E32M58-115
52 E35M49-230
55 Xcup28
Fresh Panicle weight
Plant height
Flowering date
SBI-04b
0 Xtxp51
14 Xtxp212
26 Xtxp41
31 E44M49-348
38 E43M49-170
Plant height
Brix
SBI-06
0 E35M49-212
12 E32M49-455
13 E35M49-205
21 E43M55-160
30 Xtxp17
35 E31M59-245
43 Xcup12
57 Dsb37
63 Xtxp265
68 Xtxp97
73 E39M49-325
90 Xtxp273
Flowering date
Plant height
Sucrose content
Sugar content
Fresh panicle weight
Tillers per plant
Tillers per plant
Stem diameter
SBI-07
0 E45M57-120
19 Xtxp312
28 E31M61-420
41 E44M60-142
52 E43M58-349
61 Xtxp227
71 E32M47-215
77 E31M59-202
88 E39M49-205
Stem diameter
Stem diameter
Brix
Sugar content
Glucose content
Plant height
Stem diameter
SBI-08
0 E44M60-250
5 Sb6-34
9 E44M60-245
21 E39M49-215
31 Xtxp321
37 E39M49-210
52 Xtxp354
60 E35M49-220
71 E32M54-95
79 E44M60-275
85 E44M49-190
91 E44M49-195
100 Xtxp292
106 E32M47-455
119 E43M49-357
133 Xtxp274
Sugar content
Plant height
Sugar content
Stem diameter
SBI-09
0 E34M50-315
17 E32M47-500
22 E31M61-210
28 Xga042
32 E43M49-495
36 E31M59-123
39 Xtxp230
44 E32M49-390
48 Xcup2
53 E43M59-152
57 E32M54-455
59 E43M59-175
65 E32M47-415
67 E34M50-195
70 E32M54-458
73 E43M58-320
79 E43M51-210
89 E44M49-370
Plant height
Sugar content
SBI-10
0 E43M55-415
9 Xtxp217
13 PepC
16 E32M58-195
19 E31M59-175
23 Xtxp270
31 E34M50-175
Tillers per plant
Sucrose content
Plant height

and 04 throughout the chapter represent the field experiments as presented in Table 1.

Flowering date

CIM detected four QTLs and one putative QTL on SBI-03, SBI-04a, SBI-06, SBI-07, and SBI-08 associated with flowering dates (LOD score $\geq$2.5). The QTLs on SBI-03, SBI-04a, and SBI-06 were found significant in various environments. The phenotypic variance explained by these significant QTLs was 33.4%, and their additive effects were 3.90, 0.62 and −2.72 days on SBI-03, SBI-04a, and SBI-06, respectively.

Plant height

Seventeen QTLs were detected in nine chromosomes associated with plant height (LOD score $\geq$3.0). Nine significant QTLs were identified on SBI-01, SBI-02, SBI-04a, SBI-4b, SBI-06, SBI-07, SBI-08, SBI-09, and SBI-10. The phenotypic variation explained by the detected QTLs ranged from 7.5 to 26.1%, whereas the additive effects ranged from −18.79 to −0.20 cm for SS79 and 3.05–9.48 cm for M71 parent. Most of the QTLs (61%) had negative additive effects suggesting that the parent SS79 had a higher contribution of tall alleles.

Stem diameter

Thirteen QTLs and one putative QTL influencing stem diameter were distributed over eight chromosomes. Nine out of 13 QTLs were significant across the environments. The QTL on SBI-03 at position 102 cM seems to be the major QTL because it was significant in three environments and explaining 13.3% of phenotypic variation. The additive effect of this QTL was −0.042 cm suggesting that the QTL allele derived from the female parent. Besides, the QTL on SBI-07 at position 30 cM also seem to be a major QTL because of its high additive effect (0.069 cm), high LOD score of 5.03 and because it explains 11.6% of the phenotypic variation.

Number of tillers per plant

Six significant QTLs were detected (LOD $\geq$ 2.5) that were associated with number of tillers per plant. A QTL on SBI-06 at position 64 was found significant in all the environments and was detected at LOD score of 2.90 using the overall mean of the data set. In total, 14 QTLs were detected on nine chromosomes explaining a phenotypic variation ranging from 6.7 to 15.7% and their additive effect ranging from −0.09 to 0.092 number per plant. Only three of the detected QTLs had positive additive effects indicating that the increase in tiller numbers was contributed by SS79.

Fresh panicle weight

At a LOD score of 5.62, a major significant QTL associated with fresh panicle weight was located on SBI-06. This QTL explained 12.9% of phenotypic variation and showed additive effect of 4.87 g/plant. Besides, four other significant QTLs were detected at a LOD score $\geq$2.5 on SBI-01, SBI-02, and SBI-04a. Together, they explained 20.3% of the phenotypic variation.

