

Identification of QTL for sugar-related traits in a sweet × grain sorghum (*Sorghum bicolor* L. Moench) recombinant inbred population

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Abstract QTL for stem sugar-related and other agronomic traits were identified in a converted sweet (R9188) × grain (R9403463-2-1) sorghum population. QTL analyses were conducted using phenotypic data for 11 traits measured in two field experiments and a genetic map comprising 228 SSR and AFLP markers grouped into 16 linkage groups, of which 11 could be assigned to the 10 sorghum chromosomes (SBI-01 to SBI-10). QTL were identified for all traits and were generally co-located to five locations (SBI-01, SBI-03, SBI-05, SBI-06 and SBI-10). QTL alleles from R9188 were detected for increased sucrose content and sugar content on SBI-01, SBI-05 and SBI-06. R9188 also contributed QTL alleles for increased Brix on SBI-05 and SBI-06, and increased sugar content on SBI-03. QTL alleles from

R9403463-2-1 were found for increased sucrose content and sucrose yield on SBI-10, and increased glucose content on SBI-07. QTL alleles for increased height, later flowering and greater total dry matter yield were located on SBI-01 of R9403463-2-1, and SBI-06 of R9188. QTL alleles for increased grain yield from both R9403463-2-1 and R9188 were found on SBI-03. As an increase in stem sugars is an important objective in sweet sorghum breeding, the QTL identified in this study could be further investigated for use in marker-assisted selection of sweet sorghum.

Keywords Sweet sorghum · Sugar · AFLP · SSR · QTL

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Introduction

Particular varieties of *Sorghum bicolor* (L.) Moench, known as ‘sweet sorghums’ or ‘sorgos’, accumulate 10–25% sugar in expressed stalk juice near the time of grain maturity. Glucose and fructose are the predominant reducing sugars in the juice, and sucrose is the predominant disaccharide (Hunter and Anderson 1997). Sweet sorghums are generally characterised by wide adaptability, drought tolerance, waterlogging tolerance, saline-alkali tolerance, rapid growth and high biomass (Reddy and Reddy 2003). There is a long history of sweet sorghum cultivation with large

areas grown in China, USA, Russia, Mexico, India and Japan (Cuiyan 1998). The stalk is processed and used to produce sorghum syrup, molasses and sugar (Hunter and Anderson 1997), or can be used as forage, silage and hay for feeding animals (Cuiyan 1998). Sweet sorghum is also considered a potentially valuable source for biofuel production (Reddy et al. 2005). Thus, increasing stem sugar yields is becoming an important objective in sweet sorghum breeding.

QTL for several sugar-related traits have been previously identified in sweet sorghum. In a cross between two unrelated sweet sorghum lines, Natoli et al. (2002) generated 129 F₂ progeny lines. Using this population, they gathered phenotypic data for the sugar-related traits of sugar percentage, sucrose percentage and sugar yield, and constructed a linkage map using 144 AFLP and SSR markers. Significant QTL explaining up to 18.8% of the phenotypic variation were identified for sucrose percentage and sugar yield on chromosomes SBI-02, SBI-03 and SBI-05 (using the chromosome nomenclature of Kim et al. 2004). Yun-long et al. (2006) also created a F₂ population with 207 lines from a cross between an inbred high sugar sorghum line and an inbred grain sorghum line. The population was phenotyped for Brix and a genetic linkage map was constructed from 31 RFLP, 254 AFLP and 42 SSR markers. In this case, QTL explaining up to 25% of phenotypic variance were identified on chromosomes SBI-04 and SBI-10 (using the chromosome nomenclature of Kim et al. 2004).

Obtaining a higher sugar yield is also a major focus of sugarcane (*Saccharum* spp.) improvement programs. However, progress is hindered by the genetically complex genome of sugarcane: it is an interspecific hybrid between *S. officinarum* L. ($2n = 80$) and *S. spontaneum* L. ($2n = 40–128$), that is both polyploid ($\sim 10–12x$) and aneuploid ($\sim 100–130$ chromosomes) (Al-Janabi et al. 1994a). Among the cultivated grasses, the closest relative of sugarcane is sorghum (*Sorghum* spp.), a diploid species ($2n = 2x = 20$) (Al-Janabi et al. 1994b).

Comparative mapping between sugarcane and sorghum using RFLPs has revealed a high level of synteny (Dufour et al. 1996, 1997; Grivet et al. 1994; Guimaraes et al. 1997; Ming et al. 1998, 2002a). Furthermore, Ming et al. (2002b) aligned sugarcane linkage groups from two different sugarcane populations with a sorghum map to assist in the evaluation

of QTLs affecting sugar-related traits from different sugarcane maps. Using this approach, 62 sugarcane QTLs for these traits could be inferred to map to nine sorghum chromosomes.

The objective of this study was to identify QTL for 'sugar-related' and other agronomic traits in a large recombinant inbred sweet \times grain sorghum population and to compare the QTL with those obtained by Natoli et al. (2002) and Yun-long et al. (2006). To more directly link the sweet sorghum QTL identified in this study to sugarcane QTL, an attempt was made to map sugarcane SSRs and identify QTL for similar traits in sorghum and sugarcane.

Materials and methods

Plant material

The sorghum inbred lines R9188 and R9403463-2-1 were used by the Queensland Department of Primary Industries and Fisheries (DPI&F) as parents to develop a F₆ recombinant inbred line (RIL) population with 184 progeny lines. R9188 is a dwarf conversion of the sweet sorghum line Rio (Tao et al. 1993) and R9403463-2-1 is a DPI&F elite grain sorghum male parent or restorer line (R-line).

Field trials

Two field trials were conducted in the 2003/2004 summer at the University of Queensland Redland Bay Field Station ($27^{\circ}37' S$, $153^{\circ}19' E$, altitude 5 m). Field trial one (RB1) was sown on 14 October 2003 and harvested on 2 February 2004, and field trial two (RB2) was sown on 25 November 2003 and harvested on 22 March 2004. A randomised block design was used with eight replications of the two parental lines and two replications of all progeny lines in each trial. Both trials consisted of 6 m² plots (2 rows at 75 cm spacing by 4 m long), with 12 columns (4 m wide) and 70 rows (35 plots with two rows each), and surrounded by two border rows. The trials were over-sown, and at 14–20 days after sowing, were thinned to nine plants m⁻². The trials were regularly irrigated to prevent development of drought stress. Pesticides, insecticides and fungicides were applied at the prescribed rates to control weeds, sorghum midge (*Contarinia sorghicola*), heliothis (*Helicoverpa*

armigera), rust (*Puccinia purpurea*) and ergot (*Claviceps sorghi*).

