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Characterisation of alleles of the sucrose phosphate synthase gene family in sugarcane and their association with sugar-related traits

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Abstract Sucrose phosphate synthase (SPS) is a key enzyme in the production of sucrose. Five SPS gene families have been identified in monocotyledonous plants including sugarcane. Using SPS family-specific primers to four of the five families (we had previously characterised the fifth gene family), an approximately 400-nt region was amplified from the parents of a sugarcane mapping population, namely the cultivar Q165 and a *S. officinarum* line IJ76-514. Alignment of the sequences from both parents suggested from one to three genes per SPS gene family, with variable numbers of alleles per gene. Single-dose (SD) single-nucleotide polymorphisms (SNPs) were identified in at least one allele from each SPS gene family and mapped in Q165. For gene families SPS I–IV, SNPs from different alleles in each gene family mapped to different linkage groups within the same homology group (HG), suggesting a single gene per gene family, or multiple genes at a single locus. These map locations were syntenic with SPS gene family locations in sorghum. Two SNPs from different alleles in gene family SPS V were mapped to two different HGs, suggesting two genes in this family; one of the map locations was syntenic with the location of SPS V in sorghum. QTL analysis for sugar-related traits was undertaken with the SD and double-dose SNP markers. SNPs from SPS gene family IV were strongly associated with sugar-related traits, while SNPs from other gene families were associated with agronomic traits, such as stalk weight, diameter, and number. This study provides insight into the evolution of this important polyploid crop as well as highlights the importance of this gene family to sugar production in sugarcane.

Keywords Sugarcane · SNP · Sucrose phosphate synthase · Ecotilling · Sugar · Sucrose · Sorghum

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Introduction

Sugarcane provides approximately 80 % of the world's sugar. It has a very complex genetic structure and is both aneuploid and polyploid with chromosome numbers typically greater than 100 that can be grouped into eight homology groups (HG) ($x = 8$ in *Saccharum spontaneum*, an ancestral species of cultivated sugarcane, D'Hont et al. 1996). Each HG is estimated to contain between 8 and 14 homo(eo)logous chromosomes. Not surprisingly, most traits in sugarcane are quantitatively inherited, and QTL analysis for traits such as sugar content (Aitken et al. 2006; Hoarau et al. 2002; Ming et al. 2002; Piperidis et al. 2008; Alwala et al. 2009; Pinto et al. 2010; Singh et al. 2013) and fibre content (Hoarau et al. 2002; Piperidis et al. 2008) has indicated many QTL of individually small effect. In studies involving sugarcane cultivars, sugar-related QTL typically explain between 3 and 8 % of the phenotypic variation, although individual QTL of up to 15 % have been detected in an interspecific population (Ming et al. 2002).

Sucrose phosphate synthase (SPS; EC 2.4.1.14) is a key enzyme in the synthesis of sucrose (Quick and Stitt 1989), and its activity has been shown to be linked to several important agronomic traits. SPS activity has been shown to be correlated with leaf expansion rate (Seneweerra et al. 1995) and height (Ishimaru et al. 2004) in rice, growth rate (Rocher et al. 1989) and dry matter yield (Causse et al. 1995; Sarquis et al. 1998) in maize, and higher final sucrose content (Zhu et al. 1997) and sucrose content in the upper internodes of high sucrose progeny lines and cultivars as compared with low sucrose progeny and cultivars (Grof et al. 2007; Verma et al. 2011) in sugarcane. An SPS gene has also been shown to co-locate with quantitative trait loci (QTL) for grain yield in maize (Bertin and Gallais, 2001). Overexpression of SPS genes has been successful in a range of plants including rice, tobacco, and cotton, resulting in improved sucrose synthesis. Additional phenotypes have included improved fibre quality in cotton (Haigler et al. 2007), increased biomass in tobacco (Park et al. 2008), earlier flowering and greater flower numbers in tobacco (Baxter et al. 2003), as well as improved tuber weight and yield in potato (Ishimaru et al. 2008).

SPS is a multi-gene family in both dicotyledonous and monocotyledonous plants. While three families (A, B, and C) have been identified in dicotyledonous plants, monocotyledonous plants contain five families; they contain these three families (A = II; B = V, C = I), plus two additional, closely related gene families (D1 = III, D2 = IV) (Castleden et al. 2004). Representative sequences from all five SPS gene families have been found in sugarcane and its close diploid relatives, sorghum and maize (Castleden et al. 2004). There are five SPS genes, one per SPS gene family, in sorghum and at least seven SPS genes in maize; there are at least two genes in families IV and V in maize (Castleden et al. 2004).

We have previously characterised the SPS III gene family in the cultivar Q165 and *S. officinarum* line IJ76-514 (McIntyre et al. 2006). The pattern of SNP-defined haplotypes in a 400-nt region amplified using SPS III family-specific primers suggested two putative gene members in this family, each with multiple alleles. Two SNPs corresponding to different alleles from one of the genes segregated as SD markers and were mapped to different linkage groups (LGs) in HG 1 of Q165. One of these alleles was associated with the agronomic trait tonnes of cane harvested (TCH) (McIntyre et al. 2006).