Stem juice weight

For stem juice weight, one QTL was detected on SBI-02 between 12 and 26 cM with LOD score of 3.54. This QTL explained 7.9% of the phenotypic variation and had additive effect of −17.603 g/plants. The negative additive effect shows that the allele was derived from SS79. Stem juiciness was not segregating well in the population because both parents are juicy, and the trait is also significantly controlled by the environment.

Brix

Four out of 14 detected QTLs significantly influenced the total soluble solids in sorghum stem juice. These significant QTLs were distributed on three chromosomes where chromosome 4 had two of these significant QTLs. Two major QTLs were identified on SBI-02 and SBI-06 at position 82 and 16, respectively. These QTLs explained 21.9 and 22.3% of the total phenotypic variation, respectively. Besides, they had the LOD score $\geq$10.00 and the additive effects of −0.167 and −0.797, respectively. The negative signs of both alleles show that these alleles were derived from SS79, the parent with high sugar.

Glucose content

A total of ten QTLs and one suggestive QTL were detected for glucose content on seven chromosomes. Out of these 11 QTLs, four QTLs were found significant in various environments. The QTL detected at position 70 of SBI-07 seems to be the major QTL. This QTL had LOD score of 5.99 explaining 13.7% phenotypic variation with additive effect of −0.351 g/l. This QTL was significant in the combined analysis and in two of the four environments. Its negative additive effect shows the contribution of the female parent in glucose content. In general, the detected QTLs showed that both parents contributed alleles for increasing and decreasing glucose content.

Sucrose content

For sucrose content, seven QTLs and two putative QTLs were pin-pointed on seven chromosomes, where four QTLs were found to be significant. These significant QTLs explained 42% of the phenotypic variation where a QTL on SBI-06 explained the highest phenotypic variation (10.6%) compared to the others. Both negative and positive additive effects were observed showing the positive alleles for sucrose content of both parents.

Sugar content

A total of 15 QTLs and two putative QTLs was detected that controls sugar content in stem juice on seven different chromosomes. Out of these 17 QTLs, nine QTLs were found significant in different environments. All detected QTLs explained from 6.6 to 12.5% phenotypic variation. The additive effects of the QTLs were either negative or positive showing that the QTLs derived from both parents.

Epistatic effects and QTL × environment interaction

The digenic epistatic effects are presented in Table 5. Twenty-four significant digenic epistatic effects (*aa*) were detected for seven traits using combined analysis. This suggests that two locus interactions were widespread in the entire genome: a total of five digenic QTLs were found regarding fresh panicle weight, four for plant height and glucose content, three for number of tillers, sucrose and sugar content, and two for flowering date. There were no significant additive × additive interactions observed for estimated juice weight, brix, and stem diameter.

Epistasis between the loci affecting both sucrose and sugar content was found between SBI-01 and SBI-4b, and SBI-01 and SBI-06. The total phenotypic variation explained by digenic interactions was as follows: 8.7% for flowering dates (2 pairs), 21.4% for plant height (4 pairs), 25.4% for fresh panicle weight (5 pairs), 18.7% for number of tillers (3 pairs), 21.7% for glucose content (4 pairs), 19.1% for sucrose content (3 pairs), and 17.8% for sugar content (3 pairs). In general, the total phenotypic variation explained by such interactions was smaller than the corresponding main effects. Nevertheless, the importance of additive × additive interaction effects in total genetic effects may well be trait-dependent.

In this study, interactions between QTL and environments were analyzed for all traits (Supplementary Table S1). Significant QTL × environmental interactions were observed for sugar content on SBI-01, fresh panicle weight on SBI-01 and SBI-06, tiller number per plant on SBI-07 and SBI-10, and flowering date on SBI-06. No significant QTL × environment interactions were observed for the other traits studied.

Pleiotropic effects and co-localization

To identify a linkage group as a hotspot, we considered QTL clusters that have at least one QTL explaining more than 5% of the phenotypic variance with both paternal and maternal effects as reported by Rae et al. (2009) in poplar. QTLs for all traits co-localized in all linkage groups except for linkage groups 3 and 5 (Supplementary Table S1; Fig. 1). Hotspots can be declared on SBI-01, SBI-02, SBI-06, SBI-07, and SB1-09 because all traits co-localized on those linkage groups and more than one QTL has been found per trait.