Flowering time (FT) after planting was recorded when 50% of plants in the plot had 50% flowering (which occurs when anthers have burst on more than 50% of the panicle). After flowering, height was measured from the ground to the top of the panicle for each plot.

At 6 weeks after anthesis, a 2 m × 2 row section was removed from each plot and the main stems and tillers were counted and a fresh weight (FW) recorded for each. A proportional subsample of plants and tillers was chosen to make a total subsample size of 20 stems. The subsample was partitioned into panicles, leaves and stems and the FW for each portion was recorded. All panicles were bagged for drying and grain yield determination. A second subsample of leaves was selected, bagged and FW recorded before drying, and the remaining leaves discarded. All the stems were milled in a small, single-stem, motor-operated sugar press. The FW of all milled stems was recorded before a subsample of milled stems were selected, bagged and FW recorded before drying, and the remaining milled stems discarded. The percentage of soluble solids in solution (Brix) was measured from a sample of the extracted juice by a hand-held refractometer (Atago, Japan). A second juice sample was immediately frozen in liquid nitrogen for later sucrose, glucose and fructose analysis by High Pressure Liquid Chromatography (HPLC). Data on the dry weights of plant parts were calculated on a per m⁻² basis to estimate biomass: total dry matter = (DW main stems + DW tillers)/harvest area; and, grain yield = total DW plants m⁻² × DW grain ratio.

HPLC

Analysis of sugars used the methodology and equipment described by Albertson and Grof (2007), with the exception that sucrose, glucose and fructose were measured from a sample of the juice extract that had been diluted approximately 1,000 times with a mechanical diluter (Hamilton digital diluter; Hamilton Company, Nevada, USA) then passed through a 0.2 µm filter (Filterpur S, Sarstedt, Nümbrecht, Germany). From these sugar measurements, sugar content, sucrose content, glucose content, fructose content, sugar yield and sucrose to sugar ratio could be calculated: sugar content = % sugar present in the

juice sample; sucrose content = % sucrose present in the juice sample; glucose content = % glucose present in the juice sample; fructose content = % fructose present in the juice sample; sugar yield = FW stems m⁻² × sugar content; sucrose yield = FW stems m⁻² × sucrose content; and, sucrose to sugar ratio = ratio of sucrose content to sugar content.

Data analysis

To adjust for any spatial variation field trends, RB1 and RB2 were individually analysed using a model in the form discussed by Cullis and Gleeson (1991). Data from both trials were then pooled and analysed together, with trials assumed to have fixed effects in a mixed model analysis. The random effects for the across-trial model can be written in a general form: trait = trial + corgh(trial):genotype + (trial-specific extraneous variation + trial-specific natural variation). The corgh(trial):genotype term in the model is fitted as a random term that accounts for a genetic variance for each trial and the genetic correlation between the two trials. By fitting this term and accounting for the correlation between trials, information from both trials could be most efficiently combined to estimate a BLUP (best linear unbiased predictor) average for each genotype. Trial-specific natural variation included fitting of spatial autoregressive models to account for field trends for each trait, while factors fitted for trial-specific extraneous variation included design factors (replicate, row and column) where needed.

The genetic variance from each site was given in the model by fitting the corgh(site):genotype term as a random effect. The broad sense heritability (H²) for each site can be calculated by 1-avsd²/(2*gen.var). The avsd (average standard error of difference) for each site was calculated from the standard errors for each of the BLUPs (best linear unbiased predictions) that were produced as a result of fitting the site:genotype term as a random effect. All computations were performed using the statistical package S-Plus and models were fitted using the SAMM procedure (Butler et al. 2003).

DNA isolation

Leaves were harvested from 4-week-old field-grown plants, snap frozen in liquid nitrogen and lyophilised

in a freeze drier. Genomic DNA was extracted using a CTAB method (Hoisington 1992). The DNA concentration was determined by fluorometry with a Fluoroskan Ascent FL fluorometer (Labsystems, Minnesota).

Generation of AFLP markers

AFLP analysis was carried out according to Vos et al. (1995). All PCR reactions were performed on a Gene Amp PCR System 9700 (PE Applied Biosystems, California). The PCR products were mixed with an equal amount of loading dye, denatured at 95°C for 10 min and 3.5 µl was run on a 5% denaturing polyacrylamide gel with 1×TBE buffer at 100 W for 2–2.5 h after a pre-run at 160 W for 30 min. The gels were dried in a gel dryer (BioRad, California) for 40 min at 80°C and exposed to Kodak X-Omat X-ray film for 4 days. A total of 38 AFLP selective primer combinations were used in this study. Segregating polymorphisms were scored using A (absent parent 1 and present parent 2) and B (absent parent 2 and present parent 1).

Microsatellite markers

Sorghum SSR primers identified by Texas A&M University (Bhatramakki et al. 2000; Kong et al. 2000) were screened across the parental lines and 42 *Xtxp* primers were chosen to give good genome coverage (2–4 SSRs per chromosome). All 70 sugarcane SSR primers for loci mapping in sugarcane by Aitken et al. (2005) were also screened over the parental lines and sugarcane primer pairs detecting polymorphism were identified and run across the entire sorghum RIL population. PCR reactions were carried out in a total of 20 µl containing 25 ng of DNA as previously described for sorghum (Tao et al. 1998) and sugarcane (Aitken et al. 2005). Segregating polymorphisms were scored using A or B to denote presence of the parent 1 or 2 fragments respectively, and H for the presence of both fragments.

Map construction

Linkage analysis was conducted using MultiPoint software (www.multiqtl.com). In preliminary data

analysis, markers with highly distorted segregation ($\chi^2 > 16$) were deleted. The RIL selfing population setting was selected and a maximum threshold r_f value of 0.35 was used to initially group the markers into clusters. Multipoint linkage analysis of loci within each cluster was then performed and marker order was further verified through re-sampling for quality control via jack-knifing (Mester et al. 2003). Markers that could be ordered with a jack-knife value of 90% or greater were included as ‘framework’ markers, with any remaining markers causing unstable neighborhoods being initially excluded from the map. Following a repeated multipoint linkage analysis with the reduced set of markers for each cluster to achieve a stabilised neighbourhood, the previously excluded markers were attached by assigning them to the best intervals on the framework map. Within these ordered individual clusters, the SSR locations were compared to previously published sorghum maps (Bhatramakki et al. 2000; Menz et al. 2002) in order to allow the clusters to be assigned to sorghum chromosomes, SBI-01 to SBI-10 according to recent nomenclature (Kim et al. 2004). Clusters belonging to the same chromosome were merged and the software RECORD (Van Os et al. 2005) used to determine the best marker order within each chromosome. The software package MapChart (Voorrips 2002) was used to graphically represent the LGs in this map.