In this paper, we report on the characterisation of the remaining four SPS gene families in Q165 and IJ76-514. Using SPS gene family-specific primers to amplify from the parents of our sugarcane mapping population, we used the pattern of SNP-defined haplotypes to determine the number of gene members and putative number of alleles in each SPS gene family. SNPs that segregated as SD markers were identified and mapped, and their association with sugar-related traits was determined.

Materials and methods

Plant material and phenotypic data

The sugarcane mapping population used in this study was developed from a cross between IJ76-514, a *S. officinarum* ($2n = 80$) clone collected from Iryan Jaya in Indonesia, and Q165 ($2n = 120$), an Australian cultivar and elite parent, and contained 227 progeny. IJ76-514 was used as the female. The population was planted in two field trials in 2000 and 2001 and evaluated for eight traits, as described in Aitken et al. (2006, 2008). All traits were measured and calculated using standard Australian sugar industry procedures

(Bureau of Sugar Experiment Stations (BSES) 1984). Of the eight traits, three were sugar-related traits—Brix, pol, CCS. Brix is an estimate of total dissolved solids in juice. Pol is a measure of the rotation of polarise light as it passes through juice and commonly used in sugar industries as an estimate of sucrose purity. In the Australian sugar industry, CCS is an estimate of commercially extractable sucrose and is determined from a standard function of brix, pol, and fibre (BSES 1984). The remaining five traits were agronomic traits—fibre, stalk weight, stalk number, stalk diameter, and tonnes of cane per hectare (TCH).

Identification of SNPs within SPS gene families I, II, IV, and V in sugarcane

As described in McIntyre et al. (2006), sugarcane ESTs encoding SPS genes, including those listed in Castleden et al. (2004), were retrieved from the dbEST database at NCBI (www.ncbi.nlm.nih.gov) and aligned to identify regions that differentiated the five SPS gene families. The most divergent regions were also examined for their proximity to introns, to increase the likelihood of finding introns, and for the size of the amplified product; short products were favoured to ensure complete primer extension and to minimise PCR-mediated recombination (Cronn et al. 2002). The SPS gene family-specific primer sequences are indicated in Supplementary Figure 1. The primers amplify an approximately 400-nt region from exon 9 to exon 11, encompassing two short introns, with the exception of primers for SPS gene family V which amplify from exon 8 to exon 11.

The SPS gene family-specific primers were used to amplify from Q165 and IJ76-514 genomic DNA as described in McIntyre et al. (2006). Two PCRs were performed using the same DNA sample in different reaction mixes at different times for each sugarcane parent. Amplification products were purified, cloned, and sequenced as described in McIntyre et al. (2006). SPS sequences from each SPS gene family were aligned separately for each parent. The sequence of each SNP was visually inspected. To reduce the effect of PCR and sequencing errors, a SNP and a haplotype were accepted in each sugarcane line only if it was observed at least twice in both PCRs. A consensus sequence was generated for each SPS gene family amplicon.

Mapping of SPS gene family SNPs in sugarcane and QTL analysis

For each gene family, the frequency of each SNP in the sequenced fragments was calculated and SNPs that occurred at frequencies less than 30 % were noted. These frequencies were used as a guide because only single-dose (SD) markers can be mapped easily and accurately in sugarcane populations with approximately 200 progeny, as present in IJ76-514 × Q165 mapping population; double-dose (DD) markers can also be mapped if the SD-based map is large. A SD SNP would be expected to be present on only one of the 8–14 chromosomes in each homology group in one parent only. If each SPS gene allele is recovered with equal frequency, then a SD SNP would appear in approximately 8–12 % of the fragments sequenced from one parent only and would be present in approximately 50 % of the progeny. Similarly, DD SNPs would appear in approximately 20–25 % of the fragments sequenced from one parent only.

Primers were designed to target the low-dose SNPs. The frequency of the selected SNPs in the 227 progeny of the IJ76-514 × Q165 mapping population was determined as described in McIntyre et al. (2006). Each SNP was evaluated for its potential as a SD or a DD marker using the Chi-square test. SD markers segregate 1:1 when present in only one parent, or 3:1 when present in the heterozygous form in both parents (biparental simplex marker: BPS). DD markers segregate 11:3. The DD markers were incorporated into the Q165 map using the methods described in Aitken et al. (2007). However, it should be noted that the assignment of markers as SD or DD can be difficult (Baker et al. 2010). Sugarcane is highly aneuploid and polyploid, and consequently, all progeny in a population are very likely to have a slightly different chromosomal composition. Thus, chromosome numbers for a given chromosome are very likely to vary across the population and affect segregation numbers and marker dosage determination. All SPS marker data were incorporated into the latest mapping data set for this population (Aitken et al. 2014b).

Putative QTL were detected using a one-way analysis of variance to identify significant marker-trait associations (MTAs) between the presence or absence of a marker and the 11 traits as described in Aitken et al. (2006, 2008). SD and DD markers from both Q165 and IJ76-514 were used for this analysis.

Comparative mapping of SPS genes in sugarcane, sorghum, and maize

The nucleotide sequence of each sugarcane and sorghum SPS gene EST tabled in Castleden et al. (2004) was obtained via NCBI. A BLAST search was conducted between each EST sequence and the genome sequence of sorghum (version 2.1) in Phytozome version 9.1 to determine the sorghum genome location of the five SPS gene families. A BLAST search was also conducted using the approximately 400 nt consensus sequence for each sugarcane SPS gene family. Comparative mapping of the five SPS gene families in sorghum, maize, and sugarcane was undertaken using Phytozome version 9.1 (www.phytozome.org), Gramene (Liang et al. 2008) (http://gramene.org/), and the sugarcane map in Aitken et al. (2014a, b).