Several QTLs that influence multiple traits were observed in this study (Supplementary Table S1; Fig. 1). A major QTL with multiple effects was detected in linkage group 6 with a support interval 12–18 cM. This QTL has been detected for all traits except for glucose and number of tillers per plant, and was significant for five out of six traits. The additive effects of this QTL differed per trait where the female parent contributed positive allele for flowering date, plant height, total soluble solids, and sucrose, but the male parent contributed positive alleles regarding fresh panicle weight and estimate of sugar content.

Other QTLs with pleiotropic effect have been located on various linkage groups such as *E44M48-140* on SBI-04a, *E35M49-212* on SBI-07, *Xtxp51* on SBI-04a and *Stgnhsbm36* on SBI-02. Most of the QTLs for sucrose were also detected either for Brix, or total sugar content or both.

Discussion

The growing importance of bioenergy-derived sugar crops such as sorghum and sugarcane is likely to create local as well as global environmental and economical challenges of fossil fuel usage. Similar to sugarcane as reported by Lingle (1997), our sorghum lines also differed in sucrose content. However, the accumulation mechanisms during growth in our materials are widely unknown, whereas genotypic differences of sucrose enrichment have been described in sugarcane (Lingle 1997). As sugar accumulation and content are quantitative traits controlled by polygenes and significantly affected by the environment, suitable strategies and success of breeding can be based on partitioning the agronomic traits and sugar components into various sub-traits as, e.g., has been done in the case of drought tolerance (Kebede et al. 2001; Hausmann et al. 2002). In the current study, agronomic traits related to sugar content such as flowering date, plant height, number

Table 5 Digenic epistatic loci associated with sugar-related traits

Traits	Chromosome	Left marker	Chromosome	Left marker	Additive effect[a]	Intercept	R^2 (%)[b]
Flowering dates	SBI-4a	*E32M58-115*	SBI-07	*Xtxp227*	−1.437	105.36	3.0
	SBI-4a	*E32M58-115*	SBI-08	*Xtxp354*	−0.023		5.7
Plant height (cm)	SBI-01	*E44M60-364*	SBI-06	*Xtxp17*	11.197	149.44	6.3
	SBI-01	*E44M60-364*	SBI-09	*E32M54-458*	8.855		5.1
	SBI-01	*Xtxp279*	SBI-05	*E31M61-130*	8.255		5.3
	SBI-01	*E44M48-225*	SBI-02	*Stgnhsbm36*	−8.263		4.7
Stem diameter (cm)	No significant interaction found						
Number of tillers	SBI-01	*E43M48-150*	SBI-02	*Xtxp304*	−0.113	2.21	7.5
	SBI-03	*E44M60-152*	SBI-06	*Xtxp265*	−0.098		5.7
	SBI-04a	*E45M59-290*	SBI-06	*E43M55-160*	0.09		5.5
Fresh panicle weight (g/plant)	SBI-01	*Xtxp279*	SBI-09	*E43M49-495*	4.768	59.89	5.5
	SBI-02	*E44M49-145*	SBI-07	*Xtxp227*	4.291		5.8
	SBI-05	*E32M47-198*	SBI-07	*Xtxp227*	−3.564		3.9
	SBI-06	*E35M49-205*	SBI-07	*E45M57-120*	4.493		5.5
	SBI-06	*E35M49-205*	SBI-08	*E39M49-210*	−4.985		4.7
Estimated juice weight (g/plant)	No significant interaction found						
Brix	No significant interaction found						
Glucose content (g/L)	SBI-01	*E43M62-275*	SBI-07	*Xtxp227*	−0.192	7.95	4.9
	SBI-03	*E31M61-367*	SBI-05	*E32M50-130*	−0.241		6.9
	SBI-03	*E44M60-135*	SBI-05	*E43M53-348*	−0.229		5.4
	SBI-07	*E45M57-120*	SBI-09	*E43M51-210*	0.207		4.5
Sucrose content (g/L)	SBI-01	*E39M49-160*	SBI-4b	*Xtxp51*	−4.531	114.85	6.1
	SBI-01	*Xtxp279*	SBI-06	*E35M49-205*	−6.12		6.2
	SBI-06	*E35M49-205*	SBI-07	*E44M60-142*	5.441		6.8
Sugar content (g/L)	SBI-01	*E39M49-160*	SBI-4b	*Xtxp51*	−4.52	130.15	5.5
	SBI-01	*Xtxp279*	SBI-06	*E35M49-205*	−6.016		6.7
	SBI-02	*Xtxp286*	SBI-07	*E43M58-349*	−4.382		5.6

[a] Negative additive effects indicate that the SS79 allele increases the value of the trait

[b] Partial phenotypic variance explained (percentage)

of tillers, stem diameter, fresh panicle weight and stem juice, and sugar components such as glucose, sucrose, brix, and total sugar content were investigated.