QTL analyses

QTL analyses were performed on the adjusted trait values from RB1 and RB2 using the Windows QTL Cartographer Version 2.0 software package (Wang et al. 2004). The traits processed were: (i) sugar-related (sucrose content, glucose content, fructose content, sugar content, sucrose yield, sucrose to sugar ratio and Brix), and (ii) agronomic (height, flowering time, total dry matter and grain yield). To identify marker-trait associations, and the potential position of a QTL, single marker analysis (SMA) was used and all possible associations were noted for $P > 0.05$. To confirm the location of these QTL, composite interval mapping (CIM) was undertaken with all default settings maintained. QTLNetwork-2.0 (Yang et al. 2005), using the default settings, was used to identify epistasis between markers.

Results

Analysis of phenotypic data

The seasonal conditions were normal for this site with a mean daily temperature of 24.5°C and mean daily short-wave solar radiation of 23 MJ m⁻². Prior to the mean flowering date of the progeny, the mean temperature of trial RB1 was 22.1°C, compared to 24.7°C in RB2. Mean FT of the RIL progeny set was one day earlier in RB2 (Table 1), although wet sowing conditions resulted in a slight delay in emergence of RB2 (2–3 days later than RB1), so time from emergence to flowering was shorter in RB2. Conditions during the flowering to harvest period (mean of 25.8°C across trials) were cooler in RB2 and by more than 2°C in the first and last weeks of this period (data not shown). These lower temperatures and overcast conditions resulted in slower ripening of grain in RB2, such that the period from flowering to harvest was longer in RB2 (48 days) than RB1 (41 days).

The main difference between the trials was the higher sucrose content and sucrose yield that could have resulted from a longer grain-filling period, allowing extra sucrose to accumulate in RB2. In both trials, the population showed a significantly wider range of variation than that of the parents for glucose content, fructose content, total dry matter and grain yield (Table 1), indicating that transgressive segregation occurred. In both trials, broad-sense heritabilities (H^2) were high (Table 1), indicating the presence of genetic variation for the traits and good control of within-trial environmental variation and experimental error. Genetic correlations (r_g) between the two trials for each individual trait were also high indicating that crossover genotype by environment interaction was relatively low for the traits analysed.

All sugar-related traits (sucrose content, glucose content, fructose content, sugar content, sucrose to sugar ratio and sucrose yield and Brix), were highly correlated ($P < 0.001$) with each other, based on BLUPs fitted across both trials (Table 2). Plant height was significantly correlated with sucrose content, sugar content, glucose content, fructose content, sucrose yield and Brix. Grain yield was negatively correlated with sucrose content, glucose content, fructose content, sugar content and Brix. FT and total dry matter were significantly correlated with sucrose

yield. Total dry matter was also highly correlated with grain yield.

Map construction

Using MultiPoint, the 247 polymorphic bands produced by 38 AFLP primer pairs, 42 *Xtxp* microsatellite and 10 sugarcane microsatellite markers were investigated for segregation distortion and 19 markers were removed. The remaining 228 markers were distributed on 16 LG (Fig. 1) of which 11 could be assigned a chromosomal location based on the positioning of the *Xtxp* SSRs in Menz et al. (2002). The remaining five LG could not be assigned to any specific LG because they contained only AFLP markers. Ten markers remained unlinked, including three AFLP markers, three *Xtxp* SSR markers and four sugarcane SSR markers. The LG ranged in length from 1.7 cM with two markers (LG-U3) to 314.4 cM with 31 markers (chromosome SBI-03). SBI-10 was the most densely populated chromosome with 22 markers over 159.9 cM. The map is 1879.2 cM in length excluding the unassigned LGs and 2012.9 cM including the unassigned LGs. On average there is one marker every 8.94 cM (including the unassigned LGs).

QTL analyses

The MultiPoint map was used in the QTL analyses to identify markers associated with the sugar-related (Table 3) and agronomic traits (Table 4). Sucrose content variation was associated with at least five genomic regions (SBI-01, SBI-05 (at least two regions), SBI-06 and SBI-10). In at least four regions (SBI-01, SBI-05 (at least two regions) and SBI-06) favourable alleles were derived from the sweet sorghum parental line R9188 in both RB1 and RB2, whilst in SBI-10 the favourable allele was contributed by R9403463-2-1, but this was only detected in RB2. Higher glucose content, in both RB1 and RB2, was associated with SBI-07 alleles from R9403463-2-1, while higher fructose content was associated with SBI-06 alleles from R9188 and SBI-07 alleles from R9403463-2-1 that co-located with those controlling glucose content. Sugar content variation was associated with five genomic regions (SBI-01, SBI-05 (at least two regions), SBI-06 and SBI-10) and for all five regions favourable alleles were contributed by

Table 1 Phenotypic trait data for the 11 traits investigated in two field trials (RB1 and RB2)

Trait	Units		RB1						RB2						Genetic correlation (rg) between sites
	RB1		F6 RIL progenies		Parental lines		Genotypic H ² variance		F6 RIL progenies		Parental lines		Genotypic H ² variance		
	Mean	Range	Mean	Range	R9188	R9403463- 2-1	Mean	Range	Mean	Range	R9188	R9403463- 2-1	Mean	Range	
Sucrose content	%		6.50	3.67–9.53	8.50	2.60	3.35	0.84	7.60	4.64–10.67	8.50	3.60	2.93	0.80	0.70***
Glucose content	%		0.54	0.20–0.88	0.60	0.59	0.02	0.78	0.66	0.31–1.0	0.77	0.69	0.02	0.80	0.84***
Fructose content	%		0.38	0.15–0.61	0.49	0.39	0.01	0.72	0.45	0.17–0.73	0.56	0.43	0.01	0.80	0.80***
Sugar content	%		7.50	4.23–10.88	9.50	3.50	3.76	0.83	8.70	5.76–11.76	9.80	4.70	3.17	0.82	0.73***
Sucrose yield	g/m ²		59.00	25.16–93.52	65.00	23.00	663.91	0.88	109.00	56.41–162.59	99.00	44.00	1931.77	0.90	0.84***
Sucrose to sugar ratio			0.87	0.80–0.95	0.89	0.73	0.00	0.79	0.86	0.77–0.96	0.86	0.73	0.00	0.69	0.65***
Brix	%		10.40	7.42–12.59	11.60	5.40	2.82	0.86	11.60	8.93–14.40	12.60	7.80	2.63	0.82	0.70***
Height	cm		129.00	114.36–146.06	112.00	111.00	408.78	0.95	138.00	120.33–156.74	116.00	123.00	427.17	0.95	0.97***
Flowering time	days		70.00	64.95–74.42	68.00	74.00	13.44	0.91	69.00	66.20–71.69	66.00	74.00	15.00	0.94	0.94***
Total dry matter	g/m ²		992.00	727.86–1256.78	850.00	1088.00	19930.77	0.80	1071.00	773.38–1369.21	937.00	1140.00	19318.72	0.78	0.83***
Grain yield	g		425.00	277.93–568.83	372.00	507.00	4095.40	0.75	438.00	275.79–599.76	368.00	539.00	10024.99	0.83	0.78***