Results

Identification of haplotypes within SPS gene families I, II, IV, and V in sugarcane

Alignment of 157 Q165 and 157 IJ76-514 sequences revealed 41 SNPs and/or small indels in the 395-bp SPS I gene fragment, which defined 11 and four haplotypes in Q165 and IJ76-514, respectively (Supplementary Figure 1a, Table 1); no haplotypes were common to both lines. The haplotypes were recovered at frequencies ranging from 2.6 to 17.1 % in Q165 and from 11.0 to 42.2 % in IJ76-514. Three distinct patterns of SNPs in the Q165 haplotypes (A, B, C) and two in IJ76-514 (D, E) suggested that SPS gene family I contains either divergent classes of alleles of a single gene or multiple genes.

For SPS II, alignment of the 70 Q165 and 52 IJ76-514 sequences revealed only 10 SNPs and/or indels in the 359-bp fragment (Supplementary Figure 1b, Table 2a). A total of seven and six haplotypes were identified in the two parents, respectively, with haplotype frequencies ranging from 5.7 to 22.9 % in Q165 and from 9.6 to 26.9 % in IJ76-514. One of the haplotypes was common to both parents. The pattern of SNPs suggested that only one SPS gene family II gene is present in sugarcane.

The results for SPS gene family III have been published previously (McIntyre et al. 2006). In brief, 93 and 66 sequences were aligned in Q165 and IJ76-514, respectively. Ten SNPs were identified within the 417-bp fragment, which defined eight and six haplotypes in the two parents at frequencies ranging from 2.3 to 35.2 %. Three haplotypes were common to both parents. The pattern of SNPs suggested that two genes were present in SPS gene family III (McIntyre et al. 2006).

For SPS gene family IV, 11 SNPs and/or indels were identified in the 343-bp fragment after alignment of 199 Q165 and 144 IJ76-514 sequences (Supplementary Figure 1c, Table 2b). The pattern of SNPs suggested that SPS gene family IV is represented by a single gene in sugarcane. Eight and three haplotypes were present at frequencies that ranged from 2.0 to 27.6 % and 6.3 to 63.2 % in Q165 and IJ76-514, respectively (Table 2b). The most frequent haplotype was common to both lines.

Similarly for SPS gene family V, eight and five haplotypes were identified from the pattern of eight SNPs in the 607-bp fragment after alignment of the 99 and 169 sequences from Q165 and IJ76-514, respectively (Supplementary Figure 1d, Table 2c). The haplotypes varied in frequency from 5.6 to 28.6 % and 3.6 to 35.5 in Q165 and IJ76-514, respectively, and four of the haplotypes were common to both lines. The similarity of the haplotypes also suggested that a single gene was present in SPS gene family V (Table 2c).

Map location of SPS gene families in sugarcane and sorghum

Of the 80 SNPs and/or small indels identified in the amplified fragments, 22 were shown to segregate as SD, BPS or DD markers in Q165 and IJ76-514 (data not shown). Fifteen of the 22 were from Q165 (Table 3), and seven were from IJ76-514 (data not shown). Of the 15 low-dose markers from Q165, 10 segregated as SD, one as BPS, and four as DD markers; all but one have been mapped (Table 3). The seven low-dose markers from IJ76-514 were either unlinked or mapped to unassigned LGs (data not shown).

In Q165, 11 haplotypes were identified in SPS gene family I, which formed three distinct haplotype groups (A, B, C) (Table 1). Four SD, one BPS, and three DD SNPs were mapped; these SNPs were present in one or more of the haplotype groups. The eight low-dose

Table 1 SNPs and haplotypes in SPS gene family I

			T/C[1]	G/A	A/G	G/C	A/G	A/C	A/-	C/T[2]	T/A	CCG/-AG	C/-	G/-	G/T	TA/-GG	TTG/---	G/A	G/C	GA/C-/GG	(CTG)$_{2/1}$	G/T	G/T
	H'type Grp	H'type	SNP[3] 54	63	69	80	81	84	85	86	87	89	96	97	99	102	106	109	113	120	124	139	146
Q165	A	1																					
	A	2																					
	A	3																					
	A	4																					
	B	1																					
	B	2																					
	C	1																					
	C	2																					
	C	3																					
	C	4																					
	C	5																					
IJ76-514	D	1																					
	D	2																					
	D	3																					
	E	1																					

			G/C	T/G	G/A	TAG/--	C/A	T/C	G/C	G/C	CG/TA	GC/AA	T/A	A/G	G/C	C/T	C/T	C/G	C/T	A/G	CC/GA	C/T	
	H'type Grp	H'type	148	150	153	154	163	174	191	193	220	230	235	244	277	283	286	289	295	303	325	343	Freq[4]
Q165	A	1																					11.2%
	A	2																					6.6%
	A	3																					9.2%
	A	4																					2.6%
	B	1																					8.6%
	B	2																					11.8%
	C	1																					3.3%
	C	2																					6.6%
	C	3																					17.1%
	C	4																					13.8%
	C	5																					9.2%
IJ76-514	D	1																					31.8%
	D	2																					14.9%
	D	3																					11.0%
																							42.2%