To date, there are only a few studies on the identification of QTL for sugar-related traits in *Sorghum bicolor*. Our own results support some of the results reported this far. For example, several QTLs associated with flowering date were detected on linkage groups (LGs) SBI-01, SBI-04, SBI-06, and SBI-10 (Ritter et al. 2008), SBI-06, SBI-09 (Murray et al. 2008), and SBI-01, SBI-03, SBI-06, and SBI-08 (Feltus et al. 2006). Besides, Chantereau et al. (2004) found few QTLs controlling photoperiod response on LGs C, F, and H that correspond to SBI-01, SBI-02, and SBI-10 in our study. In addition, Pereira and Lee (1995) detected eight QTLs associated with plant height by interval mapping and single factor analysis on four different linkage groups, which correspond to our LGs SBI-06, SBI-07, SBI-09, and SBI-10. Supporting our study, their QTLs accounted for 9.2–28.7% phenotypic variation. In 168 F_7 RILs of sorghum, Srinivas et al. (2009b) located only one major QTL on SBI-06 associated with fresh panicle weight, explaining 14.7% of the phenotypic variation having a LOD score of 4.9. Just like in our study, Murray et al. (2008) and Ritter et al. (2008) in two independent studies used different populations derived from crosses between a grain and a sweet sorghum type, respectively. Using 184 recombinant inbred lines (RILs) Ritter et al. (2008) reported 5 QTLs associated with Brix, 2 QTLs with glucose content, 11 QTLs with sucrose content and 10 QTLs associated with estimated sugar content. Their QTLs clustered on LGs SBI-01, SBI-05, SBI-06, and SBI-07. We have located two significant QTLs associated with estimated sugar content on chromosome 6, while Ritter et al. (2008) detected four QTLs on the same chromosome. Murray et al. (2008) used 176 RILs and reported QTLs associated with Brix on SBI-03, SBI-06 and SBI-07,

and QTLs associated with glucose content on SBI-02, SBI-05, and SBI-09.

Six maturity genes are known to alter flowering time and period in sorghum: Ma_1, Ma_2, Ma_3, Ma_4, Ma_5, and Ma_6 (Aydin et al. 1997; Quinby 1967). The first four genes cause long days to inhibit flowering but allow early flowering under short days, while the last two can inhibit floral initiation regardless of day length. Collinearity between rice chromosomes 7 and sorghum chromosome 2 (in our study) has been observed by Paterson et al. (1995, 2005). In rice, chromosome 7 has been reported to carry photoperiod sensitive genes *Se-2* and *El* (Laurie 1997; Yano et al. 1997). Unfortunately, we have not found a QTL on chromosome 2; chromosome 3 is shown to harbor significant QTL associated with flowering date in three environments and also to the overall mean.

Plant height of sorghum was reported by Quinby and Karper (1954) to be controlled by four independent genes: *Dw1*, *Dw2*, *Dw3*, and *Dw4*. The dwarf gene *Dw2* has a pleiotropic effect on grain yield and panicle length (Graham and Lessmann 1966), and *Dw3* exerts pleiotropic effect on the number of tillers and panicle size (Casady 1965). The QTLs for plant height detected in the present study also co-localized with QTLs and had a pleiotropic effect on other morphological and sugar traits (SBI-01, SBI-02, SBI-04, SBI-06, and SBI-07). Congruently, Pereira and Lee (1995), and Rami et al. (1998) observed co-localization of QTL for plant height and stem diameter with other morphological attributes and concluded that these QTLs can be *Dw* loci. Although the markers reported in the latter two studies are different from ours to verify, this hypothesis is a possible explanation for the results of our study.

Several alignments between sugarcane and sorghum chromosomes has been done in recent years (Dufour et al. 1997; Guimarães et al. 1997; Ming et al. 1998, 2001), which is important to determine collinearity or orthology of sugar-related QTLs. Al-Janabi et al. (1994) suggested that sorghum and sugarcane diverted approximately five million years ago. The map created by Ming et al. (2002) showed that six of the 13 homologous groups identified in the *Saccharum–Sorghum* consensus map are closely corresponding while the remaining showed a varying level of synteny. Jordan et al. (2004) reported that stalk number and suckering (underground branching or tillering) influence sugar yield in sugarcane. They aligned the maps of sugarcane generated by Grivet et al. (1996) with those of sorghum generated by Paterson et al. (1995) and Boivin et al. (1999). Using the sugarcane RFLP probes, they identified QTLs for rhizome number, rhizome distance, regrowth, and seedling tillers on linkage groups SBI-01, SBI-02, SBI-03, SBI-06, and SBI-07 based on the chromosome nomenclature of sorghum by Kim et al. (2005). The biggest cluster of QTLs was found on chromosome 1. In the current study, QTLs associated with number of tillers were detected on all chromosomes except chromosome 8.