Significance levels are indicated by *, ** and *** for $P < 0.05$, 0.01 and 0.001

Table 2 Genetic correlations based on BLUPs fitted across both trials

Trait	Total dry matter	Grain yield	Brix	Days to flowering	Height	Sucrose content	Sugar content	Sucrose to sugar ratio	Sucrose yield	Glucose content
Grain yield	0.679***									
Brix	0.071	−0.286***								
Flowering time	0.401***	0.049	−0.005							
Height	0.355***	−0.137	0.533***	0.164*						
Sucrose content	0.144*	−0.215**	0.946***	0.039	0.571***					
Sugar content	0.144*	−0.250***	0.950***	0.055	0.597***	0.987***				
Sucrose to sugar ratio	0.095	0.149*	0.500***	−0.157*	0.114	0.582***	0.490***			
Sucrose yield	0.464***	−0.121	0.728***	0.328***	0.726***	0.792***	0.812***	0.306***		
Glucose content	−0.033	−0.448***	0.370***	0.139	0.385***	0.275***	0.387***	−0.541***	0.400***	
Fructose content	−0.052	−0.416***	0.387***	0.040	0.407***	0.302***	0.409***	−0.510***	0.383***	0.957***

Correlations greater than 0.5 are bold. Significance levels are indicated by *, ** and *** for $P < 0.05$, 0.01 and 0.001

R9188. Higher sucrose yield was associated with three genomic regions (SBI-05, SBI-06 and SBI-10) in both RB1 and RB2, with two genomic regions from R9188 (SBI-05 and SBI-06), and one from R9403463-2-1 (SBI-10). Higher sucrose to sugar ratio was associated with one marker on SBI-07 in RB1, contributed by R9188, as well as two other markers on SBI-08 (one each from RB1 and RB2), contributed by R9403463-2-1. The final sugar-related trait evaluated was Brix. Regions on SBI-05 and SBI-06 were identified as associated with high Brix content, in both RB1 and RB2, and all regions came from R9188.

The remaining traits scored on the population were agronomic traits (Table 4), including height, FT, total dry matter and grain yield. Height was associated with four genomic regions in both RB1 and RB2 with two regions derived from R9188 (SBI-05 and SBI-06), and two regions (SBI-01 and SBI-03) from R9403463-2-1. For FT, four genomic regions were identified (SBI-01, SBI-04, SBI-06 and SBI-10), with two regions derived from R9188 (SBI-01 and SBI-10) and two from R9403463-2-1 (SBI-04 and SBI-06). Three of the four regions (SBI-04, SBI-06 and SBI-10) were identified in both trials. Total dry

matter was associated with three genomic regions (SBI-01, SBI-06 and SBI-10). The regions on SBI-01 and SBI-10 were contributed by R9403463-2-1. The two separate regions on SBI-06 were each contributed by one parent. The final agronomic trait analysed was grain yield, which was associated with three genomic regions (SBI-02, SBI-03 and SBI-10). The genomic region identified on SBI-02, and two markers identified on SBI-03 from RB1, were from R9188, whilst the genomic region identified on SBI-10, and the marker identified on SBI-03 from RB2, were from R9403463-2-1.

Using QTLNetwork-2.0, epistasis was identified between markers for the sugar-related and agronomic traits (Tables 5 and 6). Epistasis between the same regions on SBI-03 and SBI-05 affected sucrose content and sugar content variation, whilst epistasis between SBI-03 and SBI-08 affected sucrose yield variation. Multiple regions were involved in epistatic interactions which affected sucrose to sugar ratio (SBI-02 and SBI-08, and two regions on SBI-03 and SBI-09), Brix (two regions on SBI-03 and SBI-05, and SBI-03 and LG-U1), fructose content (two regions on SBI-01 and SBI-03, and SBI-01 and SBI-06), and glucose content (two regions on SBI-01