[1] First nucleotide is most common nucleotide in sequences recovered from Q165. Lightly shaded box represents second nucleotide indicated

[2] Darker shaded box represents third nucleotide(s) indicated

[3] SNP position in amplified fragment

[4] Frequency of sequence haplotype recovered in 152 Q165 and 154 IJ76-514 sequences

SNPs mapped to six different LGs within HG 2 (Fig. 1; Table 3). Two SNPs mapped near each other on two LGs within HG 2 (LG72b and LG1a). One pair of SNPs was detected in the same haplotype (SPSI193 and DSPSI286 in haplotype C4), while the other pair of SNPs was present in different haplotypes (SPSI230 in haplotype C2 and SPSI235 in haplotypes A3, C3-5) (Fig. 1; Table 1). One of the mapped SNP markers, DSPSI235, segregated as a DD marker but was present in multiple haplotypes (Table 1). This observation suggests that either chromosomal segregation distortion is affecting the dosage calculation for this SNP or one/some of the haplotypes are not real. Comparative mapping within the sugarcane genome and between sorghum and sugarcane suggests that all sugarcane SPS gene family I alleles map to homologous locations in sugarcane and to a syntenic location in sorghum on chromosome Sb-05 (Fig. 1, Supplementary Table 1) (Aitken et al. 2014b). After running a BLAST search of the sorghum and sugarcane ESTs from Castleden et al. (2006) and of the three sugarcane SPS gene family I haplotype group consensus sequences (Supplementary Figure 1) against the sorghum genome sequence (www.phytozome.org), the results also suggest that a single gene is present in SPS gene family I in both sorghum and sugarcane and that the map locations in both species are syntenic (Supplementary Table 1).

A single SD SNP was identified in SPS gene family II in Q165, which maps to a LG within HG 6 (Fig. 1; Tables 2a, 3). Again, comparative mapping between sorghum and sugarcane suggests that this region is syntenic with a region on sorghum chromosome Sb-09 which is the location of sorghum SPS gene family II (Aitken et al. 2014a, b). The results of the BLAST search also suggest that a single gene is present in this SPS gene family (Supplementary Table 1).

A SD and a DD SNP were identified in SPS gene family IV in Q165, and both SNPs were mapped to LGs in HG 7 (Fig. 1; Tables 2b, 3). Both comparative

Table 2 SNPs and haplotypes in SPS gene families II, IV, and V

(a) SNPs and haplotypes in SPS Gene Family II

		C/T[1]	G/T	T/-	C/-	T/-	T/C	T/-	T/-	C/T	G/A	
	H'type	52[2,3]	103	108	109	110	115	116	117	148	330	Freq[4]
Q165	1							■	■			22.9%
	2	■						■				20.0%
	3							■	■	■		17.1%
	4			■				■	■			15.7%
	5	■						■	■			12.9%
	6	■										5.7%
	7			■	■			■	■		■	5.7%
IJ76-514	1	■		■	■	■		■				26.9%
	2	■		■	■	■	■					26.9%
	3		■									13.5%
	4			■	■	■					■	13.5%
	5							■				9.6%
	6	■										9.6%

(b) SNPs and haplotypes in SPS Gene Family IV

		T/C[1]	T/C	C/A	A/T	C/A	TTT/---	A/-	T/-	T/-	A/G	T/C	
	H'type	23,61[2,3]	147	220	233	235	253	263	266	267	283	295	Freq[5]
Q165	1					■							14.1%
	2				■								14.6%
	3	■			■								18.1%
	4			■									13.1%
	5					■	■	■	■	■			7.5%
	6	■											3.0%
	7	■				■							2.0%
	8												27.6%
IJ76-514	1					■					■	■	30.6%
	2		■										6.3%
	3												63.2%

(c) SNPs and haplotypes in SPS Gene Family V

		C/T[1]	T/C	T/C	T/A	T/C	A/G	A/G	C/T	
	H'type	23,40[2,3]	51	85	132	230	306	316	532	Freq[6]
Q165	1			■					■	7.60%
	2			■						25.70%
	3								■	28.60%
	4									10.50%
	5						■			6.70%
	6		■							9.50%
	7	■		■	■				■	5.70%
	8	■		■		■		■		5.70%
IJ76-514	1			■					■	3.55%
	2			■						21.89%
	3									35.50%
	4								■	34.32%
	5		■						■	4.73%

[1] SNP nucleotides. First nucleotide = most common nucleotide in Q165 sequences at SNP position

[2] SNP position in amplified fragment

[3] Unshaded box = most common nucleotide. Shaded box = second nucleotide. Frequency of sequence haplotype recovered in 70 Q165 and 52 IJ76-514 sequences

[4] Frequency of sequence haplotype recovered in 199 Q165 and 144 IJ76-514 sequences

[5] Frequency of sequence haplotype recovered in 99 Q165 and 169 IJ76-514 sequences