Ming et al. (2001) detected 36 sugar content QTLs in sugarcane using two interspecific segregating populations derived from *S. officinarum* and *S. spontaneum*. Sugar content was defined as sucrose at 96% purity, calculated based on Brix and Pol values. Through alignment, the detected QTLs corresponded to eight regions on linkage groups A, B, C, D, F, G, and I of the sorghum genome. By searching for linked markers in http://www.gramene.org and also using the maps of Ming et al. (1998), these linkage groups seem to correspond to LGs SBI-01, SBI-02, SBI-03, SBI-04, SBI-06, SBI-09, and SBI-10. Although in our study the QTLs for brix, sucrose, and sugar content were located on these linkage groups, the exact positions of the QTLs cannot be aligned for comparative analysis. However, as Ming et al. (2001) concluded, a much smaller number of ancestral genes may account for the observed QTLs between the two species. These original genes are assumed to have been multiplied by the duplication of chromosomes that characterized sugarcane genome evolution since its divergence from a common ancestor shared with sorghum.

In our study additive × additive interaction was found in seven out of ten characters studied. There were no significant additive × additive interactions observed for estimated juice weight, brix, and stem diameter. Epistasis is difficult to detect due to most of the experimental variation being assigned to main effects (Purcell and Sham 2004) and epistasis in both negative and positive direction is important for the breeding populations even if the net effects are not observed (Routman and Cheverud 1997). According to Whitlock et al. (1995) detecting epistasis has limitations due to genotype × environmental interactions and linkage disequilibrium. Regarding flowering date, one significant additive × additive interaction between SBI-04 and SBI-08 could be detected with the effect of −1.44 together explaining 3.0% of the phenotypic variance. Ritter et al. (2008) depicted an additive × additive effect of 0.98 between SBI-04 and SBI-08. These interactions delineated in both studies favor the late flowering parents.

Uncertainty in terminology regarding epistasis often arises due to the differences in definitions and types of epistasis being reported. The epistatic interaction effect was not consistent in our study. For example, digenic pairs for plant height on linkage groups SBI-01 and SBI-09 had a negative effect separately but together produced a positive effect in sucrose content; the interaction between SBI-01 and SBI-4b showed a strong negative effect while they showed small effects with different signs separately. The simplest epistatic interactions are duplicate and complementary interactions. Although it is well known that

biosynthetic pathways of sugar traits involve series of genes, no conclusions can be made as yet on the type of epistasis reported.

Analysis of epistasis is often carried out in conjunction with QTL mapping, but epistatic effects may be confounded with background genome segregation and QTL × environmental interactions (Holland 2001). In our study, we analyzed QTL × environmental interaction among the detected QTLs. One QTL has been observed for sugar content and flowering date, and two QTLs were found for fresh panicle weight and number of tillers per plant. It is obvious that QTL that interact with the environment or even stable QTL across environments are more desired for marker-assisted selection.

Sugar-related traits in sweet sorghum are mainly influenced by additive genes as in the present study more than five additive QTLs were identified per trait using composite interval mapping. The co-localization and pleiotropic effect of the QTL is also validated by the reported correlation estimates among these traits. QTLs showing multiple effects were located, while the major one was found at 14 cM on SBI-06. This QTL favored the parental allele(s) responsible for increasing the characteristic value in almost all traits except for estimated sugar content. Such a situation would facilitate the combination of favorable alleles in single genotypes. Our study proves that besides major QTLs, breeders should also consider minor QTLs, additive × additive QTLs, and QTL × environmental interactions to improve sugar-related characteristics in *S. bicolor*. These results are considered to have significant implications for biomass and bioethanol improvement programmes in sorghum and other candidate crops such as maize and sugarcane.

Acknowledgments The authors would like to thank Dr. Willy Wenzel, Potchefstroom (South Africa), for providing the RIL population his generous advice, helpful stimulation and continuous support. Excellent technical assistance of Mario Tolksdorf, Markus Kollmer and Malte Luh is kindly acknowledged. This work has been primarily supported by Deutscher Akademischer Austauschdienst (DAAD), Bonn, Agricultural Research Council (ARC) and National Research Foundation (NRF) of South Africa.