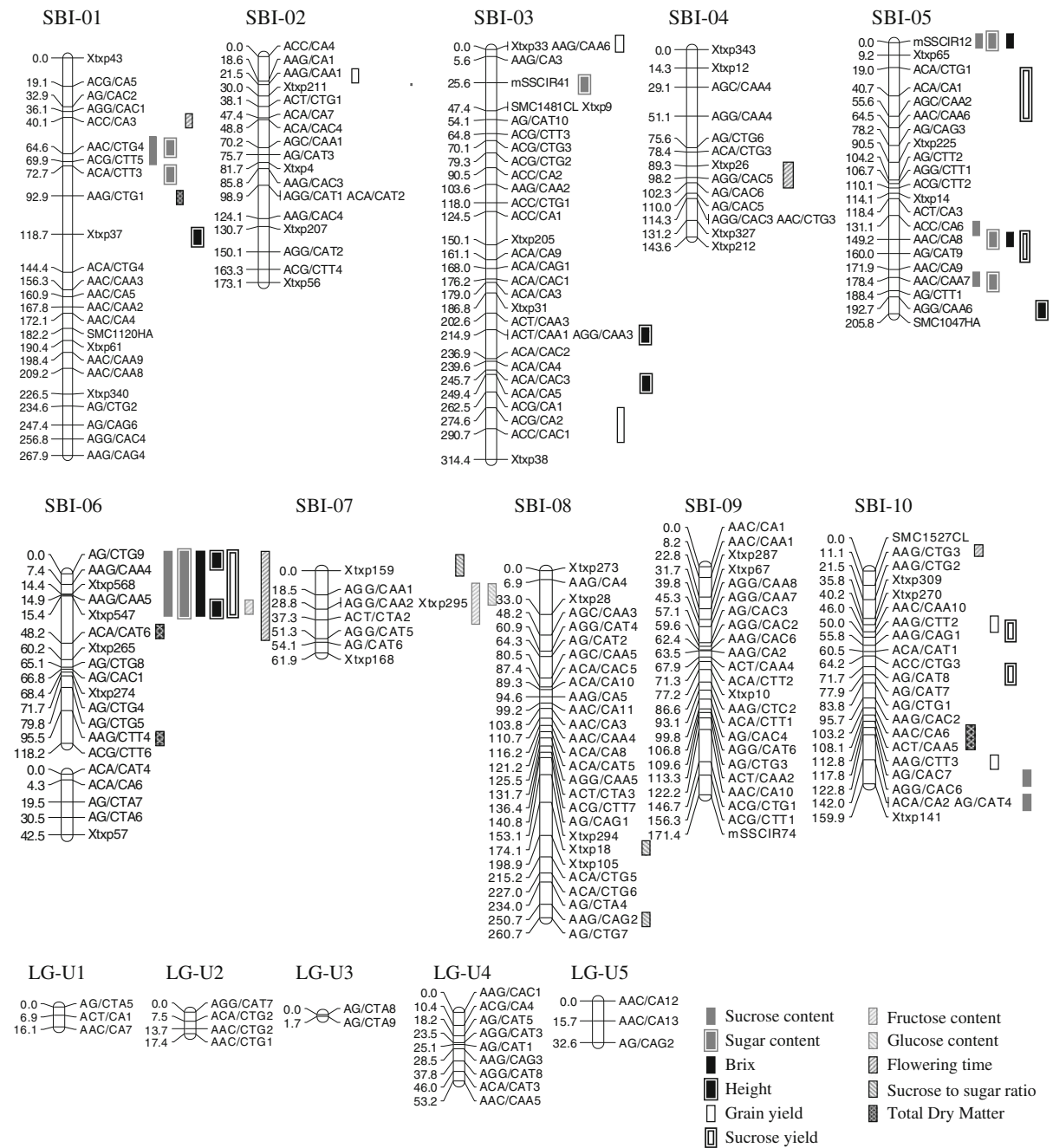


Fig. 1 A total of 228 markers were grouped into 16 LG, of which 11 could be assigned to chromosomes SBI-01 to SBI-10. The five unlinked LG are LG-U1 to LG-U5. To the right of each chromosome the QTL are shown for a LOD of 3.0 or greater

and SBI-03, and SBI-08 and SBI-10). The agronomic trait, total dry matter, only experienced epistasis between two regions (SBI-04 and SBI-07), as did grain yield (SBI-02 and LG-U4). Multiple regions were involved in epistatic interactions which affected

height (two regions on SBI-03 and LG-U4, SBI-03 and LG-U1, SBI-07 and SBI-08, and SBI-08 and SBI-10) and flowering time (SBI-01 and SBI-10, SBI-06 and SBI-10, SBI-01 and SBI-01, and SBI-04 and SBI-08).

Table 3 Molecular markers associated with the sugar-related phenotypic traits in two field trials (RB1 and RB2) based on QTL analyses carried out using CIM

Trait	Chromosome or linkage group	Marker	RB1		RB2		Phenotypic variance (R ²)	Effect of R9188 allele	Phenotypic variance (R ²)
			LOD score	Effect of R9188 allele	Phenotypic variance (R ²)	LOD score			
Sucrose content	SBI-01	AAC/CTG4	3.67	0.49	0.05				
	SBI-01	ACG/CTT5				3.07	0.41	0.04	
	SBI-05	mSSCIR12	4.76	0.81	0.15		0.61	0.11	
	SBI-05	ACC/CA6				4.56	0.50	0.06	
	SBI-05	AAC/CAA7	3.08	0.53	0.06				0.26
	SBI-06	AG/CTG9	8.29	0.90	0.17		1.00	0.27	
	SBI-06	AAG/CAA4	8.87	1.00	0.19		1.07	0.27	
	SBI-06	AAG/CAA5	8.78	0.98	0.18				0.32
	SBI-06	Xtxp547	3.00	1.05	0.24		1.14	0.12	
	SBI-10	ACA/CA2				13.62	-0.76	0.08	
Glucose content	SBI-10	AG/CAC7				3.39	-0.62		
	SBI-7	AGG/CAA1	3.54	-0.56	0.07				0.06
Fructose content	SBI-7	Xtxp295							
	SBI-06	Xtxp547	3.62	4.16	0.18				
	SBI-07	AGG/CAA1	3.56	-3.10	0.06				
	SBI-07	Xtxp295	3.79	-3.09	0.06				0.08
	SBI-07	ACT/CTA2	3.48	-2.73	0.05				0.07
	SBI-01	AAC/CTG4	3.20	0.48	0.05				
	SBI-01	ACA/CTT3				3.04	0.42	0.04	
Sugar content	SBI-03	mSSCIR41							0.10
	SBI-05	mSSCIR12	4.49	0.86	0.16		0.77	0.09	
	SBI-05	AAC/CAA7	3.19	0.57	0.06				
	SBI-05	AAC/CA8				5.99	0.65	0.10	
	SBI-06	AG/CTG9	7.81	0.92	0.16		1.06	0.23	
	SBI-06	AAG/CAA4	8.56	1.03	0.17		1.12	0.27	
	SBI-06	AAG/CAA5	8.60	1.05	0.18				
	SBI-06	Xtxp547	3.00	1.07	0.22		1.16	0.29	

Table 3 continued

Trait	Chromosome or linkage group	Marker	RB1		RB2		Phenotypic variance (R^2)
			LOD score	Effect of R9188 allele	LOD score	Effect of R9188 allele	
Sucrose yield	SBI-05	ACA/CTG1			5.41	21.56	0.16
	SBI-05	ACA/CA1	6.13	14.40	5.90	22.50	0.18
	SBI-05	AAC/CAA6	5.29	11.39			
	SBI-06	AG/CTG9	17.56	21.68			
	SBI-06	AAG/CAA4	17.59	22.04	16.26	32.27	0.33
	SBI-06	Xtxp547	16.02	22.44	15.53	32.49	0.33
Sucrose to sugar ratio	SBI-10	AG/CAT8			7.02	-22.43	0.15
	SBI-10	AAG/CAG1			7.53	-23.08	0.16
	SBI-07	Xtxp159	3.40	0.006			
	SBI-08	Xtxp18			3.66	-0.009	0.19
	SBI-08	AAG/CAG2	3.70	-0.007			
	SBI-5	mSSCIR2	3.52	0.62	4.07	0.57	0.10
Brix	SBI-5	AAC/CA8			5.13	0.57	0.09
	SBI-06	AG/CTG9	5.76	0.74	10.58	0.85	0.21
	SBI-06	AAG/CAA4	6.13	0.82	11.07	0.91	0.22
	SBI-06	Xtxp547			10.90	0.95	0.23

Any marker identified by CIM with a LOD score of 3.0 or greater was noted

Table 4 Molecular markers associated with the agronomic phenotypic traits in two field trials (RB1 and RB2) based on QTL analyses carried out using CIM