Table 3 Marker-trait associations for SPS gene SNPs

SNP	Dosage	Map location	brix02E[2]	brix02M	brixcE	brixcM	ccs02E	ccs02M	ccscE	ccscM	pol01M	pol02M	pol02E
SPSI154	SD[1]	HG2 LG57											
SPSI303	BPS	HG2 LG72											
SPSI283	DD	HG2 LG35											
SPSI193	SD	HG2 LG1a											
SPS1230	SD	HG2 LG72b											
SPSI235	DD	HG2 LG72b											
SPSI286	DD	HG2 LG1a											
SPSI325	SD	HG2 LG27											
SPSII148	SD	HG6 LG58											
SPSIII231[3]	SD	HG1 LG8											
SPSIII322	SD	Unlinked											
SPSIV147(IJ[4])	SD	Unlinked											
SPSIV220	DD	HG7 LG36	6 % 0.002	5 % 0.004	3 % 0.02	5 % 0.005	5 % 0.002	4 % 0.01	3 % 0.02	5 % 0.004	3 % 0.02	4 % 0.007	6 % 0.002
SPSIV267	SD	HG7 LG40											
SPSIV295(IJ)	SD	Unlinked	3 % 003			3 % 0.03	3 % 0.04		3 % 0.04	3 % 0.03			3 % 0.04
SPSV132	SD	HG5 LG52											
SPSV230	SD	HG3 LG10											

SNP	Dosage	Map location	polcE	polcM	SD02[5]	FB02	SW01	SW02	SW04	SWE01	SWM01	SN02	TCH04
SPSI154	SD	HG2 LG57											
SPS1303	BPS	HG2 LG72											
SPSI283	DD	HG2 LG35					3 % 0.03				3 % 0.02		
SPSI193	SD	HG2 LG1a				3 % 0.02							
SPS1230	SD	HG2 LG72b											
SPSI235	DD	HG2 LG72b											
SPSI286	DD	HG2 LG1a											
SPSI325	SD	HG2 LG27								2 % 0.05			
SPSII148	SD	HG6 LG58											
SPSIII231	SD	HG1 LG8											
SPSIII322	SD	Unlinked											5 % 0.004
SPSIV147(IJ)	SD	Unlinked										5 % 0.006	
SPSIV220	DD	HG7 LG36	3 % 0.02	5 % 0.005									

Table 3 continued

SNP	Dosage	Map location	polcE	polcM	SD02[5]	FB02	SW01	SW02	SW04	SWE01	SWM01	SN02	TCH04
SPSIV267	SD	HG7 LG40			4 % 0.007		2 % 0.05	3 % 0.02	2 % 0.04				
SPSIV295(IJ)	SD	Unlinked		3 % 0.03									
SPSV132	SD	HG5 LG52											
SPSV230	SD	HG3 LG10										5 % 0.006	

[1] *SD* single dose, *DD* double dose, *BPS* bi-parental simplex

[2] *E* early, *M* mid-season sampling

[3] SPSIII SNPs identified in McIntyre et al. (2006)

[4] IJ = SNP only found in IJ76-514

[5] *SD* stalk diameter, *FB* fibre, *SW* stalk weight, *SN* stalk number, *TCH* tonnes of cane harvested

mapping and the BLAST results suggest that there is a single gene in this family in the two species, and that the sugarcane chromosome regions are syntenic to chromosome Sb-10, the location of sorghum SPS gene family IV (Fig. 1, Supplementary Table 1) (Aitken et al. 2014a, b).

Two SD SNPs were identified in SPS gene family V in Q165 (Table 2c). These SNPs map to LGs in different HGs (HGs 3 and 5) (Fig. 1; Table 3). The region on HG 3 is syntenic with the location of SPS gene family V in sorghum (Sb-03) while the region on HG 5 is syntenic with Sb-05 (Aitken et al. 2014a). The sorghum and sugarcane sequence BLAST results indicate that the sequences map to the same region in sorghum on Sb-03. Interestingly, the sorghum sequences appear to map to two different regions on Sb-03 (Supplementary Table 1), which suggests that there may be two genes in SPS gene family V in sorghum.