References

Aitken KS, Jackson PA, McItyre CL (2006) Quantitative trait loci identified for sugar related traits in a sugarcane (*Saccharum* spp.) cultivar × *Saccharum officinarum* population. Theor Appl Genet 112:1306–1317

Aitken KS, Hermann S, Karno K, Bonnett GD, McIntyre LC, Jackson PA (2008) Genetic control of yield related stalk traits in sugarcane. Theor Appl Genet 117:1191–1203

Al-Janabi S, McClelland M, Petersen C, Sobral B (1994) Phylogenetic analysis of organellar DNA sequences in the Andropogoneae: Saccharinae. Theor Appl Genet 88:933–944

Assar HA, Uptmoor R, Abdelmula AA, Salih M, Ordon F, Friedt W (2005) Genetic variation in sorghum germplasm from Sudan, ICRISAT, and USA assessed by simple sequence repeats (SSRs). Crop Sci 45:1636–1644

Aydin S, Rooney WL, Miller FR (1997) Identification and characterization of the Ma5 and Ma6 maturity loci in Sorghum. In: Proceeding of the international conference on genetic improvement of sorghum and pearl millet, 22–27 Sep 1996, Lubbock, USA, INTSORMIL and ICRISAT, pp 641–642

Berg ES, Olaisen B (1994) Hybrid PCR sequencing-sequencing of PCR products using a universal primer. Biotechniques 17:896–901

Bhattramakki D, Dong J, Chhabra A, Hart G (2000) An integrated SSR and RFLP linkage map of *Sorghum bicolor* (L.) Moench. Genome 43:988–1002

Boivin K, Deu M, Rami J-F, Trouche G, Hamon P (1999) Towards a saturated sorghum map using RFLP and AFLP markers. Theor Appl Genet 98:320–328

Casady AJ (1965) Effect of a single height (*Dw*) gene of sorghum on grain yield, grain yield components, and test weight. Crop Sci 5:385–389

Chantereau J, Trouche G, Rami J-F, Deu M, Barro C, Grivet L (2004) RFLP mapping of QTLs for photoperiod response in tropical sorghum. Euphytica 120:183–194

Dean RE, Dahlberg JA, Hopkins MS, Mutchell SE, Kresovich S (1999) Genetic redundancy and diversity among 'Orange' accessions in the U.S. National sorghum collection assessed with simple sequence repeats (SSR) markers. Crop Sci 39:1215–1221

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus 12:13–15

Dufour P, Deu M, Grivet L, D'Hont A, Paulet F, Bouet A, Lanaud C, Glaszmann JC, Hamon P (1997) Construction of a composite sorghum genome map and comparison with sugarcane, a related complex polyploid. Theor Appl Genet 94:409–418

Fehr WR (1987) Principles of cultivar development. MacMillan, New York

Feltus FA, Hart SE, Schertz KF, Casa AM, Kresovich S, Abraham S, Klein PE, Brown PJ, Paterson AH (2006) Alignment of genetic maps and QTLs between inter- and intra-specific sorghum populations. Theor Appl Genet 112:1295–1305

Graham D, Lessmann KJ (1966) Effect of height on yield and yield components of two isogenic lines of *Sorghum vulgare* Pers. Crop Sci 6:372–374

Grivet L, D'Hont A, Roques D, Feldmann P, Lanaud C, Glaszmann JC (1996) RFLP mapping in cultivated sugarcane (*Saccharum* spp.): genome organization in a highly polyploidy and aneuploid interspecific hybrid. Genetics 142:987–1000

Guimarães CT, Sills GR, Sobral WS (1997) Comparative mapping of A*ndropogoneae*: *Saccharum* L. (sugarcane) and its relation to sorghum and maize. Proc Natl Acad Sci 94:14261–14266

Hasan M, Seyis F, Badani AG, Pons-Kühnemann J, Friedt W, Lühs W, Snowdon RJ (2006) Analysis of genetic diversity in the *Brassica napus* L. gene pool using SSR markers. Genet Resour Crop Evol 53:793–802

Hasan M, Friedt W, Pons-Kühnemann J, Freitag NM, Link K, Snowdon RJ (2008) Association of gene-linked SSR markers to seed glucosinolate content in oilseed rape (*Brassica napus* ssp. napus). Theor Appl Genet 116:1035–1049

Hausmann BIG, Mahalakshini V, Reddy BVS, Seetharama N, Hash CT, Geiger HH (2002) QTL mapping of stay-green in two sorghum recombinant inbred populations. Theor Appl Genet 106:133–142

Henderson SK, Fenn CA, Domijan JD (1998) Determination of sugar content in commercial beverages by density. A novel experiment for general chemistry courses. J Chem Educ 75:1122–1123