Trait	Chromosome or linkage group	Marker	RB1		RB2		Phenotypic variance (R ²)	Effect of allele	Phenotypic variance (R ²)
			LOD score	Effect of allele	LOD score	Effect of allele			
Height	SBI-01	Xtsp37	7.41	-12.07	7.58	-12.12	0.25	-12.12	0.24
	SBI-03	AGG/CAA3	5.27	-11.57	5.45	-12.26	0.16	-12.26	0.18
	SBI-03	ACA/CA5	3.87	-8.54			0.10		
	SBI-05	AGG/CAA6	4.23	7.50	3.97	7.48	0.09	7.48	0.08
	SBI-06	AG/CTG9	15.81	13.10	14.84	12.75	0.27	12.75	0.24
	SBI-06	Xtsp547	12.64	10.88	12.11	10.65	0.15	10.65	0.14
Flowering time	SBI-01	ACC/CA3			3.16	-0.77		-0.77	0.07
	SBI-04	Xtsp26	3.10	1.08	3.90	1.41	0.12	1.41	0.16
	SBI-06	AG/CTG9			5.90	1.32		1.32	0.17
	SBI-06	AAG/CAA4	7.22	1.28			0.19		
	SBI-06	AAG/CAA5			6.02	1.74		1.74	0.32
	SBI-06	Xtsp547	6.96	1.41	3.19	1.20	0.23	1.20	0.15
Total dry matter	SBI-06	ACA/CAT6	3.41	1.02			0.12		
	SBI-10	AAG/CTG3	5.12	-0.82	5.13	-0.90	0.09	-0.90	0.10
	SBI-01	AAG/CTG1	3.00	-66.67			0.18		
	SBI-06	Xtsp547	7.47	75.84	5.17	59.09	0.22	59.09	0.13
	SBI-06	ACA/CAT6	3.10	45.89			0.06		
	SBI-06	AAG/CTT4	3.02	-49.03	3.20	-45.92	0.10	-45.92	0.09
Grain yield	SBI-10	ACT/CAA5	11.18	-95.47	11.80	-95.07	0.31	-95.07	0.31
	SBI-10	AAC/CA6			11.12	-94.51		-94.51	0.33
	SBI-02	AAG/CAA1			4.08	71.33		71.33	0.18
	SBI-03	AAG/CAA6	4.16	-27.18			0.08		
	SBI-03	ACG/CA2			3.27	31.79		31.79	0.08
	SBI-03	ACC/CAC1			3.38	36.08		36.08	0.10
SBI-10	AAG/CTT3	5.89	-34.14			0.20			
SBI-10	AAG/CTT2	3.13	-45.10			0.14			

Any marker identified by CIM with a LOD score of 3.0 or greater was noted

Table 5 Epistatic interactions between markers for sugar-related phenotypic traits

Trait	Chromosome of 1st region involved in epistatic interaction	Nearest marker on chromosome	Position and range (cM)	Chromosome of 2nd region involved in epistatic interaction	Nearest marker on chromosome	Position and range (cM)	Estimated additive by additive effect	P-value (0.05)
Sucrose content (%)	SBI-03	ACC/CA2	(89.3) 84.3–93.5	SBI-05	ACA/CA1	(32.0) 26.0–39.0	0.569	0.000
Glucose content (%)	SBI-01	ACA/CTT3	(72.7) 70.9–76.7	SBI-03	ACG/CA2	(271.5) 265.5–282.6	-0.022	0.001
	SBI-01	ACA/CTT3	(72.7) 70.9–76.7	SBI-03	Xtp38	(311.7) 303.7–313.7	-0.029	0.000
	SBI-08	AAC/CAA4	(117.2) 113.7–125.2	SBI-10	ACT/CAA5	(108.1) 105.2–112.1	-0.027	0.000
Fructose content (%)	SBI-01	AAC/CTG4	(64.6) 56.1–67.6	SBI-03	Xtp38	(313.7) 306.7–313.7	-0.013	0.001
	SBI-01	ACA/CTT3	(72.6) 69.9–76.7	SBI-03	ACG/CA2	(270.5) 264.5–280.6	-0.014	0.000
	SBI-01	AAG/CAG4	(267.8) 263.8–267.8	SBI-06	ACG/CTT6	(117.5) 113.5–117.5	-0.015	0.000
Sugar content (%)	SBI-03	ACC/CA2	(88.3) 83.3–93.5	SBI-05	ACA/CA1	(31.0) 25.0–38.0	0.597	0.000
Sucrose yield (g/m ²)	SBI-03	ACA/CAG1	(171.0) 164.1–177.2	SBI-08	ACA/CTG6	(215.2) 210.9–217.2	-5.279	0.000
Sucrose to sugar ratio	SBI-02	AGC/CAA1	(70.2) 64.8–75.2	SBI-08	AGG/CAT4	(60.9) 57.2–62.9	-0.007	0.000
	SBI-03	ACA/CAC2	(238.9) 236.9–238.9	SBI-09	Xtp67	(27.8) 24.8–30.8	-0.005	0.007
	SBI-03	ACA/CAC2	(238.9) 236.9–238.9	SBI-09	AGG/CAA7	(48.3) 40.8–57.1	-0.005	0.000
Brix (%)	SBI-03	ACG/CTG3	(66.8) 64.8–75.1	SBI-05	mSSCIR12	(2.0) 0.0–7.0	0.222	0.002
	SBI-03	AAG/CAA2	(99.5) 91.5–103.5	SBI-05	ACA/CA1	(32.0) 26.0–40.0	0.273	0.003
	SBI-03	ACA/CAG1	(167.1) 162.1–172.0	LG-UI	AAC/CA7	(15.9) 9.9–15.9	0.345	0.000