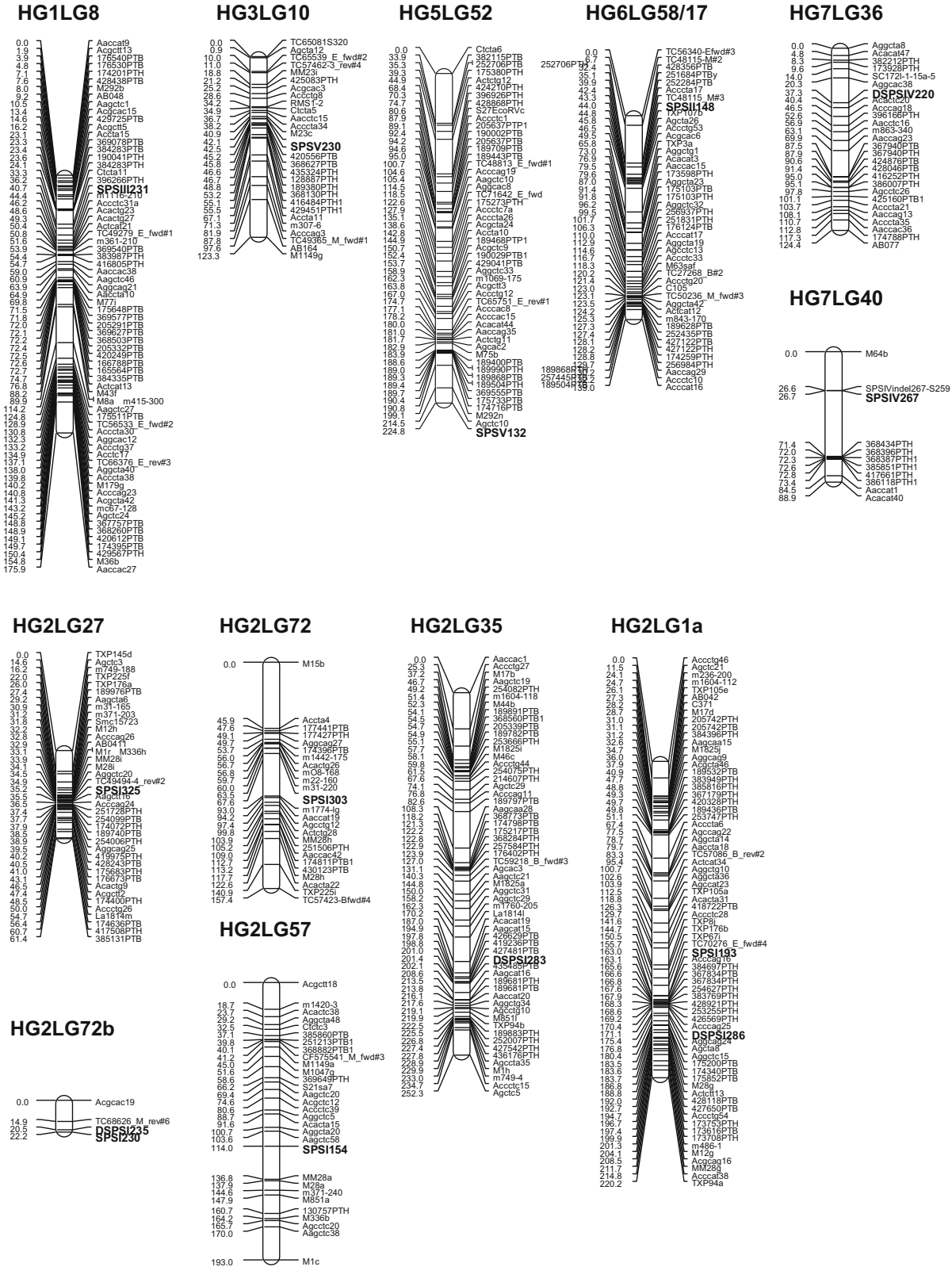
QTL analysis of SPS SNPs and sugar-related traits in sugarcane

Single-factor analysis was undertaken for the 22 low-dose SPS SNP markers in Q165 and IJ76-514. As summarised in Table 3, seven of the 15 low-dose SPS SNPs from Q165 were associated with traits as were two of the seven low-dose SPS SNPs from IJ76-514. All marker-trait associations had a positive direction of effect.

Of the eight SD and DD SNPs in SPS gene family I from Q165, three were associated with agronomic traits. Two markers were associated with stalk weight while the third was associated with fibre. Each marker explained approximately 2–3 % of the phenotypic variation (Table 3).

The SPS II SD SNP from Q165 was not associated with any trait (Table 3). One of the two SPS III SD SNPs from Q165 was associated with cane yield (TCH) and explained 5 % of the phenotypic variation (Table 3), as described previously (McIntyre et al. 2006). One of the two SPS V SD SNPs from Q165 was associated with stalk number and also explained 5 % of the phenotypic variation (Table 3).

Both SPS IV SNPs from Q165 were associated with traits (Table 3). In particular, the DD SNP SPSIV220 was associated with brix, CCS, and pol in multiple years and explained between 3 and 5 % of the phenotypic variation in these traits. A second SPS IV


HG1LG8
HG3LG10
HG5LG52
HG6LG58/17
HG7LG36
HG7LG40
HG2LG27
HG2LG72
HG2LG35
HG2LG1a
HG2LG57
HG2LG72b
SPSIII231
SPSV230
SPSV132
SPSIII148
DSPSIV220
SPSIV267
SPSI325
SPSI303
DSPSI283
SPSI193
DSPSI286
SPSI154
DSPSI235
SPSI230

◀**Fig. 1** Map locations of sugarcane SPS gene SNPs. Only LGs with SPS SNP markers are shown. LG maps are from Aitken et al. (2014b). D preceding the SNP marker name denotes a DD marker. Other markers are SD

SNP, SPSIV267, was associated with stalk weight and stalk diameter (in all three years) and explained between 2 and 4 % of the phenotypic variation in the trait. Interestingly, the only low-dose SPS SNPs from IJ76-514 that were associated with traits were from SPS gene family IV. One SNP was associated with brix, CCS, and pol, while a second SNP was associated with stalk number (Table 3).

Discussion

Despite the recent advances in genetic mapping in sugarcane (Raboin et al. 2006; Oliveira et al. 2007; Andru et al. 2011; Aitken et al. 2014b) and in cytogenetic analysis of sugarcane (Piperidis et al. 2010), the number of chromosomes in a homology group, or the range in numbers, in sugarcane cultivars is currently unknown. Previous studies using a variety of molecular techniques have shown that more than 100 chromosomes in cultivated sugarcane can be assigned to eight homology groups, equivalent to the basic chromosome number of *S. spontaneum* (D'Hont et al. 1996). Thus, each homology group is estimated to contain 8–14 homo(eo)logous chromosomes with the potential for 8–14 different alleles at each locus.