Holland JB (2001) Epistasis and plant breeding. Plant Breed Rev 21:27–92

Jordan DR, Casu RE, Besse P, Carroll BC, Berding N, McIntyre CL (2004) Markers associated with stalk number and suckering in sugarcane collocate with tillering and rhizomateousness QTLs. Genome 47:988–993

Kebede H, Subudhi PK, Rosenow DT, Nguyen HT (2001) Quantitative trait loci influencing drought tolerance in grain sorghum (*Sorghum bicolor* L. Moench). Theor Appl Genet 103:266–276

Kim J-S, Klein PE, Klein RR, Price HJ, Mullet JE, Stelly DM (2005) Chromosome identification and nomenclature of *Sorghum bicolor*. Genetics 169:1169–1173

Kong L, Dong J, Hart GE (2000) Characteristics, linkage-map positions, and allelic differentiation of *Sorghum bicolor* (L.) Moench DNA simple sequence repeats (SSRs). Theor Appl Genet 101:438–448

Laurie DA (1997) Comparative genetics of flowering time. Plant Mol Biol 35:167–177

Leon J, Becker HC (1988) Repeatability of some statistical measures of phenotypic stability—correlation between single year results and multi years results. Plant Breed 100:137–142

Lingle SE (1997) Seasonal internode development and sugar metabolism in sugarcane. Crop Sci 37:1222–1227

Littell RC, Milliken GA, Stroup WW, Wolfinger RD, Schabenberger O (2006) SAS® for mixed models, 2nd edn. SAS Institute Inc., Cary, NC

Mace ES, Rami J-F, Bouchet S, Klein PE, Klein RR, Kilian A, Wenzl P, Xia L, Halloran K, Jordan DR (2009) A consensus genetic map of sorghum that integrates multiple component maps and high-throughput diversity array technology (DarT) markers. BMC Plant Biol 9:13. doi:10.1186/1471-2229-9-13

Milligan SB, Gravois KA, Vischoff KP, Martin FA (1990) Crop effects on genetic relationships among sugarcane. Crop Sci 30:927–931

Ming R, Liu S-C, Lin Y-R, da Silva J, Wilson W, Braga D, van Deynze A, Wenslaff TF, Wu KK, Moore PH, Burnquist W, Sorrels ME, Irvine JE, Paterson AH (1998) Detailed alignment of *Saccharum* and *Sorghum* chromosomes: comparative organization of closely-related diploid and polyploid genomes. Genetics 150:1663–1682

Ming R, Liu SC, Moore PH, Irvine JE, Paterson AH (2001) QTL analysis in a complex autopolyploid: genetic control of sugar content in sugarcane. Genome Res 11:2075–2084

Ming R, Wang Y-W, Draye X, Moore PH, Irvine JE, Paterson AH (2002) Molecular dissection of complex traits in autopolyploids: mapping QTLs affecting sugar yield and related traits in sugarcane. Theor Appl Genet 105:332–345

Murray SC, Sharma A, Rooney WL, Klein PE, Mullet JE, Mitchel SE, Kresovich S (2008) Genetic improvement of sorghum as biofuel feedstock I: QTL for stem sugar and grain nonstructural carbohydrates. Crop Sci 48:2165–2179

Natoli A, Gorni C, Chegdani F, Ajmone Marsan P, Colombi C, Lorenzoni C, Marocco A (2002) Identification of QTLs associated with sweet sorghum quality. Maydica 47:311–322

Parth DK, Jordan DR, Aitken EAB, Mace ES, Jun-ai P, McIntyre CL, Godwin ID (2008) QTL analysis of ergot resistance in sorghum. Theor Appl Genet 117:369–382

Paterson AH, Lin Y-R, Li Z, Schertz KF, Doebley JF, Pinson SRM, Liu S-C, Stansel JW, Irvine JE (1995) Convergent domestication of cereal crops by independent mutations at corresponding genetic loci. Science 296:1714–1718

Paterson AH, Freeling M, Sasaki T (2005) Grains of knowledge: genomics of model cereals. Genome Res 15:1643–1650

Pereira MG, Lee M (1995) Identification of genomic regions affecting plant height in sorghum and maize. Theor Appl Genet 90:380–388

Purcell S, Sham PC (2004) Epistasis in quantitative trait locus linkage analysis: interaction or main effects? Behav Genet 34:143–152

Quinby JR (1967) The maturity genes of sorghum. In: Norman A (ed) Advances in agronomy XIX. Academic Press, New York, pp 267–305