Table 6 Epistatic interactions between markers for agronomic traits

Trait	Chromosome of 1st region involved in epistatic interaction	Nearest marker on chromosome	Position and range (cM)	Chromosome of 2nd region involved in epistatic interaction	Nearest marker on chromosome	Position and range (cM)	Estimated additive effect	P-Value (0.05)
Height (cm)	SBI-03	ACT/CAA3	(202.6) 197.8–205.6	LG-U4	ACG/CA4	(10.0) 4.0–14.4	-2.026	0.002
	SBI-03	ACT/CAA3	(202.6) 197.8–205.6	LG-U4	AGG/CAT3	(24.5) 23.2–24.5	-1.325	0.047
	SBI-03	ACA/CA5	(254.4) 249.4–258.4	LG-U1	AG/CTA5	(0.00) 0.0–4.0	-6.848	0.000
	SBI-07	AGG/CAA2 and Xtxp295	(28.8) 25.5–28.8	SBI-08	AG/CTA4	(243.0) 238.0–249.0	-4.512	0.000
Flowering time (days)	SBI-08	AAC/CA11	(100.2) 96.6–103.8	SBI-10	ACT/CAA5 and AAG/CTT3	(110.1) 106.2–115.8	-2.601	0.000
	SBI-01	ACC/CA3	(47.1) 39.1–55.1	SBI-10	AG/CAT7 and AG/CTG1	(89.8) 82.9–97.7	0.841	0.000
	SBI-06	AG/CTG9	(3.0) 0.0–7.0	SBI-10	AG/CAT7 and AG/CTG1	(89.8) 82.9–97.7	-1.011	0.000
Total dry matter (g/m ²)	SBI-01	ACC/CA3	(47.1) 39.1–55.1	SBI-01	AAC/CAA3	(156.3) 118.7–182.1	-0.397	0.039
	SBI-04	AGG/CAA4	(47.1) 29.1–77.6	SBI-08	AG/CTA4	(234.0) 215.2–259.7	0.979	0.000
	SBI-04	AGG/CAC5	(98.2) 94.3–100.2	SBI-07	ACT/CTA2	(37.3) 33.8–40.3	-23.431	0.000
Grain yield (g)	SBI-02	AGG/CAT1 and ACA/CAT2	(98.9) 98.9–103.9	LG-U4	AGG/CAT8	(37.8) 34.5–41.8	-20.102	0.000

Discussion

Similar QTL were detected in both RB1 and RB2 for many of the traits analysed. Thirty-two genomic regions were associated with the 11 traits from RB1 and RB2, of which 23 were identified in both trials. This is not unexpected considering the high heritability of the traits (Table 1). Due to the different planting dates of RB1 and RB2, the two trials experienced different environments. Despite the differences in environment, QTL identified in RB1 were also identified in RB2, which not only gave us greater confidence that these QTL are real, but also indicated that these QTL may be useful in breeding in due to their stability in different environments.

The identification of co-locating QTL for sugar-related traits is not surprising considering the high correlation between all of these traits (Table 2). Similar results have been reported previously, for example in sugarcane for CCS, Brix and pol (Aitken et al. 2006; Reffay et al. 2005). From a phenotyping perspective, the close relationship between Brix, sucrose content and sugar content in this study suggests that the slower and more expensive measurements of sucrose content and sugar content are not required as Brix, a simpler phenotypic trait to measure, provided similar QTL results.

QTL for sucrose content and sugar content were located to similar regions on SBI-01; QTL for sucrose content, sugar content and Brix were located to two similar regions on SBI-05, with one of these also associated with sucrose yield; and QTL for sucrose content, sugar content, Brix, sucrose yield, fructose content, plant height and FT were all located to a similar region on SBI-06. The co-location of QTL could be due to genes in these genomic locations that affect a number of traits (pleiotropy). Alternatively, these genomic regions may contain several genes, each of which affects a different trait and the co-location of QTL for different traits is simply a result of linkage.

Epistasis is the interaction amongst two or more genes to control a single phenotype. Some regions identified by QTLNetwork-2.0 as being involved in an epistatic interaction are different to the QTL identified for some traits (fructose content, glucose content, sucrose yield, grain yield and total dry matter), whilst some similar regions have been identified for the remaining traits (sucrose content,

sugar content, sucrose to sugar ratio, brix, height and flowering time). This difference is most likely due to the different algorithms used within the two different programs. For the sugar-related traits, the epistatic interactions resulted in slight increases (sucrose content, sugar content and brix) or decreases (fructose content, glucose content, sucrose yield and sucrose to sugar ratio) in the trait effect. For all the agronomic traits, the epistatic interactions resulted in slight decreases.

The population studied in this paper was a cross between a converted sweet sorghum and a grain sorghum. In sweet sorghum, carbohydrate is stored preferentially in the stems, often with little grain produced. In grain sorghum the converse is true, with most carbohydrate stored in the grain. In this study, grain yield was not strongly negatively correlated with the stem sugars, suggesting that stem sugar storage does not strongly affect final grain yield. Similar results have been reported previously in sorghum (McBee et al. 1983). Sugar content was strongly positively correlated with plant height and weakly correlated with delayed flowering. This is consistent with sweet sorghum characteristics suggesting taller plants with delayed flowering have more stem biomass (in which to accumulate sugars) and more time to accumulate photosynthate (Ferraris and Charles-Edwards 1986).

The progeny phenotypes exceeded the parental range for total dry matter, grain yield, glucose content and fructose content, which is indicative of transgressive segregation (Table 1). Thus it was not surprising to see QTL alleles enhancing traits being inherited from both parents. QTL alleles on SBI-05 from sweet sorghum parent R9188 had a major positive effect on several traits (including sugar content, sucrose content and sucrose yield with at least one of these also improving Brix) as did QTL alleles from various genomic regions of SBI-10 from grain sorghum parent R9403463-2-1 (for sucrose content, sucrose yield, FT, total dry matter and grain yield). Similarly, the QTL on SBI-01 for increased height is from R9403463-2-1, whilst R9188 is the source of the favourable QTL allele on SBI-05 for this same trait (although neither of these genomic regions were in fact significantly associated with sucrose yield variation). These results suggest that increases in sugar-related traits should be possible by combining QTL from R9188 and R9403463-2-1.

Several traits analysed in this study were also studied by Natoli et al. (2002) including sucrose content (described as sucrose percentage in Natoli et al. (2002)), Brix (sugar percentage), sucrose yield (sugar yield t ha⁻¹), FT, height and total dry matter (dry matter yield t ha⁻¹). Using the nomenclature of Kim et al. (2004), Natoli et al. (2002) identified QTL for sucrose percentage on SBI-02 and SBI-03, sugar yield (t ha⁻¹) on SBI-05, flowering time on SBI-01 and SBI-05, plant height on SBI-01 and SBI-05, and dry matter yield (t ha⁻¹) on SBI-05. No QTL were identified for sugar percentage (Brix). In both Natoli et al. (2002) and this study, the same chromosomes have been associated with sugar yield (SBI-05), flowering (SBI-01) and plant height (SBI-01 and SBI-05). By using a common marker between the two studies, *Xtxp225* on SBI-05, it is possible to determine that the same genomic regions may be associated with height variation, however, the limited number of SSRs in common between the two studies makes it difficult to establish an exact position of the QTL for FT on SBI-01. However, the only trait in common between this study and the sweet × grain sorghum study of Yun-Long et al. (2006) is Brix. Yun-Long et al. (2006) identified QTL for Brix on SBI-04 and SBI-10, and in this study, Brix QTL were found on SBI-05 and SBI-06. As Brix was not investigated in Natoli et al. (2002), there are no common genomic regions for this trait detected across these three sweet sorghum studies.