In rice and wheat, each of the five SPS gene families is present as a single locus per genome; rice also contains a pseudogene (Castleden et al. 2004). Five SPS genes are also apparently present in sorghum. However, seven SPS genes have been found in maize; gene families I–III have a single gene each, while gene families IV and V each have two genes. Sugarcane, sorghum, and maize are members of the tribe Andropogoneae, but sorghum and sugarcane are more closely related than either is to maize (Grivet et al. 1994; Sobral et al. 1994; Dufour et al. 1996).

In this study and our earlier paper (McIntyre et al. 2006), we characterised the DNA sequence of amplified products from Q165 and IJ76-514 in an attempt to identify all possible alleles and to determine how many genes were present in each SPS gene family in sugarcane. As noted previously, some haplotypes can arise by PCR-mediated intergenomic recombination (Cronn et al. 2002), and consequently, a conservative approach, similar to that undertaken by Mudge et al. (2009) of counting only sequence variants detected multiple times in independent PCRs, was used. The number of SNPs and haplotypes defined, together with mapping of SD and DD SNPs in our sugarcane mapping population and our previous (McIntyre et al. 2006) study, suggests that there is considerable allelic diversity in the sugarcane SPS gene families, especially in SPS gene family I, and that SPS gene families I–IV in sugarcane are represented by a single gene but that SPS gene family V may be represented by two genes, as in maize. All five SPS gene families map to syntenic locations in sugarcane and sorghum (Aitken et al. 2014a).

Haplotype numbers in the SPS gene families varied from 7 to 11, which is consistent with the number of haplotypes observed in other studies of allelic diversity in sugarcane (Grivet et al. 2001, 2003; Mudge et al. 2009; Moyle and Birch 2013; Zhang et al. 2013) and is close to the estimated range in number of chromosomes per homology group of 8–14. The frequency with which a haplotype sequence is obtained is expected to be an approximate indication of its allelic dosage. In the present study, the frequency with which haplotypes were recovered varied considerably, and more haplotypes were recovered than would be expected. Thus, some of the haplotypes observed may be errors arising from PCR-mediated recombination (Cronn et al. 2003), as noted previously (McIntyre et al. 2006). The genome of sugarcane is in the process of being sequenced and assembled by the International Sugarcane Sequencing Consortium (Souza et al. 2011). While some SNPs in the five SPS gene families were confirmed as present in the sugarcane genome sequence, genome coverage is currently too low to identify spurious SNPs and haplotypes (Aitken and Berkman unpublished observations); this will be a tremendous resource for sugarcane geneticists in the future.

SNP/indel numbers (8–11) and haplotype numbers (7–8 and 3–6 in Q165 and IJ76-514, respectively) were similar for SPS gene families II–V, but a much larger number of SNPs/indels (41) and haplotypes (11 and 4 in Q165 and IJ76-514, respectively) were observed in SPS gene family I. The haplotypes formed three distinct haplotype groups (A, B, C) in Q165 and two (D,E) in IJ76-514, respectively. A total of eight SNPs from the three groups in SPS gene family I were

mapped to putatively syntenic regions on six different LGs with HG 2 in Q165. This result suggests that either these haplotype groups are divergent alleles of a single gene or that they represent duplicated genes at the same (or closely linked) locus. As two SNPs from different haplotypes mapped to the same LG, it is possible that there are duplicate genes at this locus or in this chromosomal region. This question could be answered in the future when there is a more complete genome sequence for sugarcane.

The high level of allelic diversity in SPS gene family I is unusual when compared to the relative number of SNPs in the other four SPS gene families. As noted in Castleden et al. (2004) and Grof et al. (2006), this family is poorly represented in EST collections with relatively low levels of expression in rice, maize, sorghum, and sugarcane, although it is the most abundant family in wheat. Thus, the high level of genetic diversity does not appear to be related to function. HG 2 is one of two HGs in sugarcane in which two sets of *S. officinarum* chromosomes are aligned with one set of *S. spontaneum* chromosomes as the result of a simple fusion event; the two fusion events explain the difference in basic chromosome number between the two species, viz., $x = 10$ in *S. officinarum* and $x = 8$ in *S. spontanuem* (D'Hont et al. 1996). This HG appears to contain more chromosomal rearrangements than other HGs, which could suggest greater overall genetic diversity within this HG (Aitken et al. 2014a).

For SPS gene families II, III, and IV, the pattern of SNPs and the mapping of SNPs to syntenic regions of sugarcane linkage groups both within homology groups and to sorghum strongly suggest a single gene per gene family. For SPS gene family III, we had previously suggested that the pattern of SNPs in the haplotypes recovered indicated that two genes were present in this family. Given the number of SNPs and haplotype patterns in the other SPS gene families, the number and pattern of SNPs in SPS gene family III is also consistent with a single gene. For sugarcane SPS gene family V, while the small number of SNPs and the haplotypes recovered suggested a single gene, the two SD SNPs mapped to two different HGs, one of which is syntenic with sorghum. In maize, SPS gene family V also has two genes and a pseudogene, which have been mapped to maize chromosomes 3, 6, and 8 (Castleden et al. 2004). Interestingly, both gene locations for SPS gene family V in sugarcane appear to be syntenic with the gene locations for SPS gene family V in maize. In sugarcane, SPS gene family V SNPs were mapped to HGs 3 and 5. HG 3 is syntenic with Sb-03, which is syntenic with the duplicated chromosomes 3 and 8 in maize (Aitken et al. 2014a; www.phytozome.org; http://gramene.org/). The relevant region of sugarcane HG 5 is syntenic with Sb-05 and maize chromosome 6 (Aitken et al. 2014a; http://gramene.org/). Also of interest, however, is our observation that the sorghum ESTs for SPS gene family V (Castleden et al. 2004) appear to map to two different regions on SB-03, which suggests that there may be two genes in this family in sorghum as well.