Quinby JR, Karper RE (1954) Inheritance of height in sorghum. Agron J 46:211–216

Rae AM, Street NR, Robinson KM, Harris N, Taylor G (2009) Five QTL hotspots for yield in short rotation coppice bioenergy poplar: the poplar biomass loci. BMC Plant Biol 9:23. doi: 10.1186/1471-2229-9-23

Rami J-F, Dufour P, Trouche G, Fliedel G, Mestres C, Davrieux F, Blancard P, Hamon P (1998) Quantitative trait loci for grain quality, productivity, morphological, and agronomical traits in sorghum (*Sorghum bicolor* L. Moench). Theor Appl Genet 97:605–616

Ritter KB, Jordan DR, Chapman SC, Godwin ID, Mace ES, McIntyre CL (2008) Identification of QTL for sugar-related traits in sweet × grain sorghum (*Sorghum bicolor* L. Moench) recombinant inbred population. Mol Breed 22:367–384

Robertson MJ, Muchow RC, Wood AW, Campbell JA (1996) Accumulation of reducing sugars by sugarcane: effects of crop age, nitrogen supply and cultivar. Field Crop Res 49:39–50

Routman EJ, Cheverud JM (1997) Gene effects on a quantitative trait: two locus epistatic effects measured at microsatellite markers and at estimated QTL. Evolution 51:1654–1662

Rygulla W, Snowdon RJ, Eynck C, Koopmann B, van Tiedemann A, Lühs W, Friedt W (2007) Broadening the genetic basis of *Verticillium longisporum* resistance in *Brassica napus* by interspecific hybridisation. Phytopathology 97:1391–1396

Schloss SJ, Mitchell SE, White GM, Kukatla R, Bowers JE, Paterson AH, Kresovich S (2002) Characterization of RFLP probe sequences for gene discovery and SSR development in *Sorghum bicolor* (L.) Moench. Theor Appl Genet 105:912–920

Srinivas G, Satish K, Mohali SM, Reddy RN, Madhusudhana R, Balakrishna D, Venkatesh BB, Howarth CJ, Seetharama N (2008) Development of genic-microsatellite markers for sorghum staygreen QTL using comparative genomic approach with rice. Theor Appl Genet 117:703–717

Srinivas G, Satish K, Madhusudhana R, Seetharama N (2009a) Exploration and mapping of microsatellite markers from substracted drought stress ESTs in *Sorghum bicolor* (L.) Moench. Theor Appl Genet 118:703–717

Srinivas G, Satish K, Madhusudhana R, Reddy RN, Mohan SM, Seetharama N (2009b) Identification of quantitative trait loci for agronomicaly important traits and their association with genic-microsatelites markers in sorghum. Theor Appl Genet 118:1439–1454

Ungerer MC, Halldorsdottir SS, Modliszekwski JL, Mackay TFC, Purugganan MD (2002) Quantitative trait loci for inflorescence development in *Arabidopsis thaliana*. Genetics 160:1133–1152

Uptmoor R, Wenzel WG, Friedt W, Donalson G, Ayisi K, Ordon F (2003) Comparative analysis on the genetic relatedness of *Sorghum bicolor* accessions from southern Africa by RAPDs, AFLPs and SSRs. Theor Appl Genet 106:1316–1325

Utz HF, Melchinger AE (1994) Comparison of different approaches to interval mapping of quantitative trait loci. In: van Ooijen JW, Jansen J (eds) Biometrics in plant breeding: applications of molecular markers. Wageningen, The Netherlands, pp 195–204

Viola K, Davies HV (1992) A microplate reader assay for rapid enzymatic quantification of sugars in potato tubers. Potato Res 35:55–58

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 21:4407–4414

Whitlock MC, Phillips PC, Moore BG, Tonsor SJ (1995) Multiple fitness peaks and epistasis. Ann Rev Ecol Syst 26:601–629

Xu XY, Bai GH, Carver BF, Shaner GE (2005) Mapping of QTLs prolonging the latent period of *Puccinia triticina* infection in wheat. Theor Appl Genet 110:244–251

Yano M, Harushima Y, Nagamura Y, Kurata N, Minobe Y, Sasaki T (1997) Identification of quantitative trait loci controlling heading date in rice using a high-density linkage map. Theor Appl Genet 95:1025–1032

Yun-Long B, Seiji Y, Maiko I, Hong-Wei C (2006) QTLs for sugar content of stalk and sweet sorghum (*Sorghum bicolor* L. Moench). Agric Sci China 5:736–744

Zhao YL, Dolat A, Steinberger Y, Wang X, Osman A, Xie GH (2009) Biomass yield and changes in chemical composition of sweet sorghum cultivars grown for biofuel. Field Crops Res 111:55–64