Within this study, QTL for height were identified on SBI-01, SBI-03, SBI-05 and SBI-06. In previous sorghum studies, using the nomenclature for Kim et al. (2004), QTL for height have been previously shown on SBI-06, SBI-07, SBI-09 and SBI-10 (Pereira and Lee 1995), SBI-01 (Lin et al. 1995), SBI-01, SBI-02 and SBI-07 (Rami et al. 1998) and SBI-04 and SBI-07 (Klein et al. 2001). Height of the main stem was also investigated by Hart et al. (2001), and QTL identified on SBI-01, SBI-03, SBI-07 and SBI-10. By aligning genetic maps derived from two sorghum populations (that share one common parent but differ in morphological and evolutionarily distant alternate parents), additional previously unpublished QTL for stem height were identified by Feltus et al. (2006) on SBI-06 and SBI-09. Thus, the QTL detected for height on SBI-01, SBI-03 and SBI-06 in this population were in the same chromosomes that have previously been reported to contain height QTL.

The SSRs, *Xtxp37* and *Xtxp61* on SBI-01, and *Xtxp31* on SBI-03, were common markers between this map and the genetic map of Hart et al. (2001). As a result, it can be noted that the height QTL from the two different maps are in different regions of SBI-01, but possibly the same region on SBI-03. There were no markers in-common on SBI-06 between this study and Pereira and Lee (1995), on SBI-01 between this study and Lin et al. (1995), or on SBI-01 between this study and Rami et al. (1998), therefore it is not possible to determine if the genomic regions in these studies are associated with height variation in this study. By identifying the position of the SSRs on SBI-06 in this map, and the RFLPs and SSRs from Feltus et al. (2006) in the map of Menz et al. (2002), it appears that the height QTL identified on SBI-06 on this map may be the same QTL identified in Feltus et al. (2006). While there was a moderate to high correlation of height and sucrose content in our trials (Table 2), it seems that there were several QTL for sucrose content (especially SBI-01, SBI-03, top of SBI-05 and SBI-10), and many other traits, which were largely independent of height, and could be considered as useful QTL, independent of their pleiotropic effects on height.

QTL for FT were associated with markers on SBI-01, SBI-04-1, SBI-06 and SBI-10. QTL for FT have previously been reported on SBI-02 and SBI-10 (Crasta et al. 1999), SBI-09 and SBI-10 (Hart et al. 2001) and SBI-01, SBI-03, SBI-06 and SBI-08 (Feltus et al. 2006). By identifying the position of the SSRs on SBI-10 in this map, and the RFLPs from Hart et al. (2001) in the map of Menz et al. (2002), it can be confirmed that the FT QTL identified on SBI-10 on this map is not the same QTL identified in Hart et al. (2001). Similarly, by identifying the position of the SSRs on SBI-01 in this map, and the RFLPs from Feltus et al. (2006) in the map of Menz et al. (2002), it appears that the FT QTL identified on SBI-01 on this map is not be the same QTL identified in Feltus et al. (2006). However, by again using the map of Menz et al. (2002) as a link between this map and Feltus et al. (2006), the FT QTL on SBI-06 may be the same QTL identified in Feltus et al. (2006).

Ming et al. (2002b), aligned sugarcane LGs to a sorghum map to assist in evaluating QTL affecting sugar yield and related traits from different sugarcane maps using RFLP markers. QTL for sugar-related traits in sugarcane were given an inferred sorghum

position due to the synteny that exists between sorghum and sugarcane. Sugar-related QTL from four different sugarcane maps were located on SBI-01, SBI-02, SBI-03, SBI-04, SBI-05, SBI-06, SBI-07, SBI-09 and SBI-10. Those QTL identified on SBI-01, SBI-03, SBI-05, SBI-06, SBI-07, SBI-08 and SBI-10 agree with the chromosomal locations identified in this study. Unfortunately there are no markers in common between this study and that of Ming et al. (2002b), hence it is not known if the same QTL are being detected on these chromosomes.

To more directly link the sweet sorghum QTL identified in this study to sugarcane QTL, an attempt was made to map sugarcane SSRs. Of the 70 sugarcane SSRs selected from Aitken et al. (2005), only 10 were polymorphic of which six were included in the map of this sweet sorghum population. The six sugarcane SSRs were mapped to SBI-01 (SMC1120HA), SBI-03 (mSSCIR41), SBI-05 (mSSCIR12 and SMC1047HA), SBI-09 (mSSCIR74), SBI-10 (SMC1527CL). Aitken et al. (2006) reported that SMC1120HA maps to HG 4, mSSCIR41 to HG 3, mSSCIR12 to HG 2, SMC1047HA to HG 4, mSSCIR74 to HG 2, and SMC1527. The positioning of two sugarcane markers from two separate HG on one sorghum LG (such as SMC1120HA and SMC1047HA), or the positioning of two sugarcane markers from one HG on two separate sorghum LG (such as mSSCIR12 and mSSCIR7) is possible evidence of sorghum-sugarcane chromosomal rearrangements or may result from incomplete mapping of all SSR alleles in sugarcane as only single-dose alleles can be mapped (Aitken et al. 2005).

In this study, the sorghum linkage groups containing sugarcane SSRs, as well as sorghum markers associated with at least one sugar-related trait, are SBI-01, SBI-03, SBI-05 and SBI-10. In sugarcane, QTL for Brix and pol have been found predominantly in HGs 1, 2, 3 and 4 (Aitken et al. 2006). Although the sparsity of common markers makes a detailed comparison impossible, the observation that three of the chromosomes (SBI-01, SBI-03 and SBI-05) contain sugarcane SSRs that map in sugarcane to three HGs (2, 3 and 4) with strong QTL for sugar-related traits suggests that similar loci may be being detected between sweet sorghum and sugarcane. However, further markers should be required to be mapped in both sugarcane and sorghum to confirm these preliminary observations.

A genetic map has been constructed and QTL have been identified for sugar-related and other agronomic traits in the R9188 (sweet) × R9403463-2-1 (grain) RIL sorghum population. As an increase in stem sugars is an important objective in sweet sorghum breeding, the robust co-locating sugar-related QTL identified in this study, with their high trait correlation and heritability, are clear choices for further investigation for marker-assisted selection in sweet sorghum. An attempt was made to compare these QTL with the location of QTL for similar traits in sugarcane. From this comparison, it appears that the genomic regions involved in sugar accumulation in the two species are at least partially coincident and therefore sweet sorghum could be a potential genetic model for studies of its more complex relative.

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