QTL analysis for sugar-related traits has been carried out in sugarcane (Hoarau et al. 2002; Aitken et al. 2006; Piperidis et al. 2008; Alwala et al. 2009; Pinto et al. 2010; Singh et al. 2013). In the present study, SNPs from SPS gene families I, III, and V were weakly associated with agronomic traits while SNPs from SPS gene family IV were strongly associated with sugar-related traits. These SNPs mapped to HGs 2, 1, 3, and 7, respectively. In Q165, QTL for sugar-related traits have been identified on six of the eight HGs—HGs 1, 2, 3, 4, 5, and 6 (Aitken et al. 2006). Interestingly, the strongest marker-trait associations (MTAs) in this study were observed between SPS alleles on HG 7 and the sugar-related traits; yet, no associations were observed in the earlier study by Aitken et al. (2006). This may be due to the sparser genomic coverage on HG 7 and HG 8, compared to the other six HGs in the earlier map (Aitken et al. 2005), and the larger number of markers now mapped to these two HGs (Aitken et al. 2014b). In Aitken et al. (2006), the map used for the QTL analysis (Aitken et al. 2005) contained 14 and 11 markers in HG 7 and HG 8, respectively, out of a total of 910 markers. The map used in this study (Aitken et al. 2014b) contains 204 and 295 markers on these two HGs, respectively, out of a total of 2267 markers.

Several other sugarcane studies have identified MTAs for sugar-related traits (Hoarau et al. 2002; Piperidis et al. 2008; Alwala et al. 2009; Pinto et al. 2010; Singh et al. 2013). In each study, many MTAs and QTL were detected for each trait. Of these studies, only the studies of Hoarau et al. (2002) and Piperidis et al. (2008) used maps with some markers in common with the map used in the current paper. Although the number of common markers is very small (Aitken et al. 2005, 2014b), they do enable common HGs to be identified with a limited level of within-HG resolution.

Interestingly, Hoarau et al. (2002) also identified MTAs between Brix and stalk number and markers on HG III (=HG 7 in Aitken et al. 2005, 2014b). Hoarau et al. (2002) also identified MTAs between stalk diameter and markers on HGs I, IV, and X (=VI in Rossi et al. 2003) (=HGs 4, 6, 8, respectively, in Aitken et al. 2005, 2014b), and between stalk number and markers on HGs 8 and 10 (=VI in Rossi et al. 2003) (=HGs 2, 8, respectively, in Aitken et al. 2005, 2014b). Piperidis et al. (2008) identified MTAs for Brix and pol on HGs VII (=HG 1) and II (=HG 3) and for fibre and stalk weight on HGs VII (=HG 1) and VII (=HG 2). In each study, these markers each explained a similar amount of phenotypic variation—2–7 %.

The above QTL results are consistent with biochemical studies in which levels of expression of SPS have been associated with higher sucrose content in sugarcane (Zhu et al. 1997; Grof et al. 2007; Verma et al. 2010). Grof et al. (2006) also noted that the five SPS gene families had different patterns of expression in leaf and stem tissues and at different developmental stages. SPS gene families I and V were predominantly expressed in both immature and mature leaves; this expression pattern suggests the production of higher levels of sucrose in the leaves which can then be transported to other tissues for a variety of purposes, which may explain the association between these gene families and traits such as stalk weight, number and diameter, and fibre. Expression of SPS gene family II was lowest in leaves and increased down the sugarcane stem. Although no SD markers for this gene family were associated with traits, the HG to which this gene family maps has been shown by Hoarau et al. (2002) to be associated with stalk diameter, a trait more relevant to mature (lower) sugarcane internodes. SPS gene families III and IV were expressed at similar levels in young and mature leaves and stems. Grof et al. (2006) also noted that these two families were most highly represented in the sugarcane stem and speculated that these two families may contribute to the high sucrose levels observed in the stem. This suggestion by Grof et al. (2006) is consistent with the observed strong association between markers from SPS gene family IV and both sugar-related and agronomic traits in both Q165 and IJ76-514.

Sugarcane is an important crop with a complex genetic structure. Using SD and DD SNPs, we have now mapped all five SPS gene families to different sugarcane HGs and demonstrated that they are syntenic with the location of this gene family in sorghum. The haplotype patterns indicate multiple alleles per gene with a single gene per family for SPS gene families I–IV, similar to sorghum, and two genes for SPS gene family V, similar to maize. SNPs from SPS gene family IV, in particular, were shown to be associated with sugar-related traits in a sugarcane mapping population, confirming the role of this family as a significant enzyme in the production of sucrose in sugarcane.